FOOD SCIENCE & FOOD BIOTECHNOLOGY IN DEVELOPING COUNTRIES

5th International Congress 2012

Nuevo Vallarta, Nayarit. México

Book Of Proceedings

AMECA

ASOCIACION MEXICANA DE
CIENCIAS DE LOS ALIMENTOS A.C.

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Use Of Lactic Bacteria And Resazurin For Monitoring The Cold Chain On Biological Products

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Introduction

During the commercialization of biological products, the temperature is important parameter to corroborate, commonly called cold chain this is defined as the logistics system comprising personnel, equipment and procedures for storing, transporting and maintaining the correct temperature (below 4 ° C). An effective way to address this problem is the use oftime temperature indicator (TTI)however, these devices have a limited economic and technical record as only spot temperatures.(**1**) The present research proposes the utilization of lactic bacteriaas a monitor that detects the temperature changes (. When bacteria feels different temperature, the metabolism ofthe bacteria is activated and then resazurin utilized this activation (hydrogen in mitochondrial activity) (**2)** to change the color of resazurin and then the natural blue color change to pink (Fig. 1). This is correlated to the time of exposition of the product too high temperature, when more time and temperature the change of the resazurin color is more rapid.

Fig 1. Resazurinreaction

Methods and materials

The present research has the aims to investigate the conditions and concentration oflactic bacteria that produce the objective referred above. For research, we used *Lactococcuslactisssplactis*BM147 grown in a medium that contained skim milk, glucose, yeast extract and sodium caseinate, the culture was kept at 29° during 10 hours.

We absorb in a 1x2 cm. wathman filter paper Number 4, 0.2 ml. of the bacteria culture with different concentrations (between $1x10^7$ and $1x10^4$), after, the papers were dehydrated in vacuum (15 to 25 mm Hg) at room temperature during 30 min. .In the same manner the resazurin papers was prepared utilizing a solution of resazurin (0.0125mg/25ml). Two papers (one with the culture and the other with resazurin)were put together in a polietilen bag that contains an encapsulated drop of water (0.3ml). The closed bag was maintained in refrigeration until being used.

To activate the kit we press the encapsulated drop of water until it splash. Then the two-wathman papers were hydrated and put in contact the resazurin and the bacteria. Then we monitored the change in color vs. time at different temperatures (4, 8, 22° C) and different concentration of bacteria.

When the optimal conditions were found (concentration of bacteria that rapidly changes the color of resazurin to pink at 22° C and slowly at 4° C). Then we make several kits and put one on different foods (pork and bovine meat, chicken and milk) obtain in super market, these were put in a manner that the food and the kit were exposed too the same temperature for the same time. We monitored the time when the blue color changes to pink or white , the concentration of total viable bacteria in the food were counted (mesophilic std. plate count), this was done at different temperatures (4, 8, 22° C) and the time when change of color happens.

Results and discussion

The results indicate that the resazurin color changes to pink in 2 hrs. at room temperature, with a bacteria concentration of $1x10^7$, 3 hrs. with a bacteria concentration of $1x10^6$, 6 hrs. with $1x10^5$ and 15 hrs. with $1x10^4$. At 4° C with less than one day for the concentration of $1x10^7$, two days for the concentration of $1x10^6$, and more than 12 days for the concentration of $1x10^5$ y $1x10^4$ (fig. 2 and 3)

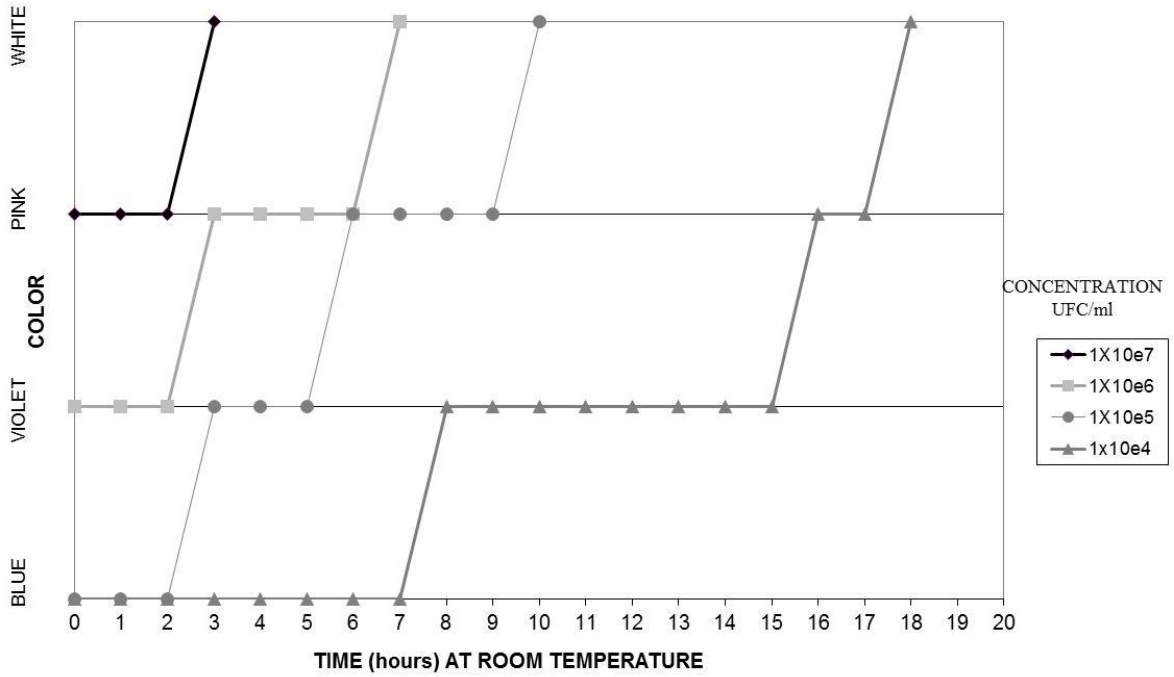

fig. 2. follow-up of the discoloration of rezasurin to room temperature with different concentrations of *L. lactis ssp. lactis*

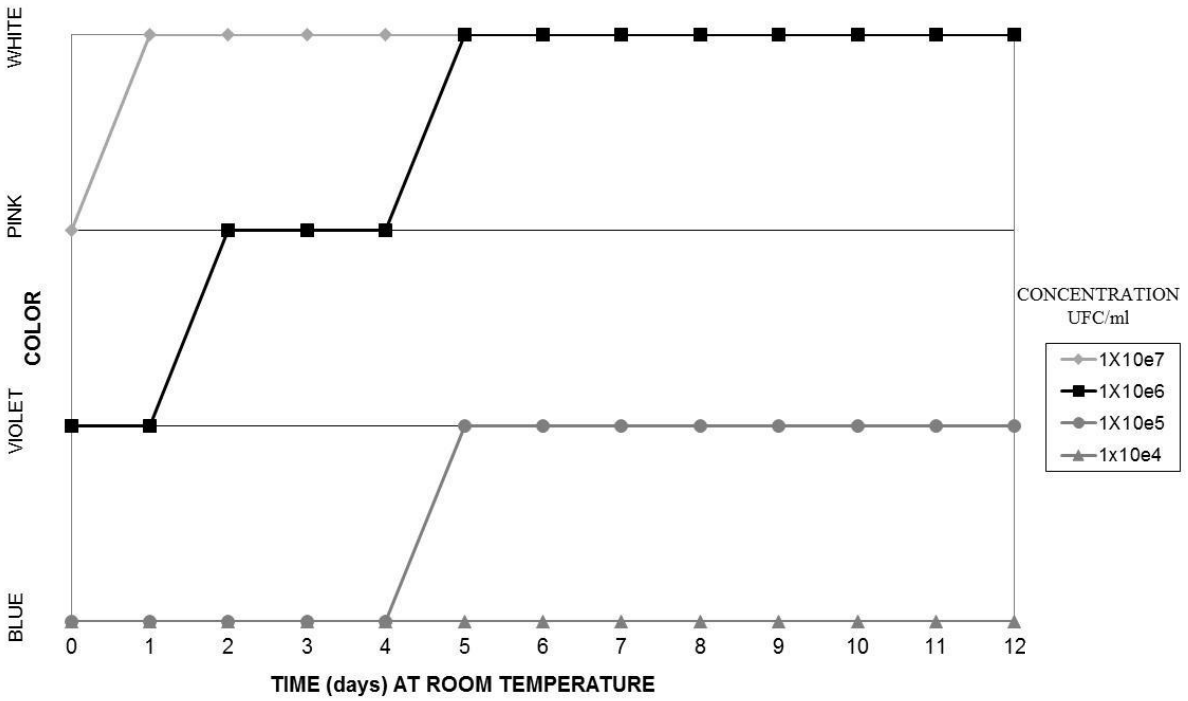

Fig. 3. follow-up of the discoloration of at 4°C with different concentrations of *L. lactis ssp. lactis*

This figure clear relates the change of color with the amount of time and temperature that bacteria were exposed.

On the other hand when the kit was put on a food, the results indicate, that we can predict the amount of bacteria present in the food with the change in the resazurin color.(Fig. 4, 5, 6)

In fig. 4 is plotted the evolution of bacterial count Vs Time at 3 different temperatures (22°C, 8°C, 4°C) of pork meat, the color of the line represent the color of the kit attached of the meat. Is clear that the pink color appears when the concentration of bacteria is between $1x10^6$ and $1x10^7$ but a different time for different temperature. 2or 3 hours at 22°C, 34 Hours at 8°C and 75 hours at 4°C (fig 5) In other investigations meat stored under refrigeration (open packing) predominantly reporting a gram-negative bacterial, not forming spores that cause putrefaction in the product. Thus, in these experiments was shown microbial load values between 10^7 a 10^8 UFC/g / g (**3**) (**4**). In fig 6 inmilk monitored was and the results be seen the samepattern.

Values of 10^6 UFC/ ml or more indicates that milk is not fit for consumption. The sensory characteristics presented are: yellow, putrid odor, presence of curds and sour taste, as reportedMunsch, A.P (**5**)AndBarbano, D.M (**6**). Who found that psychrotrophic bacteria are the main causes of the deterioration of the milk.which is consistent with what we found.

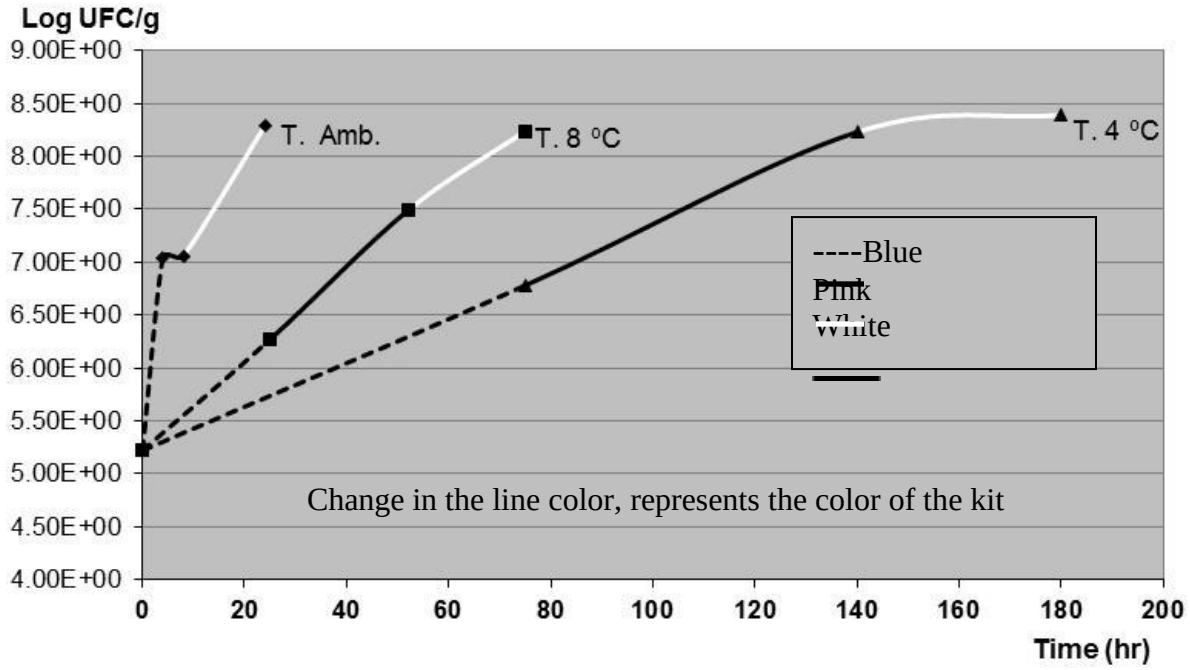

Fig 4. Relation of changes of color of kit with the microbial growth in pork meat

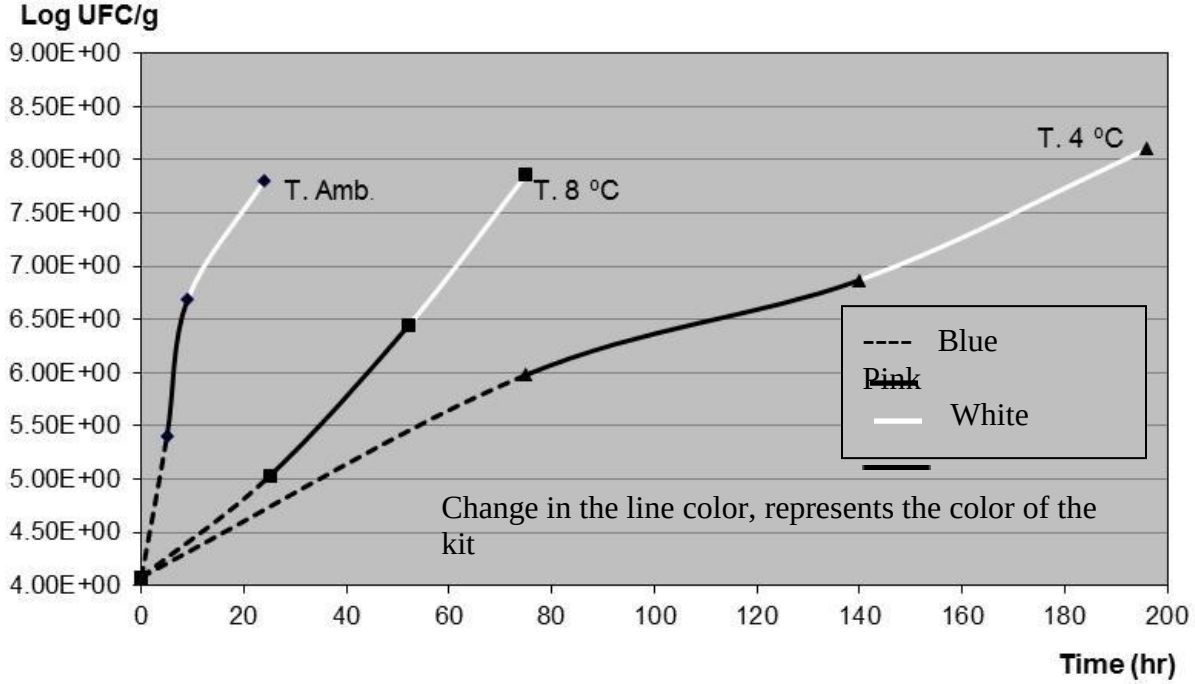

Fig 5. Relation of changes of color of kit with the microbial growth in chicken breast

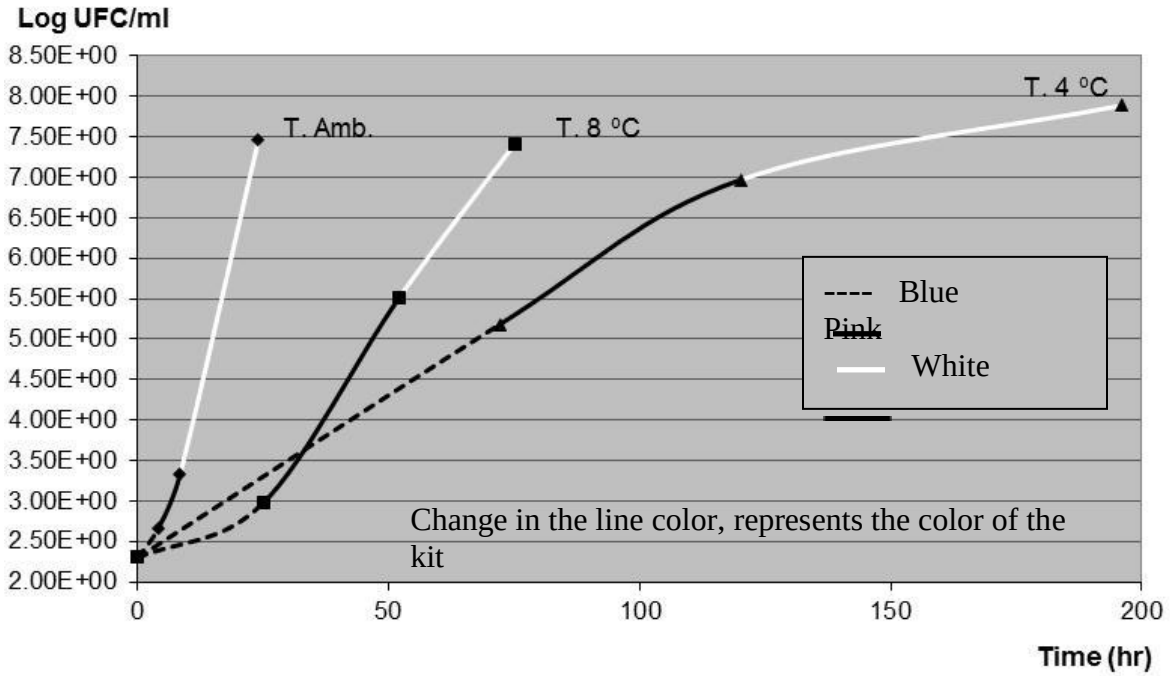

Fig 6. Relation of changes of color of kit with the microbial growth in in milk

In conclusion.With lactic bacteria and the resazurin. It is possible to detected changes in the temperature and time to which the foods were maintained and therefore, they are possible to be used in the monitoring the cold chain during the food commercialization.

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Preparation Of Betulinic Acid Nanoemulsions By High Pressure Homogenization

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Introduction

Betulinic acid (BA) is acompound from the group of pentacyclictriterpene-type lupine,found widely throughout the plant kingdom (1). BA displays wide ranges of biological activities, which includes anti-inflammatory, antimalarial, antiallergenic, hepatoprotector, hypolipidemic and anti-obesity potential by promoting weight loss. However,the most relevant of the reported activities are anti-HIV and anticancer (2-5). BA is a promising agent for the treatment of different diseases, however the potential therapeutic activity can be hampered due to its low solubility; andbothitsabsorption as well as its bioavailability is questionable.A drug delivery system is an alternative to improve the bioavailability of poorly soluble compounds; among these systems nanoemulsions have been reported as one of the most promising techniques. In this context, nanoemulsionsare thermodynamically unstable but kinetically stable systems of oil and water stabilized by an interfacial film of surfactant molecules. This system has globule sizesranging from 20 to 500 nm (6). In the present study we producedbetulinic acid nanoemulsionsprepared withphosphatidylcholineas emulsifier and different oils were employed as disperse phase, using a high-pressure homogenizer. The main objective of this work was to evaluate the influence of composition (emulsifier and oil concentration) and operational variables (pressure and number of cycles) on the characteristics of BA nanoemulsions made by high-pressure homogenization.

Materials and methods

Materials. Betulinic acid was obtained from ***Indofine*** Chemicals. Soy phosphatidylcholine (PC) with a purity >95% was obtained from Avanti Polar Lipids, Inc. (Alabaster, AL). Oils used were:Extra virgin olive oil (Crisco), corn oil (Crisco), coconut and palm oil (Sigma), flaxseed oil (Spring Valley), and medium chain oilcontaining mainly C8 and C10 (Captexfrom Abitec, Paris, IL).

Preparation of betulinic acid nanoemulsions

The aqueous and oily phases were prepared separately, the oil phase consisted of the oil in which the BA was previously dispersed by sonication and PC as emulsifier was added in concentrations of 7.5 and 10 % dissolved in Milli-Q water. Nanoemulsions contained BA at a concentration of 50 mg/L. The oil and aqueous phases were mixed in ratios of 5:95 and 10:90. The water phase was added to the oily phase and a coarse emulsion was formed using a rotor-stator homogenizer (Polytron PT-2100) at 1000 rpm for 2 minutes. After that, the coarse emulsion was passed through a high-pressure homogenizer (Nano DeBEE 45, BEE International, MA) adjusted to pressures of 68.9,137.9 and 206.8 MPa and homogenized for 5, 10 and 15 cycles. Nanoemulsions formed were stored at 25 °C for three months.

Nanoemulsion characterization

Globulesize. The mean globule size and size distribution were determined by dynamic light scattering (DLS) at 25 °C and ascattering angle of 90° using a ZetasizerNano-ZS90 (Malvern Instruments Inc). Size

distributions were reported by the polydispersity index (PdI). The samples were diluted withmilli-Q water prior the measurement.

Zeta potential measurements. Measurement of the surface charge was determined by measuring the zeta potential of the nanoemulsionusing the Zetasizer at 25 °C. The nanoemulsions were diluted with Milli-Q water.

Determination of entrapmentefficiency. The entrapmentefficiency of the nanoemulsion was determined separating the undissolvedBAby centrifugation at 11,000 rpm for 30 min. After centrifugation, the BA concentration in the nanoglobules was calculated from the final concentration of BA after preparation. The BAentrapment efficiency in the nanoemulsion was expressed as percent of the BA encapsulated by the globule in the nanoemulsion and was calculated with the following equation:

$$\text{Entrapment Efficiency } (\%) = \frac{\text{Betulinic acid in the nanoemulsion}}{\text{Total betulinic acid used to prepare the nanoemulsion}} \times 100 \quad (1)$$

The BA content was determined by HPLC with UV detection according to the method reported by Zhao *et al.* (7).

Nanoemulsion stability. The evaluation of the stability was made byperiodically measuring the globule size and PdI of the nanoemulsionsthat were stored at 25 °C for up to 3 months.

Data analysis. The experiment was conducted in duplicate and each analysis consisted of at least three measurements. In this study, a 3x3x2 factorial design was employed to investigate the influence of the variables (pressure, number of cycles and emulsifier concentration) on the globule size and the size distribution of the BA nanoemulsions made with olive and medium chain oils as the dispersed phase. All data analyses were performed using the StatisticaData Analysis Software v. 5 (StatSoft Inc.).

Results and discussion

The globule size reduction in nanoemulsions can be achieved by varying conditions in the formation variables such as type and concentration of emulsifier,dispersed phase content, and by the energy input (8). The energy input can be applied by changing the pressure or the emulsification time, or by repeating the operation for more cycles. Then, in this study we studied the effect of these variables on the characteristics of Betulinic acid nanoemulsions.

Factorial design analyses. The effect of the variables on the globule size and the size distribution was evaluated for the nanoemulsions made with olive and medium chain oils as the dispersed phase (table 1).According to factorial design analyses for olive oil nanoemulsions, for globule size all the main factors and the interactions pressure-cycle and pressure-emulsifier had effect, however the pressure was the most significant factor followed by cycles and emulsifier concentration. For the size distribution, the variable that had the highest outcome was pressure and the effect of the other variables were notsignificant. Similar results were found for globule size in medium chain nanoemulsions, although for size distribution, pressure and the interaction pressure–number of cycles had a significant effect.

Table 1. Analysis of variance for globule size and size distribution

Variable	Particle size		Size distribution	
	F- Value	P- value	F-value	P-value
P	2016.55	0.000	20.25	0.000
C	387.22	0.000	1.11	0.334
E	315.81	0.000	3.39	0.069
Interactions				
P-C	95.15	0.000	0.56	0.690
P-E	3.51	0.034	0.40	0.672
C-E	0.44	0.649	0.14	0.870
P-C-E	1.50	0.208	0.06	0.994

P (pressure), C (number of cycles), E (emulsifier concentration)

Effect of the emulsifier concentration.

In this work, when we employed 7.5 and 10 % PC and with higher pressure and number of cycles. Increasing the emulsifier concentration resulted in a decline of globule size (Table 1), that can be explained by the fact that at a concentration of 7.5 % PC, the emulsifier available to cover the globule surface was insufficient; when emulsifier concentration was increased(10 %) the globule surface was better covered and greater resistance to deformation when globules coalesced. Globule sizes obtained at 137.9 MPafor 15 cycles, and 206.8 MPafor 10 and 15 cycles, were not significantly different, producing a minimum globule size of 206 nm. Emulsions made with 10 % PC at all the pressures and number of cycles proven were less disperse, but it was not significantly differentfrom the results employing 7.5% PC.

Table 2. Characterization of globule size and size distribution of BA nanoemulsions prepared at different emulsifier concentrations

PC Concentration (%)	Globule size (nm)	Size distribution (PdI)
7.5	213.2 ± 1.5^{a}	0.086 ± 0.018^{a}
10	194.9 ± 0.9^{b}	0.081 ± 0.03^{a}

The BA nanoemulsions were prepared with a 5:95 olive oil ratio at 206.8 MPa and 5 cycles. Means with different letters in the same column are significantly different from each other (ANOVA, Tukey, $p < 0.05$).

Effect of different pressures and number of cycles on Betulinic acid nanoemulsions. We expected the mean globule size to decrease with higher pressure and number of cycles because as pressure was increased the shear forces and turbulence were also increased. Figure 1 shows the globule size of the nanoemulsions made with 10 % PC and 5% olive oil prepared at different pressures and number of cycles. The increment in the homogenization pressure resulted in a significant decrease in globule size for all pressures employed. After 5 cycles the minimum globule size of 213 nm at 206.8 MPa was obtained. As expected, an effect of the number of cycles in the reduction of globules size was observed; at 68.9 and 137.9 MPa increasing the number of cycles resulted in a significant reduction of the globule size. However, at 206.8 MPaand by applying more than 5 cycles made no significant effect on globule size reduction. By increasing pressure and number of cycles it was possible to reduce globule size at values as low as 193 nm. At 137.9 MPa and 15 cycles no significant differences were found among nanoemulsions prepared at 206.8 MPa with 5 to 15 cycles.

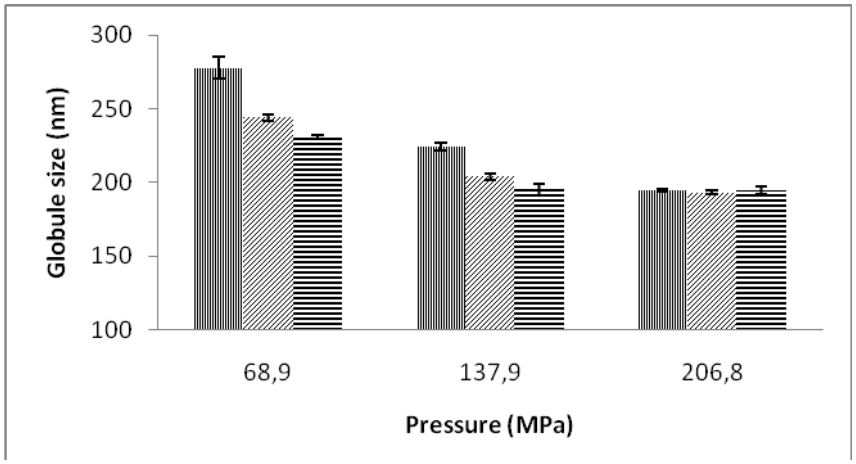

Figure 1. Globule size of BA nanoemulsions at different pressures and cycles. Key: 5 cycles, vertically striped bar; 10 cycles, diagonally striped bar; 15 cycles, horizontally striped bar. Bars indicate standard deviation.

The effect of the pressure and number of cycles on the size distribution of nanoemulsions was evaluated (Table 3). The width of the size distribution was reduced as the pressure increased; however, the effect of the number of cycles on PdI did not show a consistent pattern. No significant difference was observed between the PdI values of nanoemulsions produced at different pressures and cycles.

Table 3. Characterization of size distribution of BA nanoemulsions prepared at different pressures and cycles

Pressure (MPa)	**Cycles**		
	5	10	15
68.9	0.122 ± 0.046[a]	0.113 ± 0.029[a]	0.109 ± 0.026[a]
137.9	0.086 ± 0.024[a]	0.071 ± 0.035[a]	0.077 ± 0.018[a]
206.8	0.081 ± 0.030[a]	0.072 ± 0.011[a]	0.077 ± 0.026[a]

The BA nanoemulsions were prepared with a concentration of 10 % PC and 5% olive oil. Means with different letters are significantly different from each other (ANOVA, Tukey, $p < 0.05$).

Effect of the oil ratio. The oil concentration was increased at O/W ratio from 5:95 to 10:90 (Figure 2) and an increase in globule size was noted. At 10:90 using a 10 % PC a mean globule size of 206 nm was obtained with 137.9 MPa and 15 cycles. By increasing the oil volume the number of globules formed increased, causing more globule-globule interactions, which favored globule aggregation and in turn increased the mean globule size. When increasing the oil ratio an apparent over-processing occurred when the number of cycles was increased to 15 at 206.8 MPa. As energy input was increased higher collision frequency was produced, thus more “uncovered” globules were available to coalesce and resulted in increased globule size and dispersion. Hence, for the 10:90 oil ratio, treatments beyond 137.9 MPa and 15 cycles did not produce an improvement in globule size.

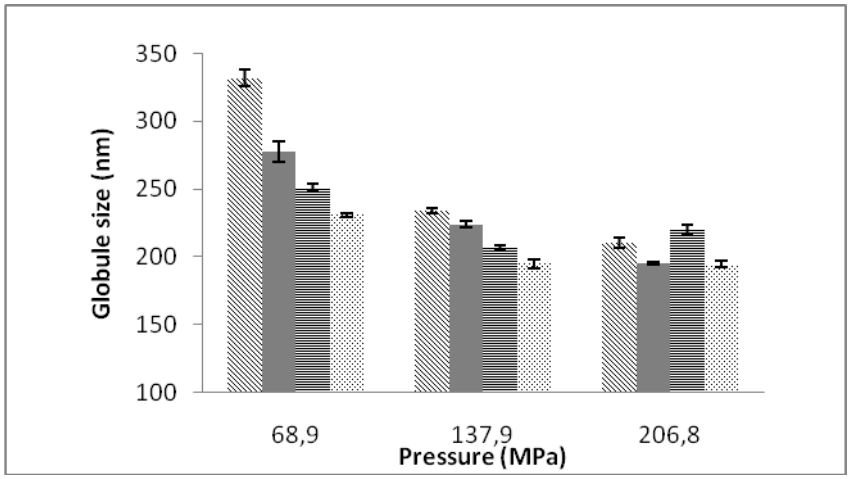

Figure 2. Globule size of BA nanoemulsions at different oil ratios. Key: 10 % oil and 5 cycles, diagonally striped bar; 10% oil and 15 cycles, horizontally striped bar; 5 % oil and 5 cycles, gray bar; 5% oil and 15 cycles; dotted bar. Bars include their standard deviation.

Increasing the oil content had an effect on the size distribution of the nanoemulsions, keeping high values of PdI compared with the oil ratio 5:95 at the same conditions (pressure and cycles), which was less favorable in terms of stability (table 4).

Table 4. Characterization of size distribution of BA nanoemulsions prepared at different oil ratios

Pressure MPa	10:90	5:95
68.9	0.124 ± 0.033^{a}	0.122 ± 0.046^{a}
137.9	0.095 ± 0.036^{a}	0.086 ± 0.024^{a}
206.8	0.091 ± 0.0186^{a}	0.081 ± 0.030^{a}

The BA nanoemulsions were prepared with a concentration of 10 % PC and 5 cycles. Means with different letters in the same line are significantly different from each other (ANOVA, Tukey, $p < 0.05$).

Influence of oil type on BA nanoemulsions. The effect of type of oil used as continue phase on globule size was evaluated. In this study we choose to use a pressure of 206.8 MPa and 5 cycles as the best conditions in the homogenizer, because less cycles were necessary to achieve the lowest globule size and the energy expenditure is reduced. Table 5 shows the results when differents oils at O/W ratio 5:95 and 10 % of PC was employed. Is observed that the change in oil resulted in a change in globule size and the size distribution, this reduction can be due to the fatty acids composition of the oils (chain length and unsaturation) as well as the viscosity (9).

Table 5. Characterization of globule size and size distribution of BA nanoemulsions prepared with different oils.

Oil	**Globule size (nm)**	**PdI**
Corn	187.6 ± 2.7^{a}	0.072 ± 0.009^{a}
Flaxseed	178.5 ± 1.7^{b}	0.075 ± 0.023^{ab}
Palm	181.1 ± 2.6^{b}	0.106 ± 0.013^{bc}
Coconut	172.2 ± 1.3^{c}	0.082 ± 0.027^{abc}
Medium chain oil	162.8 ± 1.7^{d}	0.089 ± 0.015^{abcd}

The BA nanoemulsions were prepared with at concentration of 10 % PC and 5% oil at 206.8 MPa and 5 cycles. Means with different letters in the same line are significantly different from each other (ANOVA, Tukey, $p < 0.05$).

Zeta potential of the emulsions. Negative values for zeta potential are reported when PC was used. The data are shown in table 6 for olive oil and medium chain oil at the highest pressure tested, for 5 – 15 cycles, at 10% PC and a ratio O/W of 5:95. Zeta potential values for the best conditions were -14 mV and -22.3 mV for olive oil and medium chain oil, respectively. The higher value obtained with medium chain oil was possibly caused by an over-processing and increase of temperature that may have caused partial hydrolysis of PC with formation of Lyso PC and thus produced an increase on zeta potential values (9). Values up to (+/-) 30 mV are considered suitable for good electrical stability (10); based on this, PC contributed only lightly to the zeta potential and globule stabilization.

Table 6. Zeta potential values for PC stabilized BA nanoemulsions

Cycles	Zeta Potential (mV)	
	Olive oil	Medium Chain Oil
5	-14.00 ± 1.05[a]	-22.33 ± 1.00[a]
10	-13.00 ± 0.44[a]	-18.77 ± 0.57[b]
15	-14.23 ± 0.35[a]	-48.40 ± 0.62[c]

Each value in the table represents the mean ± standard deviation of at least tree measurements from two replications. Means with different letters in the same line are significantly different from each other (ANOVA, Tukey, $p < 0.05$).

Entrapment efficiency. The concentration of betulinic acid was determined in the nanoemulsion with olive and medium chain oil like dispersed phase at 206.8 MPa and 5 cycles considered as the best conditions, obtaining a considerable entrapment efficiency of 76 and 80 %, respectively and there was no significant difference among these two values.

Nanoemulsion stability. Changes during storage were studied measuring the globule size and PdI in olive and medium chain oils at the best homogenizing conditions found previously in this work (Table 7). There were no significant changeson globule size and PdI during the 3 months of storage, the size of globule increment was 2 % at most. These results depict the stability of nanoemulsions using PC during the time designed for this work. Nanoemulsions showed overall good stability during this time, with no drastic effects on globule diameter and size distribution, both are important variablesthat control emulsion stability.

Table 7. Characterization of globule size and size distribution of BA nanoemulsions during storage time.

Week	**Globule size (nm)**		**PdI**	
	Olive Oil	Medium chain oil	Olive Oil	Medium chain oil
0	194.9 ± 0.9[a]	162.8 ± 1.7[a]	0.081 ± 0.033[a]	0.089 ± 0.015[a]
1	193.8 ± 1.5[a]	160.7 ± 1.2[a]	0.089 ± 0.034[a]	0.080 ± 0.008[a]
2	196.7 ± 1.8[a]	160.8 ± 2.1[a]	0.093 ± 0.038[a]	0.079 ± 0.021[a]
3	197.0 ± 3.8[a]	161.8 ± 1.4[a]	0.080 ± 0.015[a]	0.087 ± 0.012[a]
4	196.8 ± 1.5[a]	162.3 ± 1.1[a]	0.083 ± 0.012[a]	0.077 ± 0.011[a]
8	196.9 ± 1.9[a]	163.4 ± 2.4[a]	0.085 ± 0.029[a]	0.078 ± 0.021[a]
12	198.8 ± 4.6[b]	164.0 ± 1.7[a]	0.093 ± 0.025[a]	0.084 ± 0.016[a]

BA nanoemulsions were prepared with a concentration of 5% oil, 10 % PC, 206.8 MPa and 5 cycles. Means with different letters in the same line are significantly different from each other (ANOVA, Tukey, $p < 0.05$).

Conclusions

Globule size and distribution were influenced by emulsifier concentration, pressures and number of cycles. Reducing the oil concentration and even using a different type of oil, allowed us to achieve a reduction of globule size. Higher emulsification pressures produced marked reductions of globule size, homogeneous size distributions and the nanoemulsions showed good stability. Homogenization is suggested as a method capable to achieve the reduction of globules to nanometric scale.

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Production Of Nixtamalyzed Corn Flour By Continuous Ohmic Heating

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Introducción

Extrusion is beyond doubt one of the most promising alternative processes in the industry of nixtamalized corn flours (Bazúa y otros 1979). In this process, there is no wash water or nejayote, and it is used the whole corn grain. This process produces some important changes in the physical, rheological, structural and textural characteristics of dough and tortilla, as compared to those obtained by the traditional nixtamalization process. These changes are due to the interactions between the constituents of the corn grain and the conditions used in the process, such as temperature generated by a heating jacket, screw conveyor speed and geometry, as well as lime and humidity concentrations. That is why extrusion has not been spread in corn flour and tortilla industry (Rodríguez *et al.*, 1996).

An alternative technological process that has become increasingly popular in the food industry is the ohmic heating. Ohmic heating is a thermal process in which heat is internally generated by the passage of an alternating electrical current (AC) through a material, such as food, that offers electrical resistance. The principles of ohmic heating are quite simple: An AC is applied at both ends of the product; the heating rate is directly proportional to the square of the intensity of the electric field, the electrical conductivity, and the type of product heated (Ruan *et al*., 1999). Because heat is internally generated, the process is easy to control. Gaytán *et al*. (2011) obtained nixtamalyzed corn flours using "batch" ohmic heating with good quality characteristics; the objective of this work is obtaining nixtamalyzed corn flours using a continuous ohmic heating process determining their quality characteristics and comparing them with those of traditionally nixtamalyzed corn flour.

Materials y methods

Raw material: White corn was bought in Querétaro, Mexico, with grains 8.64, 11.72 y 4.30 mm width, length and thick, 82 kg/hL hectoliter weight, and 23 flotation indexes. These characteristics qualify the grain as apt to produce tortilla (NMX-FF-034/1-SCFI-2002) and as hard grain (NMX-F-046-S-1980). Corn was processed using the traditional nixtamalization and the continuous ohmic heating system.

Traditional nixtamalization: Corn (1 kg) was added to 2 L of water with $Ca(OH)_2$ (1%), cooked to boiling temperature during 30 min, stepping in the same liquid during 19 h, dehydrated in a flash dryer (270 °C income and 40 °C exit temperature), ground in a Pulvex mill, mesh 0.5 and stored in plastic bags at 2 $\pm$ 2 °C.

Continuous ohmic heating system: This equipment (Figure 1) was patented by Morales et al. (2010) and is divided in two zones: Transport and heating. It consists of a screw conveyor to transport corn flour, coupled to a rectangular ohmic heating cell 25, 5 and 2 cm length, width and height, respectively. At both sides of the cell are two stainless steel electrodes connected to AC voltage. The processed material exit occurs without reduction of area neither exit dye, which avoids the application of pressure over the material. The equipment has a temperature controller Watlow Mod. 981 with a K type temperature sensor,

a variable transformer voltage AC at 60 Hz (Variac), a bench multimeter HP to measure AC voltage and current and a speed controller Baldor. The temperature controller allows or avoids the passage of AC current with the appropriate potency to reach the desired temperature (maximum potency is 1500 watts).
To nixtamalize by continuous ohmic heating it was used corn ground in a Pulvex mill, mesh 1.3 micron and the amount of water stipulated for each experimental sample with $Ca(OH)_2$ (0.3 %), it was fed to the equipment and heated until the stipulated temperature (Gaytán-Martínez *et al*., 2011), material was dehydrated in a flash dryer, ground in a Pulvex mill, mesh 0.5 and stored in plastic bags at 2 $\pm$ 2 °C.
Processing of corn flours by ohmic heating: Corn grain was milled using a Pulvex mill, 1.3 mm mesh; milled corn (500 g) was mixed with 0.3 % of Ca(OH)2 and the amount of water determined (experimental design) for this particular treatment was added and homogeneously mixed. The mixture was fed in the ohmic heating equipment and processed at the transport speed and temperature indicated in the experimental design. The processed material was dried in a Flash type dryer (CINVESTAV development) at an input temperature of 270-275 °C and output temperature of 40 °C. It was milled in a Pulvex mill, 0.5 mesh and stored in plastic bag at 4 °C until analysis.

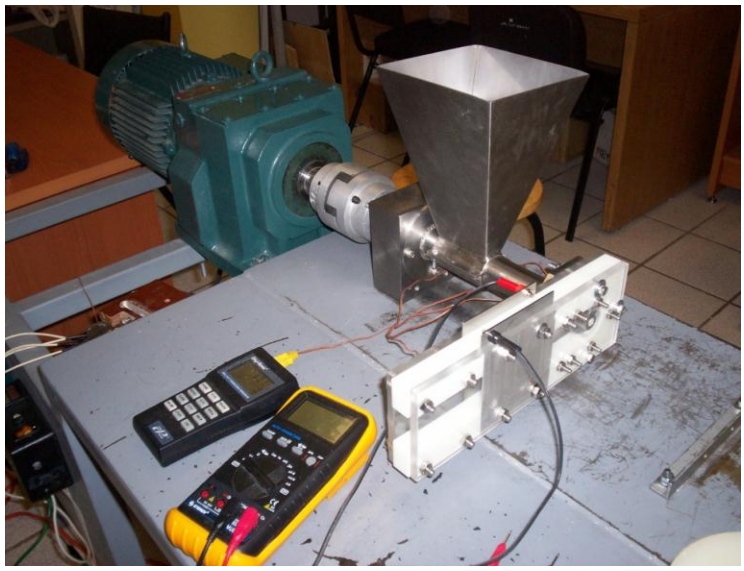

Figure 1. Ohmic heating equipment

Corn flour characterization: Water absorption (WAI) and water solubility (WSI) indices were determined by the method descripted by Anderson *et al*. (1969). To perform Dunnet's test between ohmic heated flour (OHF) and traditionally nixtamalized flour (TNF), WSI values of TNF were adjusted adding the solid compounds lost in decanted water, considering data reported by Campechano *et al*. (2011). Dynamic viscosity was determined in a Phsica MCR 1001 rheometer, according to the method descripted by Gaytán-Martínez et al. (2000). **Experimental design and statistical analysis:** Experimental design was a surface response methodology with three independent variables (Table 1) were: feed moisture transport speed and material's exit temperature), second order and five central points. It was also performed a Dunnet's test to evaluate differences between OHFs and TNF. Data were analyzed using a Minitab 14 program.

Table 1. Factors y levels used to obtain corn flour using a continuous ohmic heating equipment.

Variable	level	
	Minimum	Maximum
Feed moisture (%)	50	60
Temperature (°C)	75	85
Transport speed (rpm)	10	20

Results and discusion

Water absorption index (WAI) is one of the most important characteristics of corn flours to prepare tortillas; it is related with the capacity of flours to retain water and affects flexibility and yield of tortillas (Almeida-Domínguez *et al.*, 1996).

Processing parameters that significantly affected WAI was temperature ($p \leq 0.001$), as well as interactions between moisture-temperature and temperature-transport speed ($p \leq 0.05$). Figure 2 shows that at high moisture and temperature is when the highest WAI occurred. WAI increases because of starch modification during the thermal-alkaline processing.

Dunnet's test indicated that there are significant differences between the WAI of OHCF (2.52-3.15 g of gel/ g of flour) and TCF (2.23 g of gel/ g of flour) indicating higher values of WAI of OHCF.

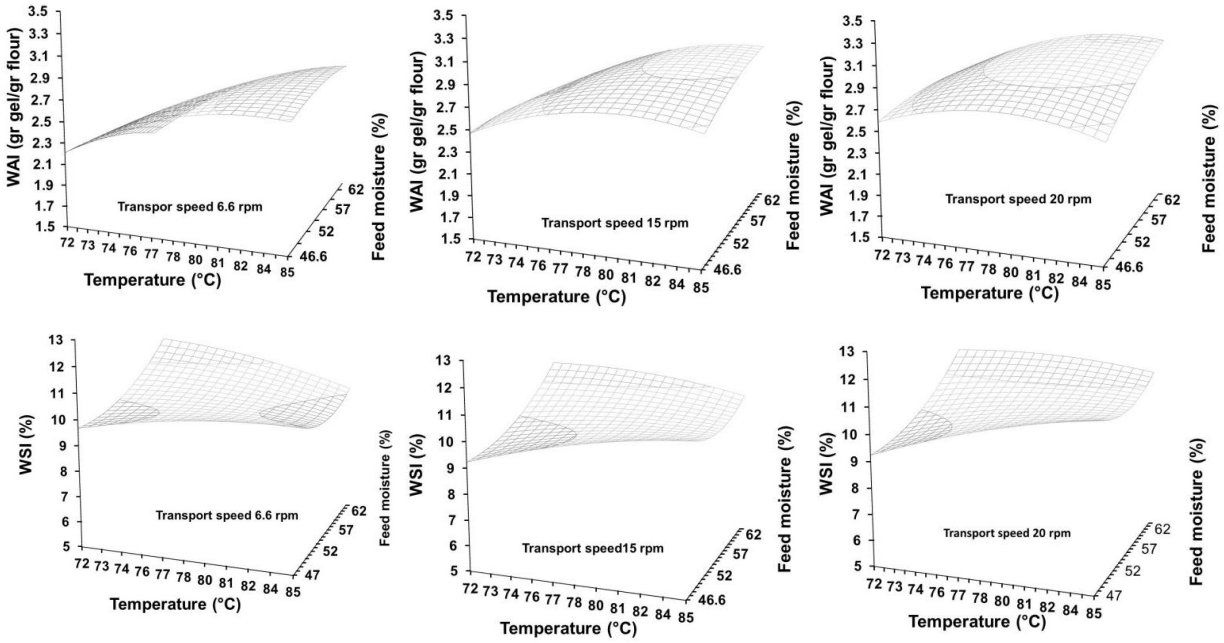

Figure 1. Water absorption index and water solutility index of flours processed by ohmic heating.

Water solubility index (WSI) indicates the fragmentation degree of starch contained in flours; this fragmentation is produced during the cooking process and it is expressed as solids soluble in water (Bressani et. al., 2001). WSI was significantly affected ($p \leq 0.05$) by temperature as well as the quadratic effect of moisture and interactions between moisture-temperature and temperature-transport speed. Samples processed at high moisture presented the highest WSI (Figure 1). OHCF (8.83 %) presented significantly higher values of WSI (Dunnet's test) tan TCF (5.96 %); however, when the loss of solids (Campechano *et al*., 2011) in washer water (nejayote) is considered (6 %) there are not significant differences ($p \leq 0.05$) between them. WSI is related with corn hardness, and thermal and alkaline cooking, which degrades corn bran. Most of the solids are solubilized during washing and for industrial processing during agitation and washing (Campechano *et al*., 2011; Rosentrater, 2006). It must be considered that some solids lost in wash water are hydrocolloids, which are present in OHCF because there is not wash water in this process improving textural characteristics of tortillas elaborated with these flours.

The process variables influencing significantly ($p \leq 0.01$) the viscosity profile of OHCF were moisture and temperature, as well as their quadratic effects.

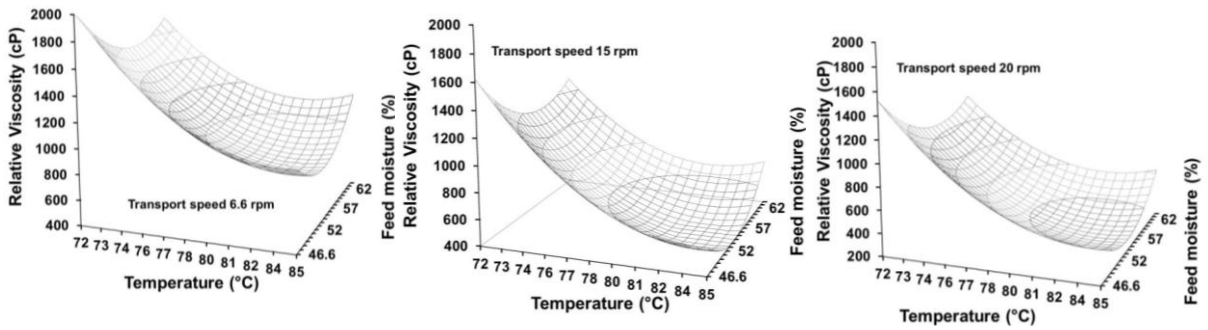

Figure 2. Viscosity profile of ohmic heated corn flours.

Dunnet's test showed a significant difference ($p \leq 0.05$) between OHCF (392-2000 cp) and TCF (5064 cp), reflecting the differences of the heating and milling between both processes. Gaytán-Martínez *et al*. (2012) reported that during ohmic heating of corn flours, the mixture corn-water-lime is heated homogenously because heat is generated internally by the alternative electric current applied; there are produced structural changes in starch, which traduces as a low viscosity. On the other hand, milling is an obligated operation before continuous ohmic heating, while the hole grain is used in nixtamalization. After nixtamalization, there are produced some changes in corn starch due to the nixtamal grinding when a stone-grind is used, because of friction between stones.

Conclusions

Continuous ohmic heating allowed obtaining of corn flour with similar characteristics to those processed by the traditional nixtamalization technique; so, continuous ohmic heating may be considered as an excellent alternative technology to solve those problems associated with polluting effluents, water waste, and dissipation of processing energy. It has also the advantage of a processing short time and the contribution of the whole grain nutrients and fibre.

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Changes In The Content Of Ascorbic Acid, Total Polyphenols And Total Carotenoids In 'Ataulfo' Mango Treated With High Hydrostatic Pressure

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Introduction

High hydrostatic pressure (HHP) is a food preservation process relatively new; is considered a non-thermal technology applied in the food industry because pressurization is carried out at room temperature (1). High pressure processing has not much studied yet on extracting useful compounds which are sensitive to heat from many food resources; therefore, the high pressure extraction process could definitely contribute to wide ranges of food industries for improving extraction yields correlated to their biological activities (2). The production of 'Ataulfo' mangoes in Mexico is important because it is one of the main varieties that are exported (3). Currently there are no reports on the effect of HHP on the nutritional components of whole Ataulfo mango postharvest; therefore, the aim of this study was to evaluate the effect of APH on some antioxidant compounds of 'Ataulfo' mangoes postharvest.

Materials and methods

Plant material. Were used (*Mangifera indica* L) 'Ataulfo' mangoes at physiological maturity.The fruits were transferred to the laboratory, washed and immersed in a solution of 2 - (4-thiazolyl)-1H-benzimidazole (20 mg/l) for 5 min to prevent fungal growth. Then they are dried and immediately used for this study.

High hydrostatic pressure treatment. The mangoes were divided into seven groups of 50 fruits, then each batch is subjected to different pressure treatments: 15, 30 or 60 MPa, for 10 or 20 min. A control group remained untreated pressurization. Hydrostatic pressure treatments were applied using an isostatic press; model CIP42260 (Avure Autoclave Systems, Erie PA USA). The fruits were covered with a film of high-density polyethylene before being introduced into the treatment chamber to avoid direct contact with the pressurizing liquid. Pressurized mangoes stored at 25±1 °C and 85-90% relative humidity (RH) until senescence.

Ascorbic acid analysis (AA). Was analyzed using the technique of Suntornsuk et al (4). It was weighed 10 g of mangoes pulp and homogenized with 25 ml sulfuric acid (2 N), 25 ml of distilled water and 3 ml of starch as indicator. The mixture was titrated with a solution of iodine (0.05 N). Reported in mg ascorbic acid per 100 g fresh weight (FW).

Total carotenoids analysis (TC). 2 g of mango pulp were weighed, it was added 7 ml of acetone:petroleum ether 80:20 (v/v) and 0.5 g of $MgCO_3$ and the mixture was homogenized for 1 min. The homogenate was centrifuged at 15.000 rpm for 30 min at 4 ° C. The supernatant was placed in a separator funnel, adding 15 ml of NaCl (20%). The aqueous phase was drained and the organic layer was diluted to 10 ml with petroleum ether. Absorbance was measured at 448 nm in a spectrophotometer UV/Vis (Jenway model 6705). The quantification was done using a calibration curve as a standard of β-carotene. The result was expressed as mg β carotene per 100 g dry weight (DW) (5).

Extractable phenols analysis (EP). For extraction, 10 g of pulp was homogenized with 96% ethanol and sonicated in a sonication equipment Cole-Parmer model 8891 for 30 min. Subsequently the sample was centrifuged (centrifuged Hermle Labortechnick model Z36HK) at 11500 rpm, 10 ºC for 10 min. The supernatant was concentrated to a volume of 4 ml; then was added 5 ml Folin Ciocalteu's reagent and 20 ml of sodium bicarbonate 20%. The reaction was allowed for 30 min and absorbance was measured at 760

nm in a spectrophotometer UV/Vis. To quantify the EP was carried out a calibration curve with standard chlorogenic acid. The result was expressed as mg chlorogenic acid per 100 g DW (6).

Antioxidant capacity or antiradical efficiency (AE). Was performed using the method of Sánchez-Moreno et al (7). To obtain the extract antioxidant, 5 g of pulp was placed under stirring with 10 ml of absolute methanol for 2 h. Then centrifuged for 30 min at 4 ° C and 15000 rpm. A solution 25 mg/l of the radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH·) was prepared. Were taken 5, 10, 20, 30, 40 and 50 µl of antioxidant extract and these were reacted separately with 3.5 ml of the solution of DPPH·. Absorbance was measured at 515 nm every minute for 5 min in a spectrophotometer.

Statistical analysis. Data were analyzed with a design of randomized complete block with factorial 3 x 2 (hydrostatic pressure of 15, 30 and 60 MPa x the treatment time of 10 and 20 min), where the blocks were the days of storage; for the analysis of variance using the general linear model (GLM) of SAS (The SAS System for WindowsTM. Version 6.11). Two replicates were performed and each analysis was done in triplicate.

Results and discussion

*Ascorbic acid (AA).*In Figure 1 shows AA content of control fruits and pressurized fruits. The control fruits showed an initial AA content of 134.98 mg/100g FW and in the days following it was synthesized with 149.14 mg/100 g FW (day 11), and then decrease when the fruit were senescent. In pressurized fruits, the AA content was affected significantly ($p \leq 0.05$) by the level of pressure and time (Fig. 1). The fruits with 15 MPa for 10 and 20 min not showed alteration in the synthesis of AA, there was no statistical difference compared with the control, this might be because these treatments did not alter the metabolism of AA. In fruits subjected to 30 MPa (10 and 20 min) showed that from 8 to 11 days storage, there was a higher content of vitamin C than the control; while in the fruit with 60 MPa (10 and 20 min) increased the AA content was even higher from day 5 of storage. It is clear that the fruits with higher levels of pressure and time increased the synthesis of AA to a greater extent, this probably because oxidative stress is higher and the increase of AA is to reduce this stress by being an antioxidant (8). On the other hand, Torres et al (9) processed orange juice at pressure levels 400, 500, 600 Mpa for 15 min; they found that during the process was obtained 94.5% retention of vitamin C in all pressure treated samples. In whole fruit, it was observed that HHP could also retain vitamin C and increase; the treatment can be used in puree mangoes.

Total carotenoids (TC). In Fig. 1 shows changes in the total carotenoids. In the control fruits, TC increased during the days of storage and the internal color of 'Ataulfo' mangoes was developed normally until the fruit ripened. The HHP and the application time significantly affect the contents of TC ($P \leq 0.05$). In pressurized mangoes at 15 and 30 MPa, TC was greater 10 min than 20 min, for fruit with 10 min of pressurization was observed from day 5 to day 14 of storage, a higher TC content (121.22 y 98.67 mg β-carotene/100 g DW, respectively) compared to control fruits (68.65 mg β-carotene/100 g DW) and pressurized fruits for 20 min (79.57 y 66.10 mg β-carotene/100 g DW). In treated mangoes at 60 Mpa for 10 and 20 min, the synthesis of TC did not increase during fruit ripening. In 'Ataulfo' mangoes pressurized to 30 and 60 MPa (10 and 20 min) showed internal and external physical changes, there was a spongy tissue and the presence of internal cavities near the peduncle (10). It is possible that the application of lower hydrostatic pressure in ‘Ataulfo’ mangoes, was facilitated the extraction of total carotenoids.

Extractable phenols (EP). The effect of HHP on EP is shown in Fig. 1. In non-pressurized fruits (control) the initial content of extractable phenols was 105.83 mg chlorogenic acid /100 g DW and subsequently the concentration of phenols decreases to 38% of the initial concentration (63.53 mg chlorogenic acid/100 g DW) until day 14 of storage. This behaviour is characteristic mangoes fruit, because the concentration of phenolic compounds is greater in green fruit, and decreases during maturation (11). The level of pressure and time significantly affect EP ($P \leq 0.05$). Treated mangoes at the three levels of pressure for 10 min, showed a greater increase in phenolic concentration from day 8 of storage than control fruit, the EP was higher in treated fruits at 15 MPa. The same behaviour was observed in EP for treated fruit for 20 min (Fig. 1), but the treated fruits at 60 MPa for 20 min synthesized higher content of EP, during the first days after application of pressure. Prasad et al (12) reported that HHP exhibited higher extraction effectiveness

on phenolic content, and higher antioxidant and anticancer activity with shorter extraction time in Longan Fruit. Possibly the high pressure was effective to improve the extraction of phenolic compounds also may reduce the activity of the polyphenol oxidase although the results depended on the pressure and time of application.

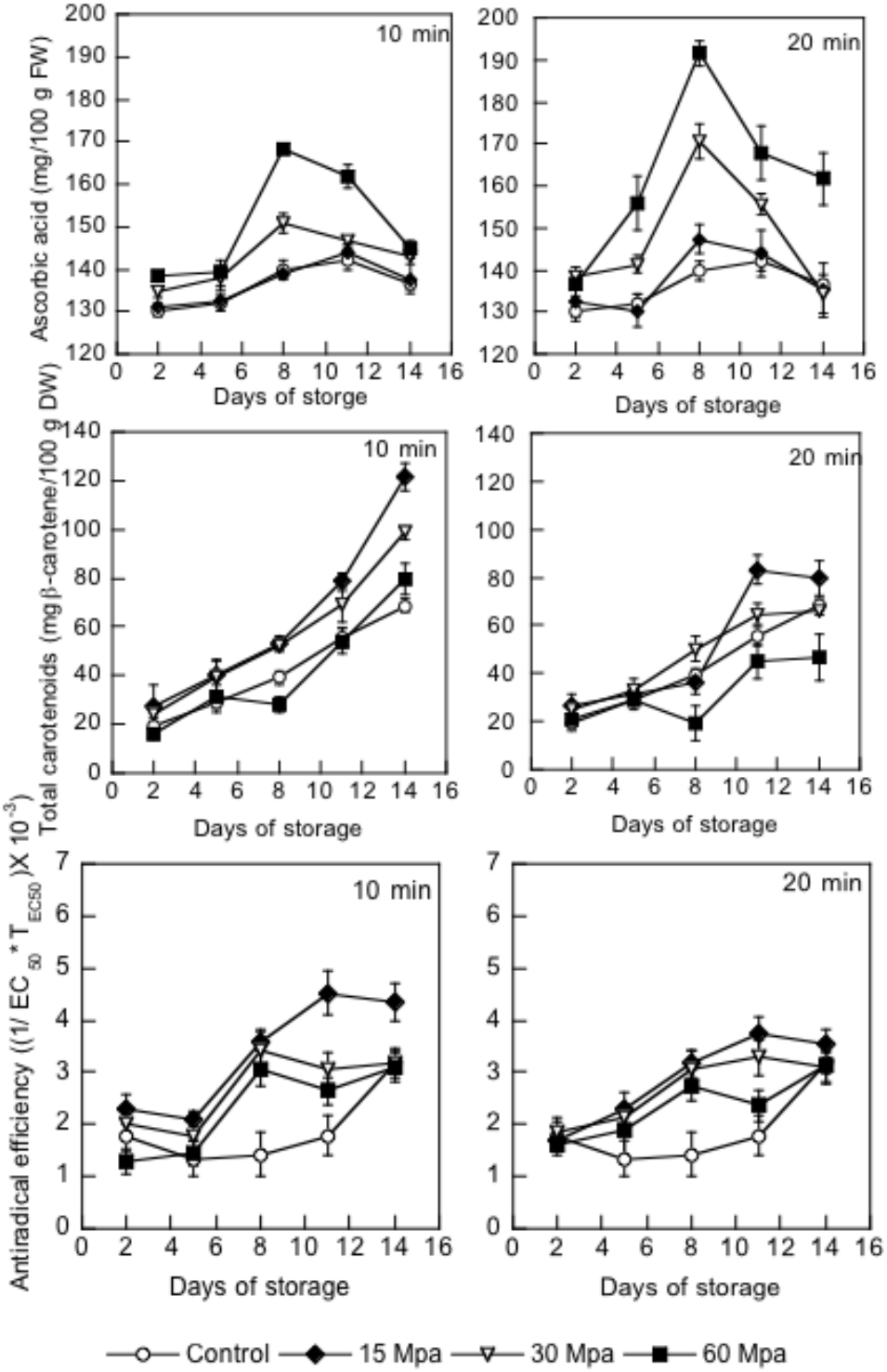

Figure 1. Changes in ascorbic acid, total carotenoids, extractable phenols and antioxidante capacity in 'Ataulfo' mangoes treated with HHP (15, 30 and 60 MPa) for 10 and 20 min, during postharvest storage at 25 ° C.

Antioxidant capacity or antiradical efficiency (AE). In the control fruit, AE reached its maximum efficiency at day 14 of storage (see Fig.2) and the antioxidant capacity of this extract is in the middle nevel (7). For fruit pressurized to 15 MPa for 10 min (Fig. 2), maximum AE was also presented on day 14 of storage and this increase coincided with the highest phenolic content and total carotenoids found. The same was for fruits treated at 15 MPa for 20 minutes but less AE. For fruit with 30 and 60 MPa (10 and 20 min), the maximum EA occurs on the day 8 and remains until day 14, with lower antioxidant capacity than fruits with 15 MPa. All high pressure treatments had higher AE than control fruits. It is possible that tissue structural changes induced by HHP, resulted in the release into the extracellular medium of antioxidant compounds (13).

Conclusion

In the present study was found that high pressures cause changes in the content of antioxidants in 'Ataulfo' mangoes during postharvest ripening at 25 ° C. The effect of HHP in mangoes fruit depended on the level and time of the pressure. The fruits treated with less pressure and time (15 MPa for 10 min) increased extractable phenols content, total carotenoids and antioxidant capacity than control fruit during storage time. The high pressure extraction technology provided a better way of utilizing mangoes fruit pericarp as a readily accessible source of natural antioxidants in food and pharmaceutical industries.

Acknowledgments

The authors gratefully acknowledge the financial support of Programa de Mejoramiento del Profesorado (PROMEP), and CONACyT of México through the grant 237840. The valuable contribution of fresh mangoes for the study by Mr. Abelardo González Cabrera is truly appreciated.

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Microwave Dehydration Of Mango Pulp, Process Optimization (*Mangifera indica L.*) Using The Response Surface Methodology

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Introduction

The most common dehydration method employed in the food industry to date is the hot air drying; nevertheless, this method has some disadvantages such as cost, product quality, etc. In recent years, microwave drying has appeared as an alternative to improve the quality of dehydrated products [1]. Response Surface Methodology (RSM) is the most popular optimization method used in recent years [2]. In this study, RSM was used to optimize operating conditions of the dehydration process of mango pulp in a microwave oven and desirability function used as the methodology for the optimization. Ripe mango (*Mangifera indica L.*) variety Paradise of low quality considered not able to be commercialized as fresh was used. A central composite design was used to develop models for the responses by Design-Expert Software (version 5.0.4). The independent variables were the oven's power (317 - 505) Watts and dehydration time (8 - 13) min, while investigated responses were temperature of mango pulp at output (ºC), water activity (Aw) and the parameter of color "a" [3]. To visualize the combined effects of two factors on any response, the response surface and contour plots were generated for each fitted model as the function of two independent variables, while keeping the other variable at the central value. The optimum dehydration zone was located within the intervals from 317 to 505 Watts and from 7.5 min to 13 min for oven's power and dehydration time, respectively. The following criteria was established for the different variables: 70.9 to 120 ºC for temperature, 0.3 to 0.5 for Aw and 14 to 16.7 forthe parameter of color "a". Applying the desirability functions the optimum conditions of dehydration were oven's power of 505 W and dehydration time of 9.6 min. The optimum parameters found could be used as a guide for escalation process for microwave mango dehydration [4].

Materials and methods

Raw material: ripe fruits of mango (*Mangifera indica L.*) Paradise variety (Petacón) considered of lower quality for fresh commercialization, and was obtained from the central of supplies of Mexico City. The fruits were washed, disinfected, and the peel and seed were removed.

Dehydration of mango pulp in microwaves: microwave oven SHARP R-530ES Household Microwave oven 1200 Watts of output. Hundred grams of pulp were used in each lot.

Measurement of the real power of the oven: the established method IMPI - 2 [5] was followed for the measurement of the real power of the microwave oven.

Optimization of the dehydration of pulp of mango by response surface methodology: A central experimental design composed was conducted using the software Design-Expert (version 5.0.4) with two independent variables at three levels and five replicates of the central point [2]. The power of the oven and the time of dehydration were chosen as independent variables.

The preset variables: oven power and dehydration time, they were variables based on preliminary tests. The intervals of the independent variables are shown in table 1.

Table 1. Independent variables and levels of the experimental design

Variables	Code	Level		
		-1	0	1
Oven power (Watts)	X_1	317.3	425.8	505.2
Dehydration time (min)	X_2	8	10	13

The dependent variables assessed were the temperature at the oven exit (ºC), water activity (Aw) and the parameter of color "a".

- *Temperature at the oven exit (ºC)*. Mango pulp temperature was recorded immediately after completing its dehydration in the microwave using an infrared digital thermometer gun type. A reading was taken around 3 s to get an average of the temperature reached at the end of the process of dehydration.

- *Water activity (Aw).* Once dehydrated mango pulp was obtained, it was cooled to room temperature, and was milled and sifted until passed mesh 40. Water activity was determined in the powder using a Rotronic HygroPalm Aw1, expressing the result as the average of three repetitions.

- *Color*. Color measurements were made by triplicate using the Color Reader DR-10, Konica Minolta. The evaluated variables were read in the CIELAB scale, where L** = brightness; a** = from green color to red color; b ** = from blue color to yellow color (Figure 1).

Data were processes using a multiple regression analysis of second grade and the response surface methodology was applied in the experimental region. It was obtained a prediction for each evaluated characteristics (dependent variables) through statistical analysis, and second order equations were obtained, which contain linear, quadratic terms, and interaction. Each contour graph was generated as a function of two independent variables, while the third variable remained constant. The independent variables were found in the axis X and Y, while the response variable is located in the Z axis.

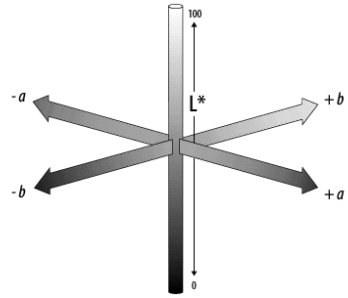

Figure 1. CIELAB color scale

Results

Experimental design process of dehydration of mango in microwave pulp

The values obtained of dependent variables according with the experimental design of 13 runs for microwave dehydrated mango are shown in table 1.

Table 1. Independent variables and dependent variables responses for different conditions of dehydration of mango pulp in oven microwave

	Independent variables		Dependent variables responses		
Run	Oven power (level) X_1	Dehydration time (min) X_2	Temperature at the oven exit (ºC) Y_1	Water activity (Aw).* Y_2	Color *"a"** Y_3
1	-1	-1	70.9	0.97	8.63
2	1	-1	82.8	0.881	15.0
3	-1	1	99.6	0.776	16.9
4	1	1	169.6	0.239	13.1
5	-1.414	0	81.6	0.919	11.1
6	1.414	0	132.6	0.323	14.9
7	0	-1.414	78.8	0.936	9.77
8	0	1.414	153.3	0.282	15.0
9	0	0	96.4	0.722	17.5
10	0	0	100.8	0.549	16.6
11	0	0	99.6	0.635	17.9
12	0	0	100.3	0.624	18.9
13	0	0	105.1	0.536	17.3

* Arithmetic mean of three repetitions

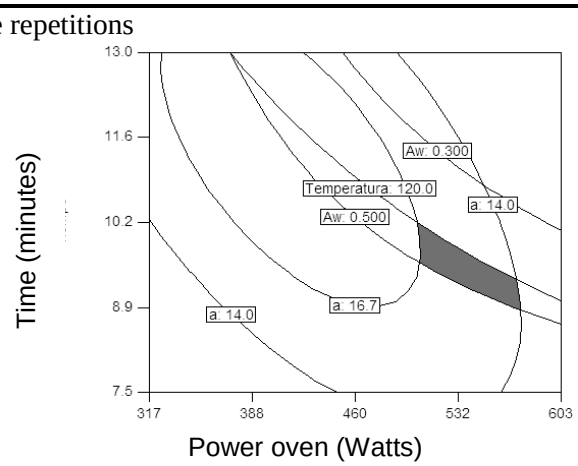

Figure 2. Surface response used for the dehydration of mango in microwave oven

Discusion

The dried mango presented a wide range of temperature in the conditions tested. The lower temperature found at the end of the microwave treatment was 70.9 and the higher was 169.9 ºC (Table 3). The central points of the design had an average value 99.4 ° C.

a model prediction of R^2 was selected. Statistical analysis showed that the data can be adjusted to a quadratic model, with a determination coefficient (R^2) of 0.985.

The final dehydration temperature of mango pulp could be described trough a mathematical model that involves the effects of the oven power and time heating (Equation 1):

$$\sqrt{T} = 12.58 - 1.58P - 0.78t + 0.046P^2 + 0.0296t^2 + 0.215Pt \qquad (1)$$

Where T is the final dehydration temperature (ºC); P is the oven power (Watts) and t is the time heating (min). This model explains the 98% of the total variation experimental.

Surface response analysis of water activity (Aw)

Table 1 shows the experimental results of the water activity of the mango dehydrated in microwave oven, which varied in the range from 0.239 to 0.97; the central points of the design had an average value of 0.613. The regression model obtained for water activity was an exponential model of second order with an R^2 of 0.952.

The 2 equation describes the effects of the power of the oven and heating time on the water activity (Aw) for mango pulp dehydrated in microwave oven:

$$\ln(Aw) = -1.67 + 0.74P + 0.26t - 0.012P^2 - 0.005t^2 - 0.098Pt \qquad (2)$$

Where Aw is the water activity of the dehydrated mango; P is the oven power (Watts) and t is the heating time (min). This model explains the 95.2% of the total variation experimental.

Surface response analysis of color “a”

In order to evaluate the color on the dehydrated mango in microwave oven, brightness parameters were measured "L", "a" and "b"; however, the results obtained for the "L" and "b" parameters showed no significant difference ($p > 0.05$), therefore it was only considered the parameter "a" (changes from green color to red color). The experimental results of the parameter of color "a" varied in a range of 8.63 to 18.9, while the central points of the design had an average of 17.64. Statistical analysis showed that the data can be adjusted to an exponential model of second order with an R^2 of 0.980. The equation of the mathematical model describing the effects of the oven power and heating time on the color of the mango dehydrated in microwave is shown below:

$$\ln(a) = -3.98 + 1.72P + 0.75t - 0.147P^2 - 0.023t^2 - 0.073Pt \qquad (3)$$

Where "a" is the color parameter; P is the oven power (Watts) and t is the heating time (min).

Optimization of the dehydration process using microwave

The optimization of the mango dehydration process in microwaves was firstly made graphically by the superposition of the graphic outline of the three dependent variables (Figure 2). The area shaded in figure 3 corresponds to a set of points that meet the desired conditions for carrying out an optimal process of dehydration. The optimal dehydration process was carried out to a power of 505.2 Watts during a time of 9 minutes 36 seconds; the run was of high desirability.

Acknowledgements

This research was financed by Institute Polytechnic National (IPN, Mexico).

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Effect of Dahlia Root Inulin on Growth of *Lactobacillus acidophillus*

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Abstract

Dahlia is a wide-spread genus of the Compositae family of Mexican origin. It is perennial with edible tuberous roots. In prehispanic cultures they were valued for the nutritious inulin stored inside them.
Inulin was extracted from Dahlia roots. Growth on phenol red broth of *Candida kefir and Candida tropicalis* as indicator strains was used in order to verify that root extract was inulin. The inulin prebiotic effect was evaluated by means of growth of *Lactobacillus acidophillus* in MRS broth supplemented with 3 % of Dahlia inulin, then inoculated on MRS agar. L. *acidophillus* was enumerated after anaerobic incubation at 37 º C for 72 h. Chicory commercial inulin was used as control. IR spectroscopy was used in order to detect functional groups corresponding to inulin structure.
Growth of *Candida kefir* and *Candida tropicalis* showed that inulin extracted from Dalia roots promoted a similar growth than chicory inulin. A change of color and gas production was observed on phenol red broth. *L. acidophilus* rate growth was greater (6.78 x 10^7 ufc/mL) in the presence of Dahlia inulin than without it (5.77 x 10^{7} ufc/mL). With regard to chicory inulin a similar growth was observed. Infrarred analysis showed a similarity between the chemical structure of Dahlia and chicory inulin.
In conclusion, under our experimental conditions, this study showed that inulin extracted from Dahlia root has similar properties to that of chicory inulin, i.e., chemical structure and prebiotic effect on *L. acidophilus*. Furthermore, Dahlia root is a potential source of inulin.
Key words: Dahlia inulin, prebiotic, *L. acidophillus*

Introduction

Inulin is a plant-derived carbohydrate with the benefits of soluble dietary fiber. It is not digested or absorbed in the small intestine, but is fermented in the colon by beneficial bacteria. Functioning as a prebiotic, inulin has been associated with enhancing the gastrointestinal system and immune system (1). Its structure is composed of linear or branched fructose polymers with chain length of 2–60 units (degree of polymerization (DP) is 2–60) usually with a terminal unit of glucose linked together by β - (2-1) glycosidic bonds (2).
The two species currently used by the industry to produce inulin belong to *Compositae*: Jerusalem artichoke (*Helianthus tuberosus*) and chicory (*Chicorium intybus*) (3). The dahlias are a small but wide-spread genus of the family *Compositae*. Its roots were valued both for the nutritious inulin stored inside them and for the antibiotic compounds concentrated in the skin of the tubers (4). The technological use of inulin is based on its properties as a sugar replacer (especially in combination with high intensity sweeteners), as a fat replacer and texture modifier. For fat replacement in low-fat dairy products inulin seems particularly suitable as it may contribute By increasing faecal biomass and water content of stools, they improve bowel habits and regularity. Likely due to their specific fermentative properties, they affect several functions involved in colonic mucosa protection and repair, which may contribute to reduce the risk of intestinal diseases (5).
Oliveira and col (6)., showed that in the presence of inulin, the concentrations of Streptococcus thermophilus (4.1±0.1 g/L) and Bifidobacterium lactis (2.6±0.2 g/L) in the St–Bl co-culture were about 35 and 53% higher (0.05) than in their respective pure cultures. These results as a whole confirm the symbiotic and bifidogenic effects already mentioned by several authors.

Because dahlia roots are a rich source of inulin, there is a potential to use them as to stimulate the growth of health promoting bacteria to be a potential novel source of prebiotic nutraceuticals for the food and dietary supplement industry, and could be an alternative to more traditional FOS and inulin sources such as chicory roots. The aim of this investigation was to hypothesize that dahlia roots have a potential of be fermented by *L. acidophilus.*

Materials and methods

2.1 Inulin extraction

1.5 kg lots of peeled tubers were chopped into fine pulp in hot water containing sodium metabisulphite to minimize browning at 95-98° C for 10 min. The resulting extract was concentrated and after subsequent filtrations, was clarified using activated carbon. Aliquots of the concentrate were weight and mixed with equal parts of ethanol (96 %), the flasks were stored at 4 °C for one day. The precipitate formed was filtered and dry at room temperature. The yield was calculated at the end of the dry process (**Unpublished results**).

2.2 Microbiological identification of inulin.

A sterile solution of phenol red broth was prepared and inoculated with 1 % of dahlia inulin, chicory; commercial inulin was used as control. Each tube contained a Durham´s fermentation tube or similar appliance. Tube No. 1 was inoculated with *Candida kefir* CDBB-L-1244 from CINVESTAV-IPN strains collection. Tube No. 2 was inoculated with *Candida tropicalis* obtained from the Facultad de Química, UAEM strains collection. The tubes were placed in an incubator at 37º C for 72 hours.

2.3 Prebiotic effect of inulin

Inulin extracted from dahlia roots, and commercially available inulin (Frutafit® SENSUS, The Netherlands) were added at the rate of 3% (w/v) to pasteurized MRS broth (Difco BD., NJ USA). Then, pasteurized MRS was inoculated (1% v/v) separately with *L. acidophilus* strains (La-5® Chr-Hansen, Denmark) and incubated for 36 h. Serial dilutions were prepared from broth-bacteria mix and viable *Lactobacillus* were enumerated by the pour plate technique using MRS agar (Difco BD., NJ USA). Plates were incubated at 37º C for 48 h. Control sample did not contain any prebiotics. Each experiment was carried out in triplicate. A growth curve test was done in order to show the influence of the two different sources of inulin on the growth of *L. acidophilus*.

2.4 Infrarred analysis

Extracted dahlia inulin and chicory inulin (Frutafit® SENSUS, The Netherlands) were used. For IR analysis, samples (1%) were pressed into pellets of KBr (200 mg). Infrared spectra (4000- 400 cm^{-1}) were recorded with an FTIR Perkin Elmer spectrometer, Spectrum 2000 (Waltham, MA USA)

Results and discussion

3.1 Microbiological identification of inulin

After the incubation time gas production and a change of color from red to yellow was visible in the media who was inoculated with *Candida kefir* coming to the conclusion that the extracted substance is inulin. According to Cruz (1999), these changes are owed to inulinase enzyme that is produced specifically by *Candida kefyr, Kluveromyces marxianus, Aspergillus sp., Penici1lium sp.* This enzyme degradates inulin giving as a result molecules of fructose and other sugars such as: sucrose, rafinose, stachyose and inulin oligosaccharides with β (2-1) and β (2-6) linkages.

It`s important to say that this study is just a qualitative test, in order to show that the obtained product was inulin, but it will take in count to other studies, such as the obtention of high fructose syrup, which has gained importance in food, drink and pharmaceutical industries.

3.2 Influence of the inulin on the growth of *L. acidophilus.*

The growth of *L. acidophilus* was evaluated in the presence of inulin at the rate of 3% for the commercial inulin (IC) and experimental inulin (IE) in MRS agar.

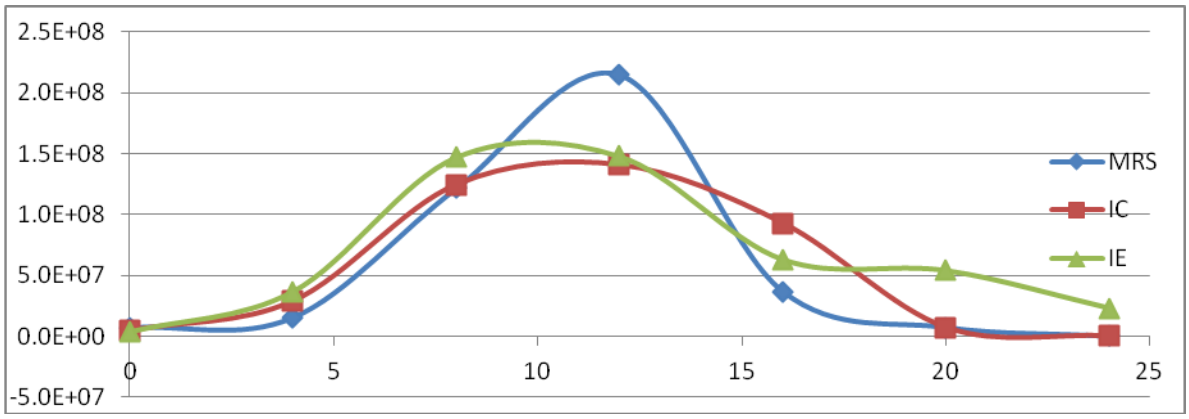


Fig 1. - Growth curve of *L. acidophilus*

The CFU of L. *acidophilus* 0, 4, 8, 12, 24 and 36 hours are given in Fig 1. Both IC (commercial inulin) and IE (experimental inulin) produced a significant increase in *L. acidophilus* (Fig. 1) compared to the control (MRS). The major increase occurred after 8 h on the media that was supplemented with any inulin for lactobacilli. According to Minaña (2007) who evaluated a different kinds of FOS, found that the growth of lactobacillus and bifidobacterias is proportional to the concentration of the substrate.

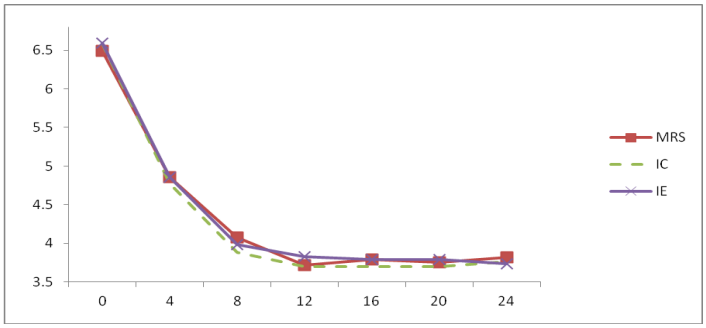
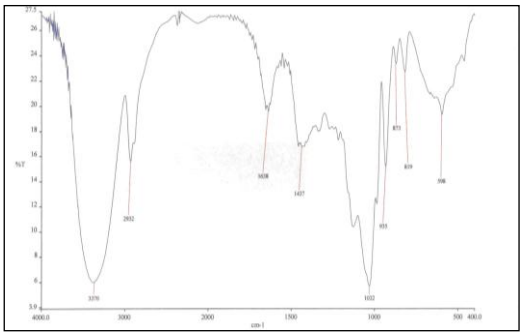


Fig 2.- pH variation

Another important parameter that demonstrate the growth of lactic acid bacteria (LAB) is the pH it was mesure after the fermentation, as seen in Fig 2 there was a direct relation between bacterial growth and changes in pH, it means that bacterial growth use the inulin as an important source of carbon, and there´s a production of short carbon fatty acids, that has been reported as beneficial in the inhibition of pathogenic bacteria.

3.3 Chemical structure

The IR spectroscopy technique was used in order to detect functional groups corresponding to inulin structure. Fig 3 and Fig. 4 belong to dahlia inulin and chicory inulin. As seen both structures are similar because the representative functional groups in the dahlia spectrum, appeared in the same region, for example OH bands showed in 3370 cm-1 and then the CH were found on 1638 cm-1 so that it is possible to consider it as representative of the compound called inulin. Higuera 2009, made a similar study comparing artichoke inulin and chicory inulin, and concludes that the structures were similar even if the extraction methods were different.



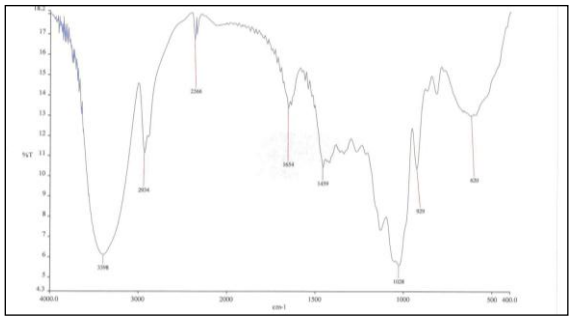


Fig 3. FT-IR for spectrum for dahlia inulin

Fig 4. FT-IR for spectrum for Chicory inulin

According to Molina and *col* .2005 (1) FT-IR spectrum for artichoke inulin was essentially identical to dahlia inulin and chicory inulin showing OH stretch (3353 cm_1) and carbonyl bands (1745 cm_1) characteristic of inulin.

Conclusions

According to these results, we can verify that inulin stimulates the growth of *L. acidophilus*, considering the polymer obtained as a prebiotic. The addition of inulin also exerted a clear effect on the growth of lactobacillus and show that there´s no a significant difference between dahlia inulin and chicory inulin. The acidification kinetics was enhanced by inulin addition to the MRS broth, in that it increased the maximum acidification rate and decreased the generation time.

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Viscosity In Extruded Corn Flour

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The traditional nixtamalization method to obtain corn tortilla is still used nowadays. This process generates high biochemical oxygen demand wastewater. An alternative to this traditional process is the extrusion, which combines heat and mechanical shear stress to obtain corn masa using less water without generating pollutants. The quality of the extruded masa is still an open issue. In the present study, the main aim is to know the behavior of the viscosity corn flour obtained from extruded dough using different processing conditions. The input factors were: particle size, moisture and temperature in order to obtain a mathematical model to predict the behavior of the viscosity. The methodology consisted in a full factorial design 3^3 with the variables: particle size (0.5, 0.8, 1.3 mm), moisture (45, 55, 65 % w/w) and cooking temperature (70, 75, 80 °C). It was used a single screw extruder with L/D = 20. Data showed that the processing parameters affecting the viscosity were the particle size and the interaction moisture – temperature. It was possible to model the viscosity profile with a quadratic model, with an adjustment of R^2 = 0.93 for the peak viscosity and R^2 = 0.87 for retrograde viscosity. Response surface and quadratic model can predict viscosity of corn flour at different input conditions.

Introduction

The nixtamalization is a process used to produce food widely consumed in Mexico. It involves cooking the corn in an excess of water with 1% to 3% (w/w) of lime [1], followed by a steeping, washing and grinding steps. The thermal-alkaline process improves the nutritional quality of the grain and gelatinizes around of 15% of the starch in the corn. The main disadvantage of the method is that it uses excessive water and produces polluting effluents. A technological alternative is the nixtamalization by extrusion. During this process, the extruder is fed with a mixture of ground corn, lime, and water. This material is transported by a screw, and cooked by electric resistances. The cooked masa exits through a die [2].

The extrusion can include several operations such as: mixing, transporting, cooking, and shaping. This process combines mechanical stress with heat to facilitate starch gelatinization [3, 4]; however, it has not replaced the traditional process because the corn flour produced by extrusion does not achieve the desired quality.

The apparent viscosity of flours provides information about the degree ofstarch modification,generally attributed to the leaching of amylose chains during the cooking process and it is reported as a quality factor in flours [5].

Materials and Methods

Commercial maize variety “maicena”, produced in 2011, wasobtained at the supply center of Querétaro. The corn was ground in a mill (Pulvex 200), with meshes of 0.5, 0.8 and 1.3 mm. The flour obtained was mixed with 0.3% (w/w) of lime and watertomoisturesof 45%, 55% and 65%, according to a 3^3 factorial experimental design, having as factors: particle size, moisture, and temperature.

Extrusion Process

For the cooking of the samplesa single-screw extruder of stainless steel (2.2 cm diameter with an L / D = 20, developed at CICATA IPN Querétaro). Extrusion was operated at 20 rpm(Figure 1).

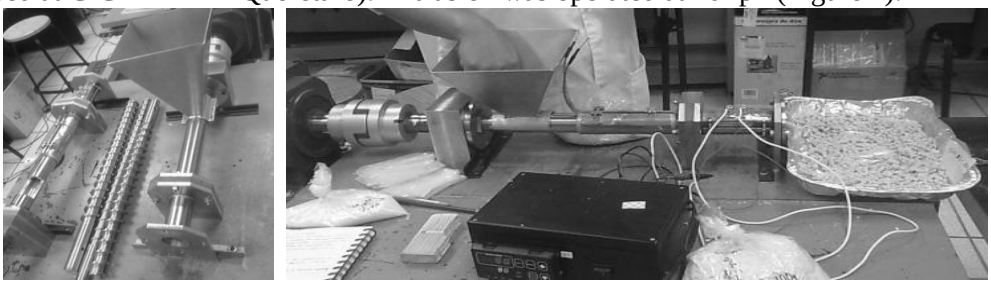

Figure 1.Single-screw extruder, technology of CICATA-IPN, Qro.

The extruded masa was ground in a Krupscoffee grinder and dried in an air recirculation oven (Binder brand), at 50°C for 24 hours. Dried samples were sieved trough mesh No. 60 (particle size <250 um).

Viscosity Profile

Viscosity was measured with a Rheometer (Anton Paar, MCR Phisyca model 101, with agitation probe STD 24-2D). Sampleswith a weight of 3 ± 0.0001 g, were conditioned to form an aqueous suspension by adding distilled water toa total weight of 21g. The temperature ramp used for the measurement was: initial time of 1 minute at 50°C, with heating from 50°C to 92°C, at a rate of 6 °C / min, and the temperature was maintained at 92°C for 6 min, ending with a cooling of 92°C to 50°C at the same rate [6].

Results and Discussion

The viscosity shows the degree of modification of the starch granules during gelatinization.In a viscosity profile analysis of nixtamalizedfoods, the initial heating starts to raise the viscosity by swelling the starch granules.Atthis point the pasting temperature is determined. The maximum viscosity is generated when mostof the granules have reached the maximum swelling; this parameter isrelated to the ability of the starch to retain water, thus affecting the quality of the products: “tortilla” or “masa”.

The maximum viscosity (Table 1) showed significant differences (P <0.05) due to the effect of particle size, followed by of the moisture and the interaction moisture-temperature.

The particle size determines the availability of the starchto reacts with moisture and calcium ions; also this parameter defines the modification of starch by the cooking temperature.

Table 1.Analysis of the effect of extrusion variables on the viscosity profile

Factor	Response variable		
	Pastingtemperature	Maximumviscosity	Viscosity of retrogradation
Particlesize	0.0001	0.0001	0.0001
Moisture	0.0474	0.0238	0.0268
Temperature	0.0092	NS	NS
Particlesize *Moisture	NS	0.0038	NS
Particlesize *Temperature	0.0246	0.0038	NS
Moisture*Temperature	0.0182	0.0023	0.0001
P values with α=0.05; NS= Non significative (P>α).			

The retrogradation viscosity is a prediction of how quickly the product will ageand it is a numerical value calculated as the difference between final viscosity and low viscosity. Physically, the retrogradation is the realignment of starch fragments. Theretrogradation valuewas significantlydifferent between treatments (P <0.05),and this difference was dominated by the particle size, moisture and the interaction of moisture - temperature.

$$Vmax = 264.35 + 2899.97P + 265.228H - 109.21T - 57.899TP - 2.6172TH + 1.523T^2 + 70.02P^2 - 0.837H^2 \quad (2)$$

$$VRetro = -23933.15 + 2984.18P + 267.69H + 534.55T - 44.51TP - 4TH - 2.27T^2 + 60.85P^2 + 0.06H^2 \quad (3)$$

This latter interaction determines the degree of starch gelatinization obtained. The necessary temperature to achieve this reaction is from 70°C to 80°C [7], but the availability of water in the material limits the gelatinization reaction in the granules. According to the above, depending on conditions, starch gelatinization may occur in extruded products, depending on the relation water / starch and temperature [8].

Knowing the factors that have the largest effectsone can to discern the quality to be obtained in an extrusion process, manipulating the input parameters to optimize the process. For this optimization a quadratic model was used Eq. (1).

$$R = a + bP + cH + dT + eTP + fTH + gPH + hT^2 + iP^2 + jH^2 \quad (1)$$

Where:

R= Response variable; T= Temperature; P= Particle size; H= Moisture content in percentage; a, b, c, d, e, f, g, h, i, j= Model coefficients

It was decided to setthe particle size for each surface response. The temperature and moisture content were varied.

Usingexperimental values and the method multiple regression, mathematical models were found for maximum viscosity and viscosity retrogradation; these are shown in Equations 2 and 3.

The response surfaces generated for maximum viscosity and viscosity of retrogradationare in Figures 2 and 3. The adjustment value and analysis of variance arein Tables 2 and 3.

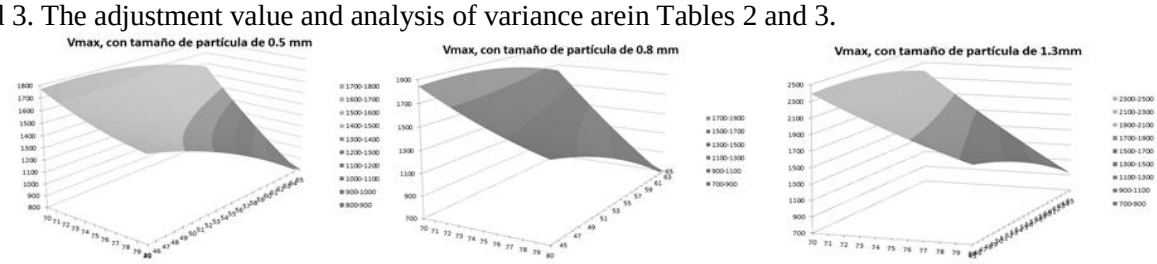

Fig. 2.Response Surfacesof maximum viscosity using different particle size.

Table 2.Analysis of variance of maximum viscosity

	Degrees of freedom	Square Sum	SquareAverage	F	Criticalvalue F
Regression	9	9684112.617	1076012.51303	33.56	7.54902E-17
Residue	45	1623181.791	36070.70647		
Total	54	11307294.41			
R^2 = 0.96, R^2adj= 0.93					

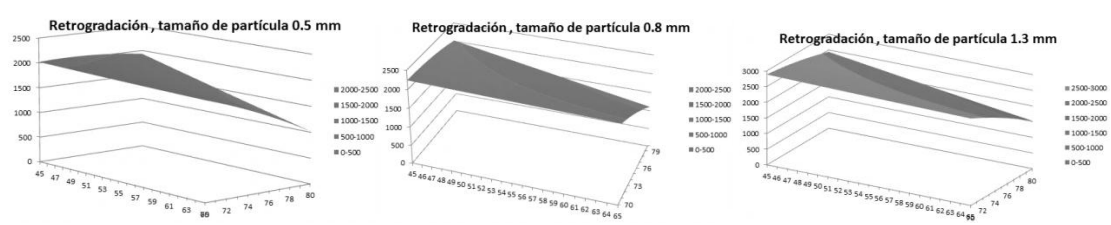

Fig. 3.Response surfacesof retrogradationviscosity withdifferent particle size.

Table3. Analysis of variance of retrogradation viscosity

	Degrees of freedom	Square Sum	SquareAverage	F	Criticalvalue F
Regression	9	12030848	1336760.89	38.39	5.89802E-18
Residue	45	1762849.66	39174.4369		
Total	54	13793697.6			
R^2 = 0.93, R^2adj= 0.87					

According to the adjusted R^2 coefficient,shown in tables 2 and 3, around 90% of the experimental data fit the model in the responses evaluated.These models can predict the quality of materials that will be obtained by extrusion.

Conclusions

The maximum viscosity and retrogradation viscosity depend mainly on the particle size, which is a factor that limits the diffusion of water into the granule. In a large particle this diffusion is slower, and therefore gelatinization is carried out only in regions which have already achieved the hydration.

The interaction of moisture-temperature showed a significant effect on the high viscosity and retrogradation viscosity. The profile variables determine the quality of thenixtamalizedproducts. These propertiescan be predicted with the quadratic model, havingas input factors: particle size, moisture content and process temperature. Theadjustmentwith these models to the maximum viscosity and retrogradation viscosity is R^2 = 0.93 and 0.87, respectively.

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Viscosity Profiles Of Corn Grits Macerated In Alkaline Solution

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Abstract

During corn nixtamalization biochemical reactions occur inside the grain, molecular interactions modify its rheological characteristics, and therefore in derived products, such as corn masa and tortilla. These changes are due to the modification of the starch during process conditions, such as calcium hydroxide concentration and the moisture content during cooking and stepping time. One indicative of changes in the components of corn is the relative viscosity in a viscoelastic amyl- profile graph. The aim of this study was to determine the effect of the stepping time on maize grits without the cooking process in an alkaline solution, on the maximum, minimum and final viscosity. The methodology consisted in crushing corn to a particle size of 4 mm and macerate it in a calcium hydroxide solution (1% w/ w), taking samples at 1, 30, 60 min and every 60 min for 10 hours, using a control corn flour, macerated without lime. Data showed a tendency to increase viscosity in the macerated samples, with and without lime, according to the stepping time, reaching a peak at 4 hours in grits with 1% Ca $(OH)_2$ and up to 8 hours in samples without lime. The lime stepped sample's viscosity varied from the 5 to 10 hours but analysis of variance and t-student test did not show a significant difference between samples (α= 0.05). It was concluded that stepping time at which maximum viscosity was reached, in samples with 1% lime, is 4 hours which may be due to physicochemical changes in the corn, caused by diffusion of calcium ions and water into the grain.

Introduction

Consumption of nixtamalizedfoods spread from Mexico to the world, which is consumed mainlyas tortillas and snacks. The quality of these products is determined by nixtamalization conditions.

A crucial step of this process is the steepingtime of the cooked corn. During this stage the gelatinized starch is recrystallized to form new structures, increasing the calcium content in corn, due to diffusion reactions of calcium ions and water into the grain. Other changes include modification of the starch granule structure and protein bodies associated [1].

It has also been reported that with increasing steeping time growths the swelling of the granules intact and the crystal structurechange [1]. Also, the quality of "masa" obtained from the hydration of flour is due to the conjunction of the steeping time and particle size. These features provide cohesiveness, plasticity and softness to the mass upon rehydration of flour for manipulation and shaping [2].

In nixtamalized flour a quality factor is the viscosity, which gives information relating to changes in the structure of the starch granule by the severity of the process. It has been reported that the dwell time has a positive effect on the quality of nixtamalizedfoods. However, the results are related to three factors: temperature, calcium hydroxide and steeping time.The effect of steeping time in the alkaline solution without heating is unknown. The aim of this study was to evaluate the effect of the steeping time or maceration on the viscosity of corn with and without calcium hydroxide, and without heating.

Materials and Methods

Samples preparation

Commercial maize variety "maicena", produced in 2011, was obtained at a supply center of Querétaro. The corn was ground in a mill (Nixtamatic), to obtain grits of 4mm. The grits weremacerated with three parts of water,adding0% and 1% (w/w) of lime.The macerated time was 10 h.Macerated samples for1, 30, 60, minutes and every hour for 10 hours were taken from the solution by using a colander. These samples were ground in a mill (Nixtamatic), dried in an oven with recirculating air at 50°C for 24 hours, ground in a Krupsgrinder coffee to obtain flour. The dried flour samples were sieved througha 60 mesh screen.

Viscosity Profile

Viscosity was measured with a Rheometer (Anton Paar, MCR Phisyca model 101, with agitation probe STD 24-2D). Samples with a weight of 3 ± 0.0001 g, were conditioned to form an aqueous suspension by adding distilled water to a total weight of 21 g. The temperature ramp used for the measurement was: initial time of 1 minute at 50°C, with heating from 50°C to 92°C, at a rate of 6 °C / min, and the temperature was maintained at 92°C for 6 min, ending with a cooling of 92°C to 50°C at the same rate.

Results andDiscussion

The maximum viscosity occurs when there is a balance between maximum reaching swelling of starch granules and amylose leaching chain [3].

According to a preliminary experiment, the viscosity in the raw milled corn reaches a value of 3600 mPa*s. For traditional method nixtamalized flour maximum viscosity is in the range from 2800 to 3000 mPa*s [4, 5].

Results show that the viscosity of the corn meal increases directly with the macerating time, even without heating. This behavior occurred in samples with and without calcium hydroxide. However, changes in the viscosity are dependent of both factors (Figure 1), butnonlinearincrease was observed.

In samples macerated with 1%, it lime was found that the viscosity increased significantly in the early stages of the macerating, from 1 min to 6 hours, at latter timershowed a viscosity peak with 7315.94 mPa*s, but this is wasnot significantly different (P> 0.05) from the viscosity measure after4 hours of macerating, which had a value of 7174.94 mPa*s. These results are shown in Table 1 and Figure 1.

Table 1.Results of profile viscosity ofgrits macerated with 1% lime

Name	Tpasting	Vmax	Vmin	Vfin	Retrogradation
X0	71.05^{b}	3176.10^{e}	1158.71^{e}	3590.37^{d}	2431.66^{c}
X0.5	72.36^{a}	4313.07^{de}	1401.94^{de}	4603.77^{c}	3201.83^{b}
X1	72.36^{a}	5167.22^{cd}	1770.66^{cd}	5108.35^{c}	3337.70^{b}
X2	72.22^{ab}	5863.77^{bc}	2023.44^{bc}	5510.15^{bc}	3486.70^{b}
X3	72.53^{a}	6797.29^{ab}	2320.60^{ab}	6414.17^{ab}	4093.57^{a}
X4	72.14^{ab}	7174.94^{a}	2392.68^{ab}	6544.21^{a}	4151.53^{a}
X5	72.24^{ab}	7262.33^{a}	2422.05^{a}	6694.25^{a}	4272.20^{a}
X6	72.22^{ab}	7315.94^{a}	2398.47^{ab}	6795.81^{a}	4397.34^{a}
X7	72.43^{a}	7143.74^{a}	2341.70^{ab}	6666.48^{a}	4324.78^{a}
X8	72.49^{a}	7195.49^{a}	2325.15^{ab}	6759.04^{a}	4433.89^{a}
X9	72.43^{a}	7304.00^{a}	2331.43^{ab}	6812.28^{a}	4480.85^{a}
X10	72.52^{a}	7280.73^{a}	2247.31^{ab}	6758.55^{a}	4511.24^{a}
Different letters indicate significantly different average (P<0.05)					

Where X0= Macerated corn 1 minuto, X0.5= Macerated corn 30 minutes, X1= Macerated corn 1 hr., X2= Macerated corn 2 h., T3= Macerated corn 3 h., X4= Macerated corn 4 h., X5= Macerated corn 5 h., X6= Macerated corn 6 h., X7= Macerated corn 7 h., X8= Macerated corn 8 h., X9= Macerated corn 9 h., X10= Macerated corn 10 h.

Another event of interest was the increased of pasting temperature (PT) in samples with 1% lime. This feature is related to the energy required to be gelatinizedthe available starches. According to the above, when increasing the dwell time in the 1% limesolution thus requires more time to achieve greater energy for gelatinization. In a related work [5], it is mentioned that an increase in PT may be duethe incorporation of calcium ions into the structure of maize and protect the starch from gelatinization, requiring greater energy to the process.

According to the literature the damaged starch granules are insoluble in cold water, but it might absorb water reversibly, returning to its original size when dry [3]; however, it was found that only the grits macerated in water treatment also provide an increase in viscosity with time, indicating changes in the starch structure. In these samplesthe viscosity reaches a maximum of 5800 mPa*s after 8 hours maceration (Table 2).

Table 2.Results of profile viscosity of grits macerated with 0% lime

Clave	TP	Vmax	Vmin	Vfin	Retrogradation
M0SC	71.98^{g}	2998.62^{i}	1313.70^{g}	3604.26^{g}	2290.56^{h}
M0.5SC	72.41^{e}	3276.97^{h}	1385.48^{fg}	3921.23^{c}	2535.75^{g}
M1SC	73.05^{b}	4008.00^{g}	1494.59^{f}	4354.30^{e}	2859.71^{f}
M2SC	73.52^{a}	4779.01^{de}	1875.02^{de}	5953.12^{c}	4078.10^{d}
M3SC	73.11^{b}	4864.02^{cd}	1743.44^{e}	5341.50^{d}	3598.07^{e}
M4SC	72.88^{c}	4898.54^{cd}	1754.46^{e}	5358.67^{d}	3604.21^{e}
M5SC	72.86^{c}	4995.35^{c}	1916.85^{d}	5946.46^{c}	4029.61^{d}
M6SC	72.19^{f}	5657.21^{b}	2084.15^{b}	6449.90^{b}	4365.75^{bc}
M7SC	72.24^{f}	5774.36^{ab}	2183.13^{b}	6686.46^{b}	4503.33^{b}
M8SC	72.67^{d}	5870.38^{a}	2422.50^{a}	7323.55^{a}	4901.05^{a}
M9SC	70.65^{h}	5448.00^{ef}	2109.5^{cd}	6075.00^{c}	3965.5^{cd}
M10SC	70.5^{h}	5197.50^{f}	1932.5^{bc}	5452.50^{c}	3520^{d}
Different letters indicate significantly different average (P<0.05)					

Where X0= Macerated corn 1 minuto, X0.5= Macerated corn 30 minutos, X1= Macerated corn 1 hr., X2= Macerated corn 2 h., T3= Macerated corn 3 h., X4= Macerated corn 4 h., X5= Macerated corn 5 h., X6= Macerated corn 6 h., X7= Macerated corn 7 h., X8= Macerated corn 8 h., X9= Macerated corn 9 h., X10= Macerated corn 10 h.

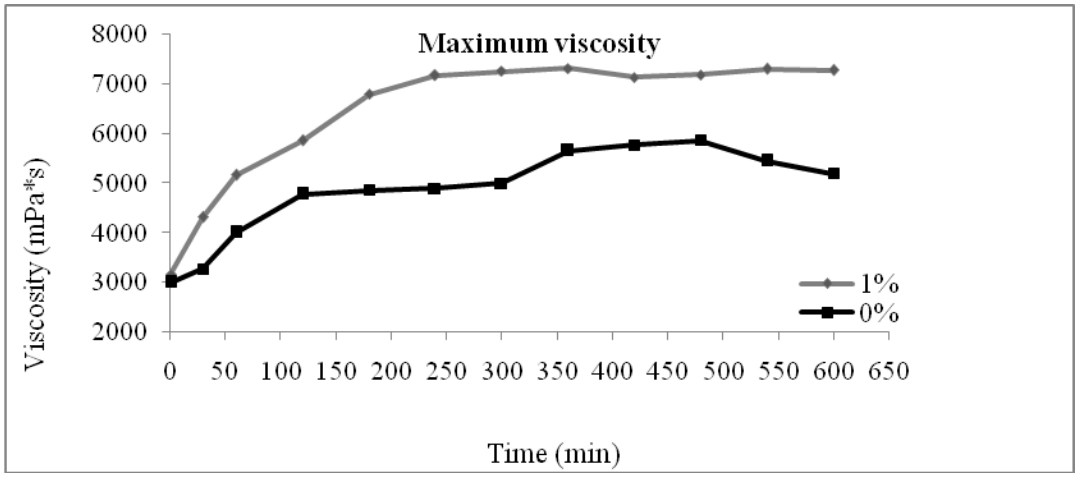

Figure 1.Trend of maximum viscosity behavior over time

Changes in maximum viscosity of macerated corn without lime can be associated to the solubilizing globulins of the starch. When they dissociate from the granules, they allow exposure thereof to swelling by water and causes a small degree of leaching out of the same chains.

Conclusions

Viscosity of the macerated corn is influenced by changes in their physical and chemical structure. When corn is broken or cracked exposing the material naturally protected by the pericarp to the external environment, it may undergo major changes in its structure, which will be reflected of in this property.

The steeping time is a significant that affects the viscosityof property with and without addition of calcium hydroxide.

The starch is changes during the first 6 hours ofsteeping with 1% ofcalcium hydroxide, reaching a maximum viscosity of 7000 mPa*s, but this is not significantly different from the viscosity of macerated for 4, 5, 7, 8, 9 and 10 hours of steeping.

The samples soaked in water without lime also show increases in viscosity, indicating that there are changes in the starch structure even without the presence of alkali; however, the maximum value reached in these conditions occurs about 8 h being 5000 mPa*s.

The kinetics of the process in the absence of calcium hydroxide is slower compared with that found in macerated corn with 1% lime.

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Optimization Osmotic Dehidratation By Vacuum Packaging Of Mango Fresh-Cut

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Introduction

The technologies of modified atmosphere packaging (MAP) apply to a wide variety of products of different nature and have a long history in the conservation of certain foods such as meat products, vegetables, milk, coffee and snacks, making them suitable for fresh and minimally processed foods [1]. These techniques are designed to maintain the sensory quality of these products and extend their shelf life, which is increased by two or three times with respect to the conservation of the product in passive atmosphere [2].

The use of vacuum to generate a modified atmosphere packaging in conjunction with osmotic dehydration involves applying a less than atmospheric pressure during the filling of products with solid and liquid phases, resulting in a beneficial effect in the process of diffusion kinetics and in fruit quality, preventing structural changes in the fruit [3]. Water loss and gain of solids whose conditions are in a state of equilibrium, increase the mobility of solute sat the beginning of the process, occurring hydrodynamic mechanisms (HDM), where as the dehydration at atmospheric pressure are not these movements. HD Mare given by the effect of pressure gradients through the combined action of capillary pressure and pressure changes in the pores of the plant tissue [4]. There sponse surface methodology (SMR) is a tool to study the interactive effects between variable sofa process, provides technical design of experiments, modeling, evaluation and optimal search desirable and in turn is able to reduce the number of experimental runs, with statistically valid [5,6].

The aim of this study is optimized by response surface method, the conditions of preparation and packaging of mango cut and packing type chamoy considering the potential effects of the process factors on the kinetics of diffusivity and firmness changes. The variables considered were: storage temperature (-7, -2, 5, 12 and 17°C), guar gum concentration (0.19, 0.33, 0.54, 0.75 and 0.89% w/w) and vacuum (332, 400, 500, 600 and 668 mbar).

Materials and methods

Materials

Guar gum, chamoy essence, chamoy flavor, glucose syrup(at 50ºBxother ingredients of thepreparationliquid medium were acquiredinDrogueríaCosmopolitaS.A.de CV. citricacid, potassiumsorbateand calcium chloride (BASF, Mexico). Themango (*Mangiferaindica*)varietyAtaulfowaspurchased in Comercial center in Cuautitlán México in a batchof 23kg. The mango was selectedaccordingtoits maturity, considering the skin color (green to yellow), firmness and soluble solidspresent.

Liquid medium preparation

Theliquid mediumwas preparedwith 70% glucose syrupat50 °Bx , 3% citric3%chamoyessence,Chamoy flavor,and 0.1 % of potassiumsorbateaspreservative. Guargumwas usedashydrocolloidin liquid medium

inat different concentrationsaccording thefactorialdesignproposed: (0.19, 0.33, 0.54, 0.75and 0.89% w / w).The dispersionofthe ingredientswas madeina stirrer ofpropeller(HeidolphGermany) at 1.500rpm, withtwo cyclesof5 minutesand a temperature of50 ± 2°C.

Diffusion kinetic

Osmotic dehydrationwas made usingmangocubes (1 x1 cm) andliquid mediumin ratio 1:3 and then packed to differentpressure conditionsinpolyethylenebagsofpolystyrene-polyethylene(11.1 x 17.5 cm), packed and stored atthetemperatureaccording factorial design by obtaining ofdiffusionkinetics,by measuringthe speedatwhichgain solid onmango cubes and lose water. Effectivediffusivityofsolidwas obtained bythefollowingcalculation basis:

$$SG = \frac{{}^{\circ}Bx_t - {}^{\circ}Bx_{\infty}}{{}^{\circ}Bx_i - {}^{\circ}Bx_{\infty}} \qquad WL = \frac{X_t - X_{\infty}}{X_i - X_{\infty}}$$

Where: SG=solid gain; $^{o}Bx_t$=Soluble solid at t (time); $^{o}Bx_{\infty}$= soluble solid at infinitum; $^{a}Bx_i$ = Initialconcentration of soluble solid; WL = water loss; Xt =moisture at time (t); X_{∞}=moisture at infinitum; X_i= initial moisture (Ferrari et.,al., 2011)

Fromthe straight part ofthe graphtimevsSGand time vsWLthe effectivediffusivity was obtainedwiththe following equations:

$$SG = \frac{8}{\pi^2}\exp\left(\frac{-\pi^2 \times De \times t}{4L^2}\right), \quad WL = \frac{8}{\pi^2}\exp\left(\frac{-\pi^2 \times De \times t}{4L^2}\right)$$

Where SG= solid gain; De = Effective diffusivity; t = time; L= charactisticlength[6].

Activation energy (Ea)

Activation energy was calculated at 0.54 % of guar gum and vacuum of 500 mbar and all temperaturesstudied (-7, 5 y 17ºC). Activation energy was calculated with de next correlation:

$$\ln D_e = \ln\ Do - \left(\frac{Ea}{R}\frac{1}{T}\right)$$

Where: De= effective diffusivity; Do = effective diffusion frecuency; Ea = Acitvationenergy (KJ/mol); R = ideal gas constant; T = temperature (K).

Results and discussion

Table1 showsthe regression coefficientsandprobability values (α = 0.05).Showing that thelinear and quadratic termofvacuumwas the variables factor thatmost influenceinthe diffusion coefficientof moistureduring osmotic dehydration processof mango. Vacuum and guar gumwere the terms that hadthe greatest influence on water loss. Forthecase of diffusionofmoistureregressioncoefficient (Table 1), indicatesthattheobtained coefficientsadequatelypredictsthebehavior of factorsinrelationtotheresponse.

The resultsto diffusionof soluble solidsshowedthat the term more significant was the linear term of vacuumandthe quadratic terms ofthetemperatureandguargum, which shows thatunder the conditionstested,there had amaximum. As reported by others authors, temperature and vacuum are factor that affect the solid diffusion [6]. The regression coefficientsobtainedadequatelypredictthe diffusion processof solidsand moisture

Table 1. ANOVA, regression coefficients and goodness of fit

Regression coefficient	Diffusion effective of moisture (D_{eff})	p-value	Diffusion effective of Solid D_{eff}	p-value
β_0 (Constant)	-37.73	0.000	-17.65	0.000
β_1 (Temperature)	-1.41	0.895	-0.61	0.884
β_2 (Vacuum)	0.11	0.008	6.89 E -02	0.056
β_3 (Gum guar)	11.72	0.412	-3.54	0.345
β_{11}	1.81 E-03	0.993	-2.72 E-04	0.001
β_{22}	-9.63 E-05	0.000	-6.97 E-05	0.934
β_{33}	-5.65	0.993	-0.33	0.000
β_{12}	1.28 E-03	0.001	5.16 E-04	0.101
β_{13}	1.30	0.138	0.65	0.000
β_{23}	-1.74 E-02	0.000	2.73 E-03	0.790
R^2	0.80		0.85	
Fit (F-value)	2.41		2.45	
Fit (p-value)	0.058[b]	0.076	0.056	

βi, regression coefficients of linear terms, βii, quadratics terms and βij interactions terms.

[a] Significant ($p < 0.05$)

[b] no significant ($p > 0.05$)

Figure2 (a-c) shown the effect of the factors studied. Figure 2(a) shows that the higher diffusivity is given by the interaction between the temperature (10 °C) and vacuum (≤ 200mbar). The Figure 2(b) shows a behavior saddle point between the temperature and thecontent of guar gum, establishing that the maximum diffusion of moisture takes place between 5 and 15 °C and concentrations of guar below 0.4%. Figure 2(c) shows an area where the minimal diffusion occurs (-4 to-2 x10^{-8} m^2/s), also was found that concentrations of 0.8% guar gum and 600mbar vacuum during packaging of the product, so at lower concentrations of guar gum (0.2 to0.4%) takes the largest moisture diffusion (-12 a -10 x 10^{-8} m^2/s), Has been recommended to use a higher concentration of guar gum at 1% w/w dispersion presents difficulties and the viscosity is very high for a liquid medium [7] and it is possible to optimize the response for diffusion coefficients values greater of -4 x10-$^{-8}$ m^2/s

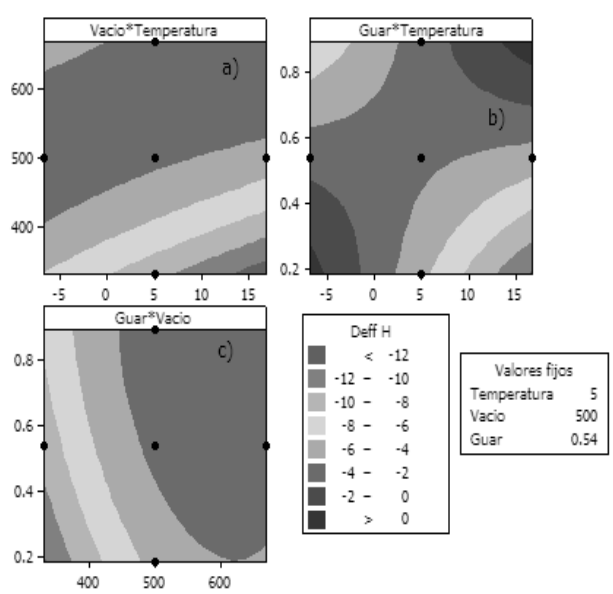

Figure 2. Effect of process variable on coefficient moisture diffusion

Figure3(a-c) shown thecontourscorrespondingto the coefficientsofsolid diffusionfromtheosmoticsolutionto the handle.Figure3(a) shows theinfluence of temperatureand vacuumonthe solid diffusion coefficient, showing thata temperature increasing 5ºC and vacuum decreasing below400 mbar, risesthe diffusion coefficientsup≈-6x 10^{-8}m/s, These resultsare explained because thetemperaturecausesincreasedkinetic energy of theliquid mediumand hencethe particles movefasterand promotesthe mass transfer process, whileguargumreplacestheairporespresent inthefruit causing decreasing the rateofmassexchange [8].
The optimization of osmotic process was conducted to maximize the moisture loss and gain solid, the optimal condition was obtained to a temperature of 5ºC, guar gum of 0.18 % and vacuum of 480 mmHg.

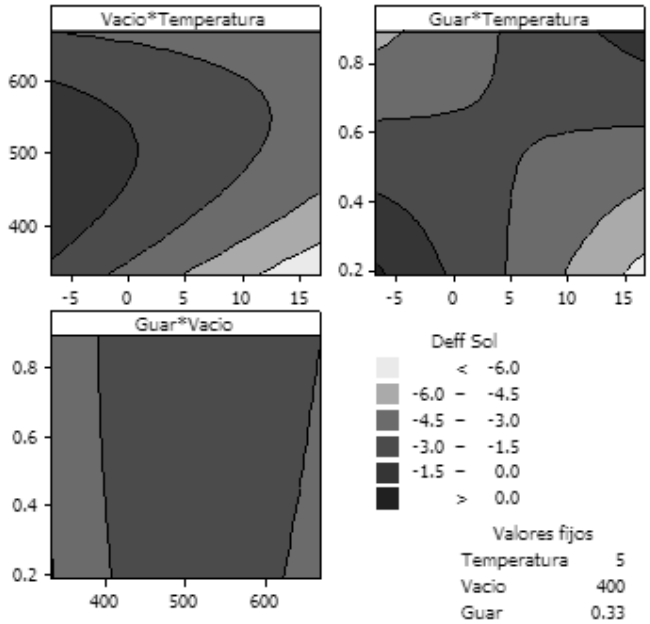

Figure 3. Effect osmotic dehydration on coefficient solid diffusion

The activation energy is a parameter that depends on the mass and temperature. The higher activation energy implies an increased sensitivity to temperature and requires a small change in temperature for the transport of solids into the membrane material give faster.The activation energies increase as the gain of solids, according to Alakaliet al. [6]. The activation energies obtained in this work with cooling temperatures between -7 and 17 ° C, were of 6,586KJ/mol in solid gain and 18,767KJ/mol in loss water. Have been reported activation energy in range of 0 to 63KJ/mol for diffusion controlled processes, so that both activation energies were obtained experimentally in this work corresponding to diffusive process.

Conclusions

The use of liquid so flow viscosity coverage and therefore with low concentrations of guar gum in combination with packaging at low vacuum promotes better solids gain.
The implementation of vacuum promotes the transfer of solids within the hand leand water loss. The vacuum is directly proportional in the diffusion rate and like wise on the activation energy.

Acknowledgements

The authors acknowledge the financial support for this work from PAPIME: PE203711 and PAPIIT: IT231511.

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Effect Of Solid Lipid Nanoparticles-Concentration And Wax Type On Edible Coating Whiteness

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Introduction

Solid lipid nanoparticles (SLN) are solid spherical submicron systems used as delivery systems and vectors in different fields. The waxes in combination with other ingredients have been applied as treatment in fresh fruit conservation. Recently, the nano-coatings have become greatinterest in the food technology in particular as treatments to increase food shelf-life.[1]. The growingof consumer demandforhigh quality products with better and new properties is an issue very important to the food research. The nanotechnologyc an solve these approaches. One resource is to user enewable biopolymers, such as polysaccharides, proteins, lipids, and compounds derived from plants and animals [2].It was found that the use of solid lipids instead of liquid oils was a very attractive idea for achieving controlled release of certain components in the feed. Solid lipid nanoparticles are submicronic dispersions with high surface area which are composed of acceptable lipis, stabilizers andwater[3]. One problem found in coating susing lipids is that when the system contact with water it acquired a white coloration on the surface, causing a poor appearance. This whiteness may be dueto a slight condensation of water vapor after the fruit has been cooled, but may also be due to various factors related to the components which form the coating [4]

Materials and methods

Beeswax (melting point = 65°C), Candeuba Wax (melting point= 85°C) were obtained from Multiceras®SA deC.V., Pluronic F-127 ®(Poloxamer 407, PF-127)(BASF, México), Xanthan Gum food grade (Drugstores Cosmopolitan®), Milli-Q ® (Millipore ®, USA-Bedfore, MD). All other reagents were of analytical grade and were used without further purification.

Preparation of Solid lipid nanoparticles(SLN)

The SLN were prepared by hot homogenization method. The wax was heated at 5ºC above the melting point by 5 min, the aqueous phase was prepared at the same temperature.The oil-in water emulsion was prepared using a Ultra-Turrax ® T-50 Basic (IKA Labortechnik) with a propeller S25 model N-25. Three cycles at10,000/5min were employed to decrease of particle size. The solid nanoparticles were formed during the cooling of the dispersion.

SLN Characterization

The particle size and the polydispersity index (PDI) were determined using a CoulterNano-Sizer® N4Plus. Measurements were made in triplicate. NL Sare diluted with water Milli-Q ® according to the volume frequency histogram.The determination was performed to angle of 273º at 25ºC [5].

ζ was evaluated for the system prepared using a Z-sizer 4 (Zetasizer Nano Series, Malvern Ltd, France) after appropriate dilution in distiller water Milli-Q®. ζ values were normalized with polystyrene standard dispersion (ζ = -55 mV). Measurements were made at 25°C in triplicate.

Morphology characterization

The morphological analysis of SLN was performed after removed the excess of stabilizer by three centrifugations at 30,000 rpm for 50 min. A drop of this concentrated suspension was spread on a microfilter membrane (200nm) and dried under vacuum at RT. They were placed onto stubs and coated with gold (~ 20 nm thickness) (Coater® JFC-1100 JEOL, Tokyo, Japan). Finally the samples were observed under a scanning electronic microscope (JSM-6400® SEM JEOL, Tokyo, Japan).

Biological Model

Lemons were used as a fruit model to compare the whiteness developed by the different coatings. They were purchased at a local marketin the municipality of Cuautitlan Izcalli. The fruit was selected taking into account a diameter of 5 cm and a brightness (Lmin= 30, Lmax= 45).

Morphology characterization

The morphological analysis of SLN was performed after removed the excess of stabilizer by three centrifugations at 30,000 rpm for 50 min. A drop of this concentrated suspension was spread on a microfilter membrane (200nm) and dried under vacuum at RT. They were placed onto stubs and coated (Coater® JFC-1100 JEOL, Tokyo, Japan) was used to coat with gold (~ 20 nm thickness). Finally the samples were observed under a (JSM-6400® SEM JEOL, Tokyo, Japan).

Nanocoatings preparation

A solution containing 0.4%of xanthan gum and 0.5% of propylene glycol was prepared and mixed with the solid lipid nanoparticles at different concentrations (30, 40 and 50%) using a mechanical stirrer (Eurostar power control-visc, IKA®WERKE) at 2000 rpm.

Nanocoating application

The coatings use dare summarized in table 1. They was applied by immersion (30s) and then dry the during 1 minusing an hand dryer (Vidal Sassoon® BrandVS773MXN1 model) with air temperature of 35°C and speed of 4 m/s. The lemons were then evaluated by whiteness index.

Table 1. CoatingCompositions.

Sample	Wax	Concentration (g/L)
1	Candeuba	60
2	Candeuba	50
3	Candeuba	40
4	Beeswax	60
5	Beeswax	50
6	Beeswax	40

Whiteness index (WI)

WI evaluation was performed by color measurements using a Minolta® CR-300. Readings were obtained at scale L *, a *, b * (CIELAB) after calibration of the apparatus, on a white plate MinoltaL *=98.11, a * = 0.22and b *=1.94.WI was calculated according to the equation:

$$WI = [(100/L)^2 + (10b^2)]^{0.5}$$

All measurementswere carried outon two sidesoflemonandthree samplesbycoating.

Statistical analysis

The resultswere analyzed using thestatistical softwareMINITAB14Inc.®, ANOVA and t-student test for mean comparison were performed.

Results and discusion

NLS Characterization

The particle sizefor theSLN of candeubawaxwas 233nmwitha polydispersity indexof0.153 and zeta potential of -39 mV.For beeswax the size obtained was 348 nm with a polydispersity index of 0.272 and zeta potential of -59mV.The polydispersity index indicates the deviation of an autocorrelation function as amono-dispersed dispersion of spheres with the same diameter [7].The difference in potential characteristics between the lipids is attributed to the chemical composition of the wax. The zeta potential allows predictions about the storage stability of the colloidal dispersion. In general, thea ggregation of the particles isless likely incharged particles (high ζ) due to the electric repulsion [8]. The results obtained indicates that submicronic nanoparticles with a low dispersion (few microspheres in the batches) and good colloidal physical stability were obtained. The Figure 1 shows particle size distribution on function of wax type, for both waxes the distribution was normal.

The Figure 2 shows the micrograph of NLS; this evidence the presence of small spheres of less thanonemicrometersize, according to the particle size distribution obtained by light scattering

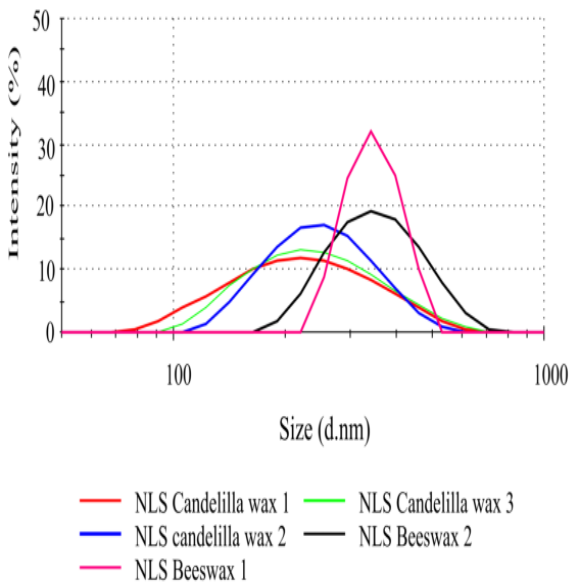

Figure 1. Particle size Distribution Of SLN

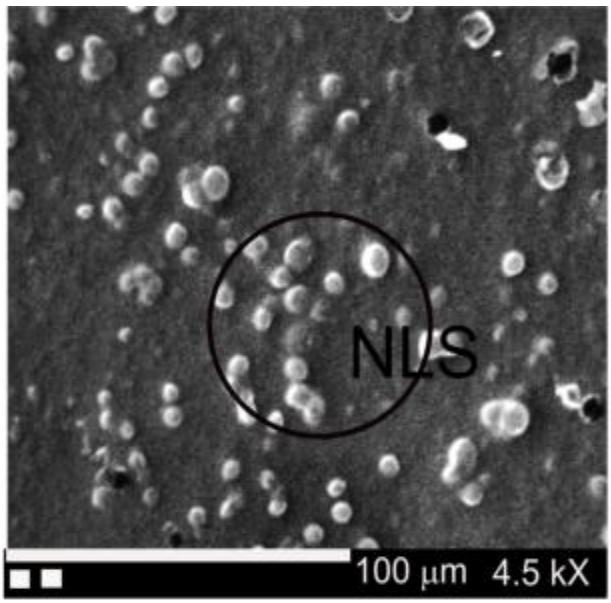

Figure 2. NLS morphology NLS of Beeswax

Whiteness Index (WI)

Table 1 shows the results of color measurement based on L*, a* and b* values It is observed that candeuba wax had a higher white component with L-values > 60 associated with whiter trend in relation to the plate calibration values. Furthermore,there was notstatistically significantdifferenceinthe function of candeuba wax concentration. On the other hand,the sampleswith beeswax showed a brightness less than 38% candeuba wax which is associated with the smaller index of whiteness coated emons shown by SLN prepared with beeswax. There was a statistically significant difference in function of the SLN proportion, thisbehavior is evidenced in the values of L *.

Table 1. Color values by lemons coatings with waxes.

Coating	L*	a*	b*
Candeuba(40 %)	68.56[a]	-15.41	44.96
Candeuba(50 %)	64.54[a]	-11.03	49.19
Candeuba (60 %)	68.95[a]	-8.39	41.23
Beeswax (40 %)	37.42[b]	-12.79	17.70
Beeswax (50 %)	40.51[b]	-13.75	19.67
Beeswax (60 %)	47.89[c]	-17.25	30.47

Different letters in same columnsare indicative significantdifference (α =0.05)

Figure 3 shows thebox plotrepresentingthe differences betweenthe use of candeuba wax and beeswax at different concentrations.

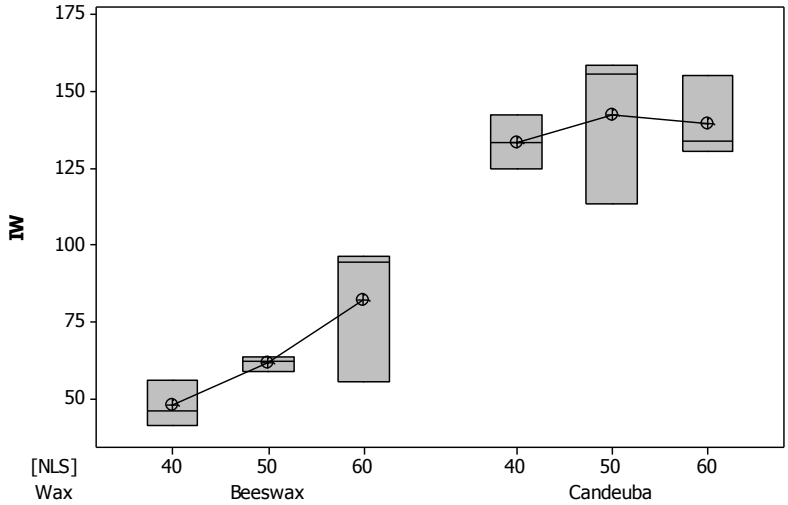

Figure 3 Comparison based on the type and concentration of wax

It confirms that the beeswax tends to decrease the WI according to theconcentration. The dispersion of the results is less at concentrations between 40 and 50% of SLN. In the case of candelilla wax there is no difference statistically significative among the concentrations but the WI is greater.

Figure 4 shows photographies of treated, with candeuba wax (40%) they had a white appearance (Fig. 4A), where as with beeswax did not show whiteness (Figure 4C), however, the two types of coating showed a white layer easily visible when they were wet ted apparently by a thickcoating (Figure 4 Band D).

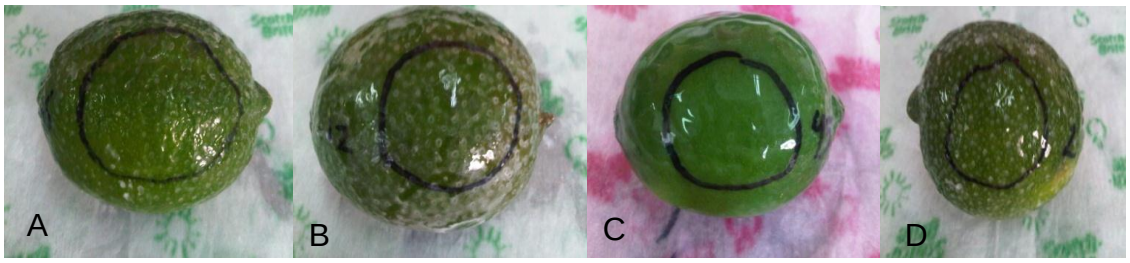

Figure 4. Lemons coated with different waxes: A Candeuba wax dry, B Candeuba wax wet, C Beeswax dry and D Beeswax wet

Conclusion

It was possible to develop wax coatings from SLN. They were capable to decrease the whiteness index on fruit surface. The composition of beeswax coatings gaveless WI. The NLS concentrations used in the formulation and the thick of the coating are determinants to the WI.

Acknowledgements

The authors acknowledge the financial support for this work from PAPIIT Ref. IT231511 and IN222411-3.

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Computational Simulation Of The Filtration Process Of Apple Juice

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Abstract

Apple juice is placed as the third most important variety of juice in Mexico´s national market. Due to this fact, hereby we present a computational model that predicts the characteristics of filtered apple juice by using data from both, the freshly produced apple juice and the filtration membrane. The process of filtration of apple juice was studied through a Brownian dynamics model and in order to find a correlation with a real system; the filtration membrane and the filtered and unfiltered juices were characterized experimentally. The pore size distribution of the membranes was studied through scanning electron microscopy (SEM) while the particle size distribution was obtained *via* dynamic light scattering (DLS). The experimental pore size and particle size distributions were compared to the theoretical distributions predicted by the model and the precision of the model was evaluated. The results from this work could be a key to the membrane selection process in the apple juice industry and quality control. It is important to mention that given the initial pore and particle size distributions, the model could also be extended to systems other than apple juice.

Introduction

Chihuahua, a state in the Northwest of Mexico, produces around the 68% of the overall apple production nationwide. (1) 70% of that production is directly sold without processing while the other 30% is mainly used for the production of juices and marmalades. (2) More than 70 million liters of apple juice were produced in 2011 corresponding to the en el 16.9% of the total production of juices in the country and placing it as the third in importance. (3)

To comply with the official quality specifications (density, total dissolved solids, appearance, color, taste, aroma and nutritional properties) established by the Codex STAN 247-2005 quality norm, the apple juice has to be subjected to various clarification, filtration and concentration processes as the juice has to remain stable and without sediments during the shelf life. Evaporation, rotative vacuum filtration, filter presses or membrane filtration, the later being the most cost effective are the most common processes in the apple juice industry (5). The membranes that are normally used in these processes are organic derived from polymers such as poly(ethylene), poly(propylene) and cellulose acetate.

On the other hand, computational simulations have been used as a very valuable tool and as a way to predict real processes. One of the simulation methods that is widely used in the study of fluids is Molecular Dynamics. This theory resolves the Newton motion equations for the atoms of the system. A variation of this method is Brownian Dynamics that is well known to describe the dynamics of colloidal particles taking into account the Brownian motion (6). Apple juice can be considered as a colloidal system and therefore the Brownian Dynamics model can be applied to it.
The motivation of this work is the lack of criteria for the selection of filtration membranes in the juice industry and its main objective is to find a correlation between the initial experimental characteristics of apple juice and a filtration membrane, to predict the characteristics of filtered apple juice. The results from

this work are believed to set the basis towards an efficient membrane selection process in the apple juice industry.

Methods

Computational simulation

The computational simulation developed in this work is based on the code previously developed by Marrufo in 2010. The filtration model is conceived as juice particles flowing through a membrane and is therefore composed of three elements; unfiltered juice, the filtration membrane and filtered juice.

The initial particle size distribution of the unfiltered juice and the pore size distribution of the membrane are the main inputs for the simulation. The size distributions are described by two parameters; b, which is associated to the width of the distribution and μ, which is related to the average size as described in equations 1 and 2 below. The particle size distribution of the unfiltered juice is characterized by the parameters b_p and μ_p. It is worth to mention that the simulations can be performed for both, filtration systems with a log-normal distribution or filtration systems with a Gaussian distribution.

On the other hand, the membrane model is defined as a series of parallel planes with a certain pore size distribution as described by the experimental parameters b_0 and μ_0. Though the minimum membrane width is calculated by the program. The minimum number of runs and the simulation time up to which the maximum number of particles have crossed the membranes are also obtained by the program (7). Finally, the simulation results quantify the particles that have crossed the membrane as given by the parameters b_f and μ_f.

$$f(x) = \frac{\sqrt{\frac{b}{\pi}}}{x \ln 10} e^{-b[Log(x)-\mu]^2} \tag{1}$$

Where b=1/(2S^2) with S as the standard deviation and μ being the logarithm of the average size, σ_0, (of both, pores or particles).

$$f(x) = \sqrt{\frac{b}{\pi}} e^{-b(x-\mu)^2} \tag{2}$$

Where $b=1/(2S^2)$ with S as the standard deviation and μ as the average size, σ_0 (of both, pores or particles).

Experimental characterization

Apple juice was extracted from golden delicious apples from Chihuahua. A first elimination of big particles was achieved after sedimentation of the juice for around an hour. The juice was then centrifuged to 4200 x g (5716 rpm) for 15 min. Cellulose acetate membranes characterized by SEM were used to filter the previously centrifuged juice (JC). The filtered juice (JCAC) was analyzed *via* DLS.

Correlation

The experimental pore and particle size distributions were used as the input for the simulation program so as to obtain the theoretical juice particle size distribution after the filtration process. Finally, in order to find a correlation between the theoretical model and the real system, the theoretical particle size distribution of the juice was compared to the experimental particle size distribution.

Results

The experimental particle size distribution parameters of the unfiletered juice (b_p and μ_p) and filtered juice (b_f and μ_f) as well as the experimental parameters of the pore size distribution of the membrane (b_0 and μ_0) are shown below in table 1.

Table 1. Experimental b and μ of both, the particle size distribution of the juice (filtered and unfiltered) and the pore size distribution of the membranes.

Filtration System	b_p	μ_p	b_0	μ_0	b_f	μ_f
JC vs JCAC	47.630	3.077	10.017	0.73	11.716	0.55

The experimental b and μ parameters of the unfiltered juice and the membranes were used as the input for the simulation program and the theoretical μ_f and b_f for the filtered juice were obtained through the simulation. The calculated and experimental values are displayed and compared in table 2 below. The % relative errors of both parameters are also presented in the table. As it can be observed, there is only a 10% error from the comparison of μ values while the error from the comparison between b values is much more significant.

According to the graphical comparison (figure 1), the theoretical and experimental distributions are centered in a very similar average particle size. There is a relative error of less than 10% in the comparison from the calculated and the experimental average particle size. However, the distributions differ in the width, associated to the parameter b. The polidispersity of the centrifuged system can be assumed as narrow and with a trend to decrease after the filtration process. However, the DLS from the experimental results indicate that the range of particles that cross the membrane in the real system is broader than the predicted system. This behavior can be attributed to some of the factors that are omitted in the simulation model like the effect of pressure, the interactions among the juice particles, the formation of particles aggregates and the fouling of the membrane.

Table 2. Comparison between calculated and experimental b_f and μ_f values for the filtration system.

Filtration system	bf Experimental	μf Experimental	bf Calculated	μf Calculated	%Relative Error b	%Relative Error μ
JC vs JCAC	11.71	0.55	48.357	0.50	312.95	9.04

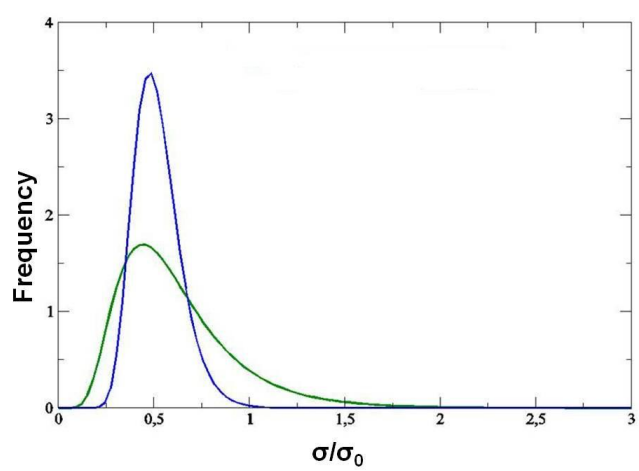

Figure 2. Comparison between calculated (blue) and experimental particle size distribution) of the JC vs. JCAC filtration system.

Conclusions

The correlation between a computational model and a real apple juice filtration system was successfully studied in this work. In order to achieve this, experimental data from apple juice and a cellulose acetate membrane was correlated to the theoretical data obtained from the simulation. By comparing both, the particle size distribution of apple juice prior and after filtration and the pore size distribution of the filtration membrane it can be said that the simulation is successful in predicting μ, a parameter related to the average particle size in the apple juice after filtration. However, there is a significant error in the prediction of the parameter b that is associated with the broadness of the distribution. This behavior can be attributed to some of the factors that are omitted in the simulation model like the effect of pressure during filtration, the interactions among the juice particles, the formation of aggregates and the fouling of the membrane.

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Mitigating Effect Of Calcium And Magnesium On Acrylamide Formation In Tortilla Chips

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Since its discovery in heated foods in 2002, the acrylamide (AA) has gained worldwide attention as a consequence of its potential toxicity at the concentrations at which it is formed during thermal food processing. Studies have indicated that polyvalent cation reduces the formation of AA in thermally processed snack foods and bakery products. In this study, It was evaluated the effectiveness of calcium and magnesium, as $CaCl_2$ and $MgCl_2$, to reduce AA formation in tortilla chips, fried during 30 sec at 190°C, using soybean oil. It was found that AA was reduced up to 70% by adding of $MgCl_2$ to the water used to make the masa, at a 0.04, 0.08 and 0.12 M concentration, compared to 52, 67 and 52% for 0.04, 0.08 and 0.12 M of $CaCl_2$, respectively. The color and fracture force of tortilla chips, added with $CaCl_2$, were similar to the control. In contrast, the tortilla chips added with $MgCl_2$ were darker and less hard than control. These findings suggest that the $CaCl_2$ and $MgCl_2$ can be used as a viable approach for the mitigation of AA formation in tortilla chip products.

Keywords: Calcium, Magnesium; Acrylamide mitigation, Tortilla chips

Introduction

Worldwide, 13% of deaths are caused by cancer (1), as consequence, factors as the presence of toxic compounds in foods have gained widespread attention. Acrylamide, a neurotoxic and probably human carcinogen molecule (2, 3) is formed in relatively high concentrations when carbohydrate-rich foods are processed at high temperatures (4). Maillard type reactions have been shown as one major reaction pathway (5, 6). Acrylamide is produced by degradation of asparagine in presence of carbohydrates, however, other reaction precursors such as lipid oxidation products have been proposed (7, 8, 9, 10). Based on the maintenance of original sensory attributes and heat processing procedures, various chemical agents have been tested for their effectiveness as food additives in reducing the formation of AA on thermally processed foods. Consumable acids, cations, phospholipids, lecithins, hydrocolloids, antioxidants and amino acids are been used as effective mitigation agents in model systems and real food samples (11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21). Among all possible above-mentioned mitigation strategies , the use of metal cations have been showed like a easy, inexpensive and viable approach for the minimization of acrylamide content, in commercial as well as in homemade foods (22). This study analyzes the acrylamide mitigating properties of metal cations by determining acrylamide formation in tortilla chips containing either calcium chloride or magnesium chloride.

Materials and methods

2.1. Materials

Deuterium-labeled [2,3,3-$^2H_3$]acrylamide were obtained from Sigma–Aldrich Company (St. Louis, MO, USA), respectively. $CaCl_2$ and $MgCl_2.6H_2O$ were all reagent grade and obtained from Merck (Darmstadt, Germany). All other chemicals were purchased from Sigma–Aldrich Company (St. Louis, MO) or Merck (Darmstadt, Germany), and were analytical grade. Nixtamalized corn flour (NFC) was produced with

commercial corn variety named Pioneer 30P16 (harvested in Guanajuato and Michoacán, México,) and commercial lime ($Ca(OH)_2$) (El Topo, Monterrey, N.L. Mexico) , commonly used in the tortilla industry. This flour was prepared by cooking 8 kg of whole maize kernels in a solution of 16L of water and 80g of calcium hydroxide. The maize was boiled in an aluminum pan for 25min and steeped 16h at room temperature (22 ± 1ºC). The steep liquor (nejayote) was removed. The cooked maize was washed with 16L of water, then ground into corn masa (FUMASA, M100, Querétaro, Mexico), and finally dehydrated using a flash type dryer (Cinvestav-AVG, M2000, Querétaro, Mexico). Before storage, the dry nixtamalized corn flour was milled using a hammer mill (PULVEX 200, Mexico DF, Mexico) with a 0.5 mm screen.

2.2. Elaboration of tortilla and tortilla chips with calcium and magnesium chloride.

The tortilla chips were elaborated of the next way: 100g of nixtamalized corn flour was rehydrated with 118mL of 0.04, 0.08, 0.12 M $CaCl_2$ or $MgCl_2$ to provide fresh masa with adequate consistency to make tortillas. For the control treatment only water was used to make the masa. The additives final concentration in masa was: 0.53, 1.07 and 1.60% (w/w) for $CaCl_2$ and 0.38, 0.76 and 1.14 % (w/w) for $MgCl_2$. The masa was flattened into thin disks of 11cm diameter and 1.0mm thickness using a commercial tortilla roll machine. Shaped tortillas were cooked on both sides for about 1.0 min by using an iron hot plate (270 ± 10 ºC). The elaborated tortillas were cut into circular pieces with an average area of $10cm^2$. Tortilla pieces were fried for 30s at 190ºC using soybean oil. After frying, tortilla chips were cooled on a paper towel to remove superficial oil and the color, breaking force, and acrylamide content determined.

2.3. Color determination in tortilla chips.

The color of tortilla chips was determined using a colorimeter MiniScan XE, model 45/0-L (Hunter Associates Laboratory, 11491 Sunset Hill Rd., Reston, Va., U.S.A.) and the CIELAB *L*, *a b* method. This parameter was reported like color total differences (ΔE) which was calculated according to equation: $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$, where L = brightness or lightness, a = greenness/redness, b = yellowness/ blueness; ΔL, Δa, and Δb = absolute differences of the values between the reference tile (white porcelain) and sample values; ΔE = total difference between reference and sample color. The reference values (calibration) were: L = 92.22, a = -0.82 and b = 0.62.

2.4. Texture determination in tortilla chips

The fracture force of tortilla chips was evaluated in freshly prepared samples, using the Texture Analyzer TA-XT2 (Texture Technologies Corp., N. Y.). The test was carried out using a 2.03 mm diameter stainless-steel probe and a platform accessory with a hollow cylindrical base with 33.5 and 10 mm external and internal diameters, respectively. The probe traveled at a velocity of 10 mm/s until it cracked the sample (distance 6 mm).

2.5. Acrylamide determination in tortilla chips

Acrylamide was analyzed as the stable 2-bromopropenamide derivative by gas chromatography–mass spectrometry (GC–MS) using the method described previously (23). The tortilla chips were ground in a lab mill and powered samples (~0.8g) were weighed, placed in centrifugal tubes, added with 20µL of internal standard solution (0.5mg/mL of deuterium-labeled [2,3,3-$^2H_3$]acrylamide in acetonitrile), 8mL of distilled water and 10mL of *n*-hexane at room temperature, and stirred for 5 min. After centrifugation at 2000x*g* for 10min, organic phases were removed, and supernatants filtered. These extracts (3mL) were treated with 0.45g of potassium bromide, 200µL of sulfuric acid (10% v/v), and 300µL of potassium bromated solution (0.1M). After 1h in the dark at 4°C, the bromination reaction was terminated by adding of sodium 1M sodium thiosulfate until solutions became colorless, and solutions were extracted with 4mL of ethyl acetate/hexane (4:1). Organic layers were recovered after centrifugation at 2000x*g* for 10 min, and were dried with sodium sulfate and evaporated to dryness under nitrogen. Each sample was dissolved in 50µL of ethyl acetate, treated with 25µL of triethylamine, and analyzed by GC–MS. The ions monitored for the identification of the analyte, 2-bromopropenamide, were $[C_3H_4NO]^+$ = 70, $[C_3H_4{}^{79}BrNO]^+$ = 149,

and $[C_3H_4{}^{81}BrNO]^+$ = 151, using *m/z* 149 for quantification. The ions monitored for the identification of the internal standard 2-bromo[$^2H_2$]propenamide were $[C_2{}^2H_2H^{81}Br]^+$ = 110 and $[C_3{}^2H_2H_2{}^{81}BrNO]^+$ = 153, using *m/z* 153 for quantification.
GC–MS analyses were conducted with an Agilent 7890GC system coupled with an Agilent 5975C MSD. In all experiments, a 30m×0.25mm i.d.×0.25μm HP5-MS capillary column was used. Working conditions were: carrier gas helium (1mL/min at constant flow); injector, 250°C; oven temperature: from 50 (1min) to 240°C at 5°C/min and then to 300°C at 10°C/min; transfer line to MSD, 280°C; ionization EI, 70 eV.
Quantification of acrylamide was carried out by preparing standard curves of this compound. Acrylamide content was directly proportional to the acrylamide/internal standard area ratio (r =0.999, p < 0.0001). The coefficients of variation at the different concentrations were lower than 10%.

2.6. Statistical analysis

All results are expressed as mean values. Statistical comparisons between two groups were made using Student's *t*-test. With several groups, one-way analysis of variance was used. When significant *F* values were obtained, group differences were evaluated by the Tukey test. All statistical procedures were carried out using the JMP 9.0 package (SAS Institute Inc., Cary, NC). The significance level is p< 0.05 unless otherwise indicated.

Results and discussion

The addition of metal cations to masa decreased the pH to an extent that depended on the amount of salt added. It has been showed that lowering pH caused a reduction in acrylamide formation (11). Initial pH value (control) was 7.8. Masa prepared with 0.04, 0.08 and 0.12 M $CaCl_2$ and $MgCl_2$ had a pH value of 7.40, 7.23, 7.18 and 7.25, 7.19, 7.14, respectively.
Tortilla chips are particularly, popular consumer snack products in Mexico, United States and other countries. In despite of tortilla chips popularity, there are few studies about acrylamide content in this type of food. Figure 1 shows the average level of acrylamide formed in tortilla chips when the frying process was performed at 190°C for 30s. The acrylamide concentration for tortilla chips without the presence of salt (control) was estimated as 1024±53μg/kg. These levels are 4-fold higher than FDA data on acrylamide concentration in foods report (24). The presence of either $CaCl_2$ or $MgCl_2$ on experimental tortilla chips resulted in decreasing of acrylamide concentration. There was a trend of decreasing acrylamide formation during frying that reached statistical significance compared to the control for the 0.04, 0.08 and 0.12 M samples with acrylamide reduced by 53, 68, 53 and 69, 70, 74% for $CaCl_2$ and $MgCl_2$, respectively. Similar results have been reported for potato strips (16), wheat based products (25, 26), cereal matrices (27) and cookies (28), where the use of metal ions derivatives in different forms are capable of reducing acrylamide levels. It has been recently suggested that adding divalent metal ions could give high-temperature stability to asparagine/matrix interactions, thereby rendering the latter species unavailable for reaction with carbonyl precursors to produce acrylamide (29). On the other hand, although the results indicated that the presence of $MgCl_2$ significantly prevented acrylamide formation in tortilla chips during frying, a bitter aftertaste was perceived in the $MgCl_2$ treated products, which was not detected in tortilla chips prepared with $CaCl_2$.
Figure 2 shows the effects of different concentrations of calcium and magnesium chloride on the color and fracture force between the samples and the control. Addition of $CaCl_2$ resulted in tortilla chips not significantly different from the control sample in terms of color and fracture force. Instead, addition of $MgCl_2$, all formulations were significantly different (p<0.05) from the control sample. As the $MgCl_2$ in the experimental masa increased from 0.04 to 0.12M, there was a trend of increasing and decreasing color and fracture force, respectively. It has been suggested in model system that browning is accelerated by the presence of $MgCl_2$ due to the increased sugar hydrolysis and to the reduction of water mobility caused by the salt counteracting the inhibitory effect of water on the reaction. In accordance with the aforementioned, starch hydrolysis increase sugars concentration that could participate in Maillard reaction

which resulted in lower fracture force values as consequence of the formation of crack in tortilla chips structure.

Conclusions

The use of salts as $CaCl_2$ or $MgCl_2$ is effective acrylamide mitigation strategy in tortilla chips production. However, although a much more detailed sensory evaluation of tortilla chips prepared with salts, the adverse effects of $MgCl_2$ on sensory properties obtained in this study are considered as a significant limitation for their applicability in the nixtamalized corn products industry.

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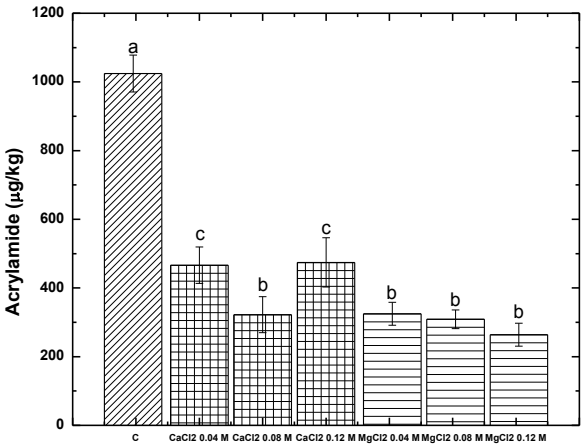

Fig. 1

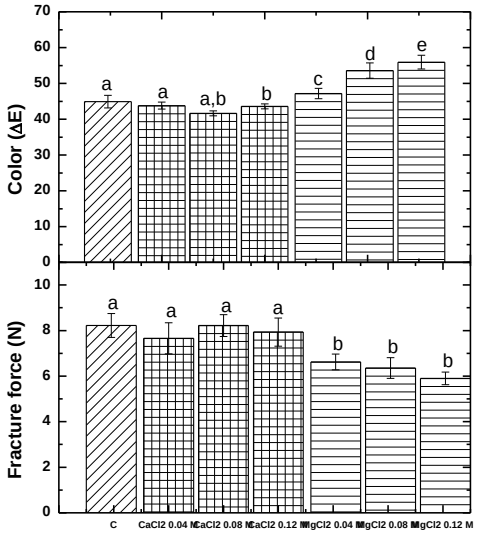

Fig. 2

Figure legends

Fig. 1. Effect of calcium chloride (crosshatched bars) and magnesium chloride (horizontally striped bars) on acrylamide content in tortilla chips fried at 190ºC for 30s. Tortilla chips without salts (striped bars) are included for comparison. Bars with different letters in the same panel has significant differences (Tukey, $p = 0.05$).

Fig. 2. Effect of calcium chloride (crosshatched bars) and magnesium chloride (horizontally striped bars) on: A) color and B) texture, in tortilla chips fried at 190ºC for 30s. Tortilla chips without salts (striped bars) are included for comparison. Bars with different letters in the same panel has significant differences (Tukey, $p = 0.05$).

Effect Of Dietary Supplementation With Vitamin D3 And 25-OH D3 In Antioxidant Activity Of The Enzymes Catalase, Glutathione Peroxidase (GPX) And Superpxide Dismutase (SOD) Of Pork

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Introduction

Oxidative processes in meat lead to quality deterioration (1). Meat has endogenous antioxidants and prooxidants and living cells have several mechanisms of protection against oxidative processes, including antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px). Catalase and glutathione peroxidase (GSH-Px) are considered the major peroxide-removing enzymes located in the cytosol (2). SOD plays an important role in protecting against damage by the superoxide anion radical (3).

Information on factors influencing the activity of antioxidant enzymes in meat is limited. Antioxidant enzyme activities differ between meat of different species (4) and muscle type. Antioxidant enzyme activity could also vary between animals of a single species. Therefore, variations in the activity of these enzymes between different genetic types could lead to differences in oxidative stability of the meat.

Methods

Sample collection

The study was conducted with *Longissimus dorsi* obtained from 80 pigs fertilis x G-Performer with an initial weight of 85 kg. The animals are randomized based on gender and weight to be assigned to one of 4 treatment provided: T1 was a control-negative, T2 was Vitamin D_3 (20,000 IU/Kg), T3 was 25-OHD_3 (500 µg/Kg) and T4 was a positive-control contained: vitamin-E(180 IU/Kg), selenium-organic (0.15-ppm) and astaxanthin (10-ppm). The pigs were fed for 35 days on site INIFAP Ajuchitlan, Querétaro. At day 36 were slaughtered TIF No. 412, (Km 8.5 Carretera San Jose Chichimequillas Alto, Querétaro, Querétaro). At 24 hours postmortem the cutting was performed in the TIF-No. 201 (TIF Apaseo, Narciso Mendoza # 408 Colonia Centro, Apaseo el Grande, Guanajuato). The left Longissimus dorsi muscle of each animal was identified and 9 chops cut into 2 cm thick cuts were packed with polyethylene bag is then closed under vacuum stored at 4 ° C in a cooling chamber for a period of up 28 days. The analysis performed on days 1, 7, 14, 21 and 28.

Sample preparation

Chopped meat samples were disrupted for 2 min whit an Ultra Turrax T18 homogenizer IKA (Staufen, Germany) homogenizer in potassium phosphate buffer (50 mM, pH 7). Homogenates were centrifuged at 10,000 rpm and 4°C (Eppendorf centrifuge 5810R, Hamburg, Germany) during 30 min and supernatant was collected and stored in an ice bath.

Determination of the activity of catalase

An aliquot of 0.1 mL of homogenate (prepared as describe above) was added to 2.9 mL of H_2O_2 (11 mM), the absorbance was read at the beginning and during the next 30 seconds to complete 2 minutes, using a spectrophotometer Varian 50 Bio (New Jersey, USA). The results were expressed as U / g of meat (2).

Determination of the activity of glutathione peroxidase

The reaction mixture was prepared by placing potassium phosphate buffer [40 mM, pH = 7, EDTA (0.5 mM) and sodium azide (1 mM)] was then added GSH (reduced glutathione, 1 mM), glutathione reductase (1.5 U / mL) and 50 µL of crude extract. The tubes were incubated for 5 min at 35 ° C after which time was added NADPH (0.15 mM) and the reaction was started by adding hydrogen peroxide (0.15 mM). Absorbance was read using a spectrophotometer Varian Bio 50 (New Jersey, USA) after 15 seconds into the reaction was monitored every 10 seconds up to a minute. We used the molar extinction coefficient (6.22 mM^{-1} cm^{-1}) to calculate the concentration of NADPH. The results were expressed as U/100g of meat (1.2).

Determination of superoxide dismutase activity

Was determined using a kit from Sigma-Aldrich SOD Co (Germany) and used according to instructions provided by the manufacturer.

Statistical analyses

Statistical analysis of the data was performed using the Statistical Package for Windows XLSTAT 2011 v. 4.02 (Addinsoft SARL, New York., USA). We applied a method of analysis of variance (ANOVA) where the source of variation was the treatment, day and treatment-day interaction, subsequently a multiple comparison of means by Duncan's test ($P \leq 0.05$).

Results

Catalase activity

This test is relevant because some studies suggest that endogenous antioxidants, especially catalase can minimize oxidative rancidity during storage because it is a very stable enzyme deactivated only by effect of the temperature (3, 4). The analysis of variance show that there is significant difference for all factors (treatment, day and treatment-day interaction). This indicates that the dose and source of vitamin D_3 had significant effect ($P \leq 0.05$) on the catalase enzyme activity.

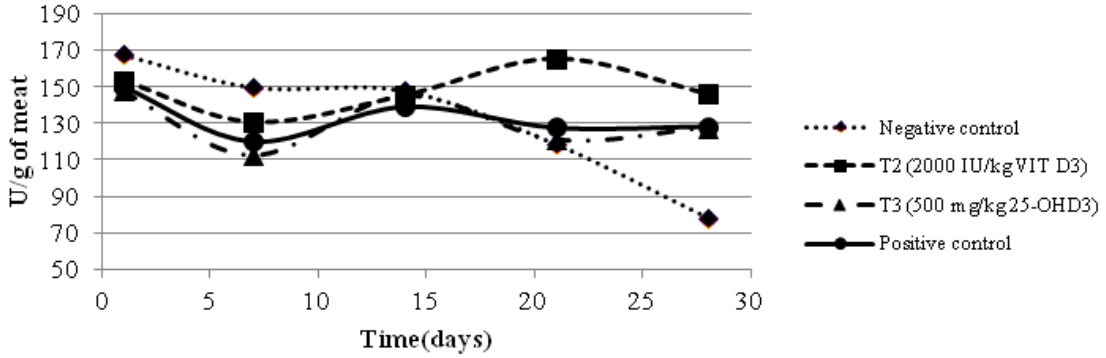

Fig. 1 Variation in the catalase enzyme activity with respect to storage time pigs cuts supplemented with vitamin D_3 and 25-OHD_3.

The activity was higher at day 1 (Fig. 1) being the negative control (167.405 U / g meat) which showed the highest activity, but treatment 2, 3 and the positive control (153.99, 150.02 and 149.56 U/g meat, respectively) showed the same activity between them. However, the activity decreases as time passes being the negative control which had the lowest activity at day 28 (78.06 U/g meat), while treatment 3 and the positive control showed the lowest activity at day 21 (127.763 and 120.834 U/g of meat).

The values of the catalase enzyme activity determined in this paper ranged between 86.235 and 182.823 U/g of meat which had the same tendency and close to those obtained by various authors (2) that are of 187 and 381 U/g meat of the difference in data is attributed primarily to the characteristics of the pigs used, since not correspond to the same features and the type of muscle used for the study. Reports on the antioxidant activity of the catalase are scarce despite that it is a critical enzyme which is stable during storage of refrigerated fresh meat (3). In general, it is considered that low concentrations of hydrogen

peroxide stimulate the activity of glutathione peroxidase while high concentrations are preferably catalyzed by catalase (5).

Glutathione peroxidase (GPX) activity

Glutathione peroxidase was determined in order to verify their presence in the pork and the effect of treatment on their activity. Analysis of variance showed significant differences for all factors (treatment, day and treatment-day interaction). This indicates that the dose and source of vitamin D_3 had significant effect ($P \leq 0.05$) on the enzymatic activity of glutathione peroxidase (GPX).

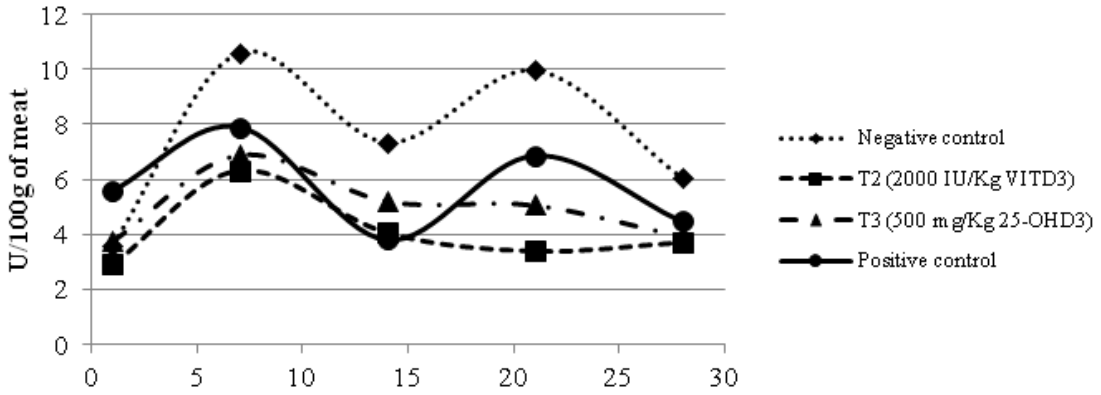

Fig. 2 Variation in the enzymatic activity of glutathione peroxidase (GPX) with respect to storage time pigs cuts supplemented with vitamin D_3 and 25-OHD_3.

The lower activity of all treatments showed day 1 (Fig. 2), the activity was increased as time passes being the largest storage at day 7 for the negative control (10.615 U/100g of meat) whereas the positive control, treatment 3 and treatment 2 showed the same activity between them (7.88, 6.89 and 6.33 U/100 g of meat, respectively) then began to decrease activity to decrease in all treatments reduced activity observed at day 28 in the treatment 2 (4.102 U/100g of meat). It was observed that the highest enzyme activity were present at day 7, which corresponds to some previous work (3.7), in addition to which an enzyme is fairly stable over time raw meat storage, and unlike of catalase does not need bigger amounts of hydrogen peroxide to display their activity. The decrease in activity from 21 days, it could be because the system glutathione GSH / GSSG ratio is one of the biggest redox systems in animal tissues and their presence decreases during storage of the exudate through the meat (6).

Superoxide dismutase (SOD) activity

This enzyme was determined in order to observe if their activity modified the effect of treatment assignment. The analysis of variance showed significant difference only for the storage time (days) on the enzymatic activity of superoxide dismutase (SOD).

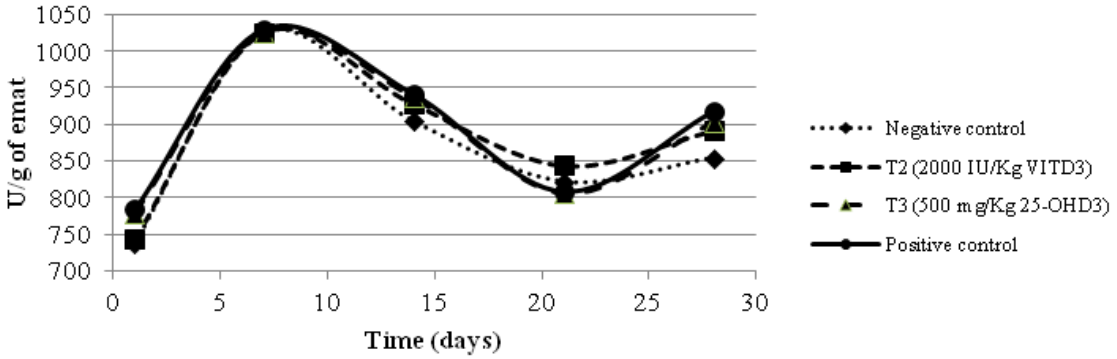

Fig. 3 Variation in the enzymatic activity of superoxide dismutase (SOD) with respect to storage time pigs cuts supplemented with vitamin D_3 and 25-OHD_3.

At day 1 (Fig. 3) all treatments showed the lowest enzyme activity, with the negative control and the treatment 2 showed lower activity (737.701 and 743.129 U/g meat, respectively) whereas the positive control and treatment 3 showed the highest activity (783.59 and 778.26 U/g meat, respectively), however the activity on day 7 in all treatments increased equally. Subsequently, the activity began to decrease with the lesser activity on day 21 being observed primarily in the positive control and treatment 3 (808.36 and 805.02 U / g meat, respectively).
In this paper the results are between 845.136 and 888.690 U/g of meat, which corresponds to what is described by some authors (2,6) where they report results between 657-928 U/g of meat, mainly Iberian pig.

Previous studies have agreed that different types of diets based on corn, sorghum and a mixture of both, the activity of SOD showed no difference in the effect of diet because this enzyme exists in three forms: Cu-Zn- SOD, Mn-SOD and Fe-SOD and probably conclude that none of the compounds present in diets may have effect on the metals which form part of this enzyme and achieve the effect by diet administered (3.7), probably due to this there was no effect of treatment provided. Plus it also depends on the type of muscle that is being used for the study, and found that increased activity in glycolytic muscles, namely, who have higher levels of oxidation, compared to muscles that have low glycolytic activity as *Longissimus dorsi* (6).

Conclusions

The correlation of SOD activity of GPX activity was positive (0261), while the catalase activity of SOD and GPX was negative (-0145 and -0105), indicating that the enzymatic activity can be a system to be coupled as a first mover endogenous antioxidants and others act later. However, no difference was observed between treatments, since in some determinations was better the negative control, which would suggest that probably both vitamin D_3 and its active metabolite 25-OH D_3 may not have lasting antioxidant effect in meat during storage time could therefore reformulate diets.

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Influence Of Freezing Temperature Oscillations In The Cryoconcentration Performance Using Dialysis-Freeze-Centrifugation And Its Recursive Usage

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Introduction

Actually, the solution concentration is a common method in different labs to around of world. Consequently, more research labs work and produce bio-molecules modified how are the proteins, peptides and anti-bodies with large range of job. This has applications on medicine, foods, industrial and academic. Protein concentration and purification methods are the key for many thechnologies that are transforming the way to learn about cellular processes. In general, biomolecule purification involves a complex series of steps where targets are selectively separated though sequential processes,[1,2]. In general, for large-scale applications, there are three main processes with common commercial application in separating solutes from aqueous solutions: evaporation, membrane technologies, and freeze concentration.[3,4]. Ultrafiltration is widely used in biotechnological industry and research laboratories; however, concentration polarization and membrane fouling remain as the usual problems present during its application[5,6,7]In general, the cryoconcentration process consists of ice crystallization, ice crystal growth, and the separation of the concentrate solution [8,9]. After the beginning of ice crystallization, the concentration of the remaining solution is increased continuously; hence, the freezing point is progressively lowered [10]. Thus when the thawing process arises the earlier droplets, the solute is eluted from the matrix, in the freeze thawing method, the concentrated solution is drained off while the ice fraction is allowed in a solid state with minimal dry matter content; however, the time required for the thawing process can be far longer than that required of freezing because of the thermal conductivity, and thus the rate of heat transfer through an unfrozen layer can be less than one-third of the conductivity and heat flow rate through an equivalent frozen layer [11].

Methods and Materials

Cryoconcentration device and procedure

The cryoconcentration procedure was based on the use of a device (fig.1)[12]. The components listed in this section referto fig 1. Scrap ultrafilters used for protein concentration (pierce Protein Concentrators) were used to develop a device crioconcentrator at laboratory scale (20 ml). For this purpose, the cellulose membrane from the ultrafilter was removed by using a solution of chlorhydric acid and sulfuric acid (ratio of 1:1, v/v) for 8 hours. After, cellulose residues were removed either the helps of a glass rod, the device was finally washed for complete cleaning. To build the component 1 a transversal cut of 3 mm in the bottom of the device was made. Component 2 is made by cutting a pierce of tube approximately 2 cm from the upper end of a tube polypropylene falcon type of 50 ml. After assembly of the device using the threaded cap (component 4) to close the device;the cap has a disc-styrofoam (component 5) to seal the sample holder device and allow its loading. This was used for the experiment of concentration reported in this study. Was designed and used for freezing protein solution and recovering the cryoconcentrated solutes by centrifugation rather than conventional thawing.

Freezing temperature oscillation

Freezing was achieved at -70ºC for 8 h by using an ultra freeze (ultraslow SANYO, USA) for the desired slow freezing. Was transferred to refrigerator to 4ºC (torrey R36-4P, Mexico) by intervals of 15, 30 and 60min.After freezing, component 1 containing the frozen sample was placed in a 50-ml polypropylene tube (component 3) and centrifuged in a Sorvall RC 6+ centrifuge (Thermo Scientific, Germany) to collect the concentrated solution. Was repeated this process, by was changed the temperature of 4ºC to -10ºC,(was used a conventional freeze, torrey CVPS-15, Mexico).

Milk whey

Fresh milk whey was prepared by acid coagulation with hydrochloric acid (pH 4.6, Sigma-Aldrich, USA). The separated whey was centrifuged (11,000g at 4ºC for 15 min), and the supernatant was filtered and stored at 4ºC, for this purpose, commercial pasteurized skim cow`s milk was used.

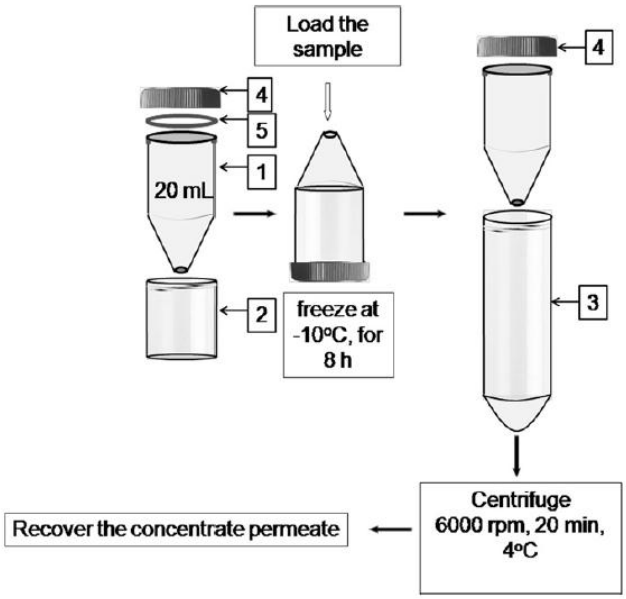

Fig.1.-Cryoconcentration device used for protein concentration based on the method of freez-dialysis-centrifugation [11]

Concentration of solutes measurement

Total soluble solid were measured using an ATAGO refractometer (model PAL-1,ATAGO.Japan)with an accuracy of ±0.1 ºBx.

Protein content

Protein concentration was determined spectrophotometrically at 595 nm (Lambda 25, PerkinElmer, USA) according to the method of Bradford. Bovine serum albumin (BSA) was used as an external standard. All protein determinations were performed in triplicate.

Dialysis

Protein solution were dialyzed against distilled water (simple/water, 1:100) for 2 h at 4ºC using dialysis tubing cellulose membrane (12kDa MWCO, Sigma-Aldrich) and with continuous stirring. A change of distilled water was made after the first hour. Later, was cryoconcentrated to -70ºC for 2h.This process repeated itself on the same sample till that was not found a significant change in the concentration.

Results and discussion

During freeze concentration process the water is segregated from the aqueous solution by crystallization and the solute is concentrated in the residual solution. In this study, the freezing and thawing principle was used to develop a procedure to concentrate protein solutions at a laboratory scale using the device described in Fig. 1, accelerating the elution of solutes by centrifugation. Fresh milk whey was used as a model to optimize temperature of freezing. The samplewas cryoconcentrated from 7.5+0.2 to 55.8+0.3ºBrix. Implying that a concentration factor of 7.5-fold was achieved (Fig. 2); recently, was obtained a result of 6.4-fold using this device but was used freezing temperature from -10ºC and your freezing time was from 8 h.was observed the behavior in the concentration at -70ºC through time, using

the cryoconcentration device where concentration was increased to 55.8ºBrix in a time of 2 hours then there was no a considerable increase in the concentration (Fig2).

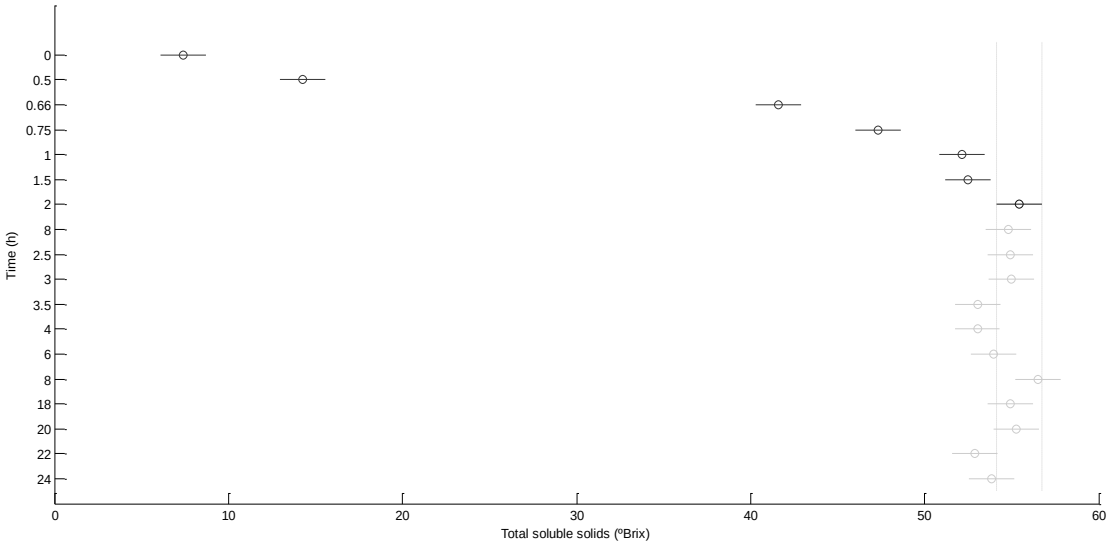

Fig 2.-Correspond to one-way ANOVA with ºBrix. This analisis revealed no significant diference between any time more than 2 hours at a temperature of -70ºC freeze.

Freezing Temperature Oscillations

The freezing temperature was oscillated from -10 to -70ºC, with stages at 4ºC (at different span of time, 15, 30, 60 min) and was observed that the freezing temperature oscillation did not occur a significant increase how was observed in the fig 3.

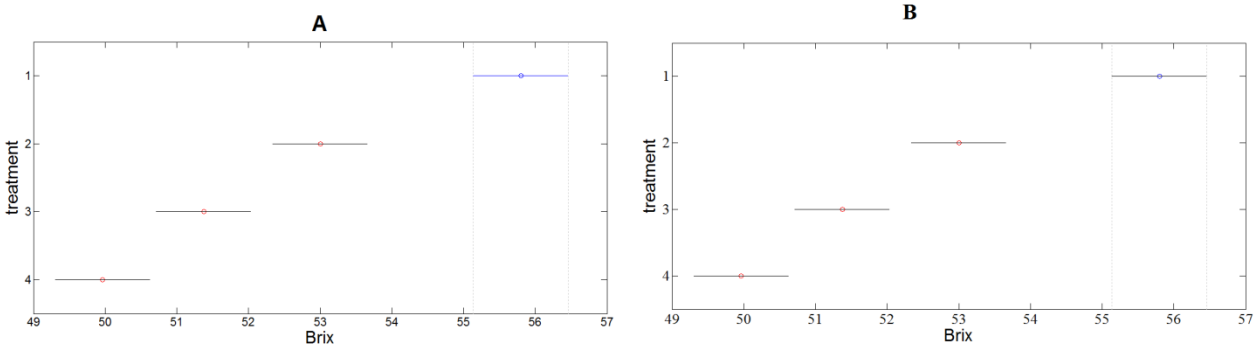

Fig. 3.-The part A4 treatment are observed representing the oscillation of freezing temperature. 1 treatment on the sample was exposed to a temperature of -70ºC for 8 h. 2 treatment on the sample was exposed to a temperature of -70ºC for 2 h then exposed to a temperature of 4ºC for 15 minutes, this process was repeated 4 times. 3 treatment on the sample was exposed to a temperature of -70ºC for 2 h then exposed to a temperature of 4ºC for 30 min, this process was repeated 4 times. 4 treatment on the sample was exposed to a temperature of -70ºC for 2 h then exposed to a temperature of 4ºC for 60 min. this process was repeated 4 times. The part B 4 treatment are observed representing the oscillation of temperature similar to part A but the temperature change of 4ºC to -10ºC.

Dialysis

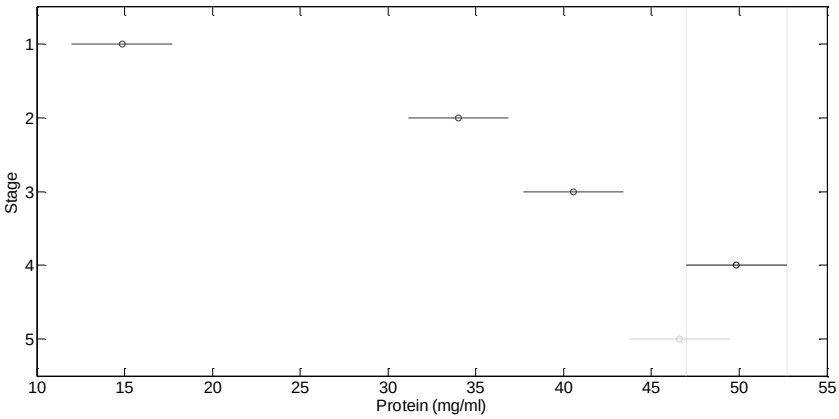

Fig. 4.- Increment of protein by stage in the cycle freezing-dialysis-centrifugation. Stage 1.-first cryoconcentration. Stage 2.- second cryoconcentration. Stage 3.- third cryoconcentration.Stage 4.- fourth cryoconcentration. Stage 9.- fivethcryoconcentration

This analisis revealed no significant diference between any times more than 2 hours at a temperature of -70ºC freeze

Was realized a recurrent dialysis and was observed that the stage increase of freezing-dialysis-centrifugation was increased of protein concentration but after fourth stage presented no significant diference between any stage more 4 stages at a temperature of -70ºC

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Use Of Life Cycle Analysis (LCA) For Environmental Impact Analysis Of Biodiesel Production In Integrated Small Farms

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Introduction

Most of the energy needs in virtually every country in the world, use non renewable energy sources, such as oil, coal and natural gas. The oil is the more significant fraction, provides (37%) of the world's energy. In the U.S., consumption leading energy, most of the oil is used in the transport sector. In fact, more than 95% of the energy used in this sector comes from oil. In this context, has been documented the environmental risk and economic dependence from this nonrenewable energy source. Taking into account that one of the options for energy generation (Biofuels) from biomass is the production of biodiesel. Some of the advantages reported in the use of biodiesel are:

- Reduce oil dependence
- Close the life cycle greenhouse emissions

However, the use of biofuels could present considerable environmental impacts. Thus, the use of biomass as a feedstock for bioenergy could have significant environmental impacts, such as land contamination and erosion of these, those ones are considered as ones of the major environmental problems, including climate change compared to, this in turn reduces the biodiversity and ecosystem degradation. Moreover, at present the production of biodiesel has a higher cost than conventional systems based on fossil energy. Therefore, in this paper we propose an alternative to the typical production of biodiesel (monocultures extension), that by using tools such as LCA (life cycle assessment) and process simulation. This by implementing a system of small farms that generate energy and economic independence among them, reducing the environmental impact and activating local economies.

Methods

As a first step scenario proposed study in which cultivation and cattle driving in model farms were based on literature data. Sought maximize the resources of the area, without exporting which could affect to environmental symbiosis and integrating the above feasibility studies in the field. This methodology represents an iterative process which seeks to obtain the stage ideal which leads to the determination of the study area.

Second step:

•Determine the location of the farms.

•Farms Dimension according to the load capacity of livestock use.

•Define the boundaries of the system under study.

•Perform material balances and energy in the system under study. This includes the biodiesel process is to implement within farms. For make the balances of the production process of biodiesel were used process simulation programs.

•Equipment sizing in the biodiesel production process and analysis economic thereof

•Determine the mass balances on farms and the interaction energy between them (for example the amount of energy in transport material between farms)

Third Stage

After obtaining the material and energy balances, was held on biodiesel LCA, which were identified in the critical stages in the process for the environment and proposes different solutions, thus reaching an overall analysis of the farm system. The final result is a proposal that has the least environmental impact ensure the viability of the same.

Results and discussion

Defining stage

To determine the cultive to use, sought one with robust agroecological requirements (Figure 1) , the cultive was selected:

Jatrofa due to the following characteristics:

• Lifetime of up to 50 years
• It grows in poor, sandy soils
• Started production from year
• 38% fat and 18% protein (cattle feed)
• Production costs low
• Do not compete with food for not being edible (Human)
• 2 tons of oil (triglyceride) per hectare
• Plant fibrous

According to agroecological properties of jatropha cultivation, it was determined that an area consistent are the suburbs of Wadley and Vanegas located in the highlands of San Luis.

System study:

Thus, the system to be studied were two small farms with energy and material interactions between them, , the characteristics of each are reported in Table 1

Tabla 1. Dimensionamiento de granjas

Vanegas	**Wadley**
40 hectares of jatrofa cultivation	25 hectares of jatrofa cultivation.
7 cows and 20 goats	12 goats y 4 cows
50 total hectares	35 total hectaras

The number of cattle was determined according to the load capacity thereof, and consider 3.25 tonnes per hectares of cake as cattle feed:

Biodiesel Production System

The Batch designed for the production of biodiesel is shown in Figure 1, the goal is to process three tonnes of jatropha oil / week in a working day of 8 hours per day. Jatropha oil (stream 1) is introduced into the reactor (R-101) and feeds excess of 6:1 (mol / mol) in methanol (stream 2). It also adds 1% catalyst (NaOH). The reactor is of adiabatic type with a reaction time of 45 minutes. In the above conditions have a 98% conversion (w / w). Subsequently, the reaction mixture (stream 3) is subjected to a first evaporation step (EV-100). In this stage is obtained methanol in vapor state, which is recirculated to the process (stream 4), and a residual mixture (stream 6) which is sent to the settling system (DE-100). Here is performed the separation of biodiesel (99.99 mol%) and glycerine with impurities (stream 7). In the same operation H3PO4 is added, which reacts with the catalyst (NaOH) for the formation of neutralizing objective Na3PO4 with this compound. Proposes the use of Na3PO4, as fertilizer on farm system, since it contains no nitrogen main cause depletions of soils. Biodiesel decantation from the system (stream 10) has a temperature of 64 ° C, the stream is treated in a heat exchanger (IC-100) to reduce its temperature to 35 ° C, temperature suitable for sending to the storage area finished product. The glycerine with impurities is processed in an evaporation system (EV-101) which aims to remove residual water contained (stream 12), obtaining a 92% mol purity glycerin at a temperature of 102 ° C (current 8). The same stream is treated in a heat exchanger (IC-101) with the aim of reducing their temperature to 35 ° C, suitable temperature for storing the product.

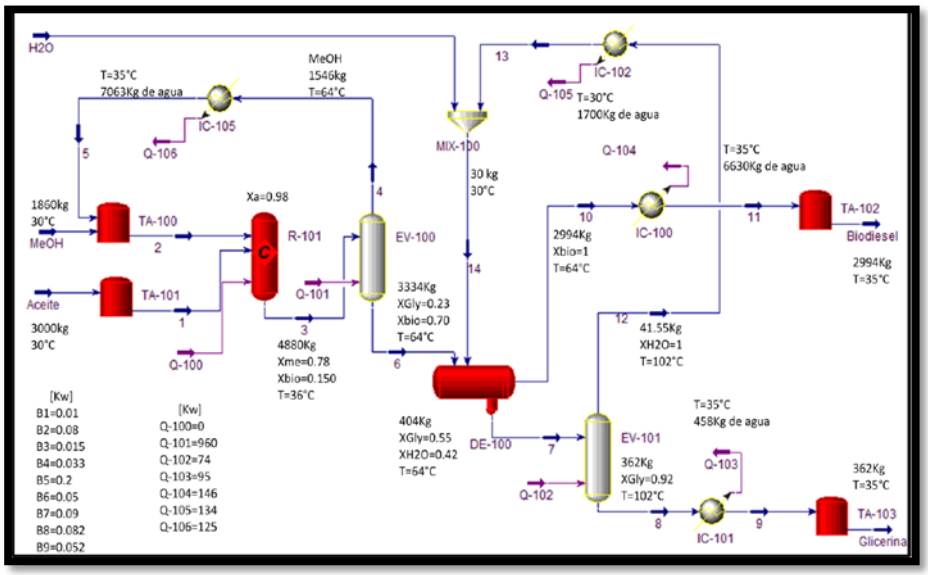

Figure 1. General diagram of biodiesel production process in the integrated farming system

To determine the total cost of the farm system was required sizing of equipment, energy costs of equipment, correlations [3] and the index update cost, performing the necessary calculations and gathering the above information was determined the cost of farm. Considering a total investment of 935 000 farm dollars, a price of $ 0.71 biodiesel / glycerin liter and 1.8 dollar / liter, the payback is estimated to be 5.3 years, without considering the above assets as byproducts farm (milk, meat, cheese, etc..).

Impact Analysis

In order to determine the viability of the system studied were compared, emissions of pollutants and greenhouse energy parameters, including: the production process of petrodiesel, the biodiesel production process from vegetable oils plant BIONOR and integrated farming system proposed in this paper.

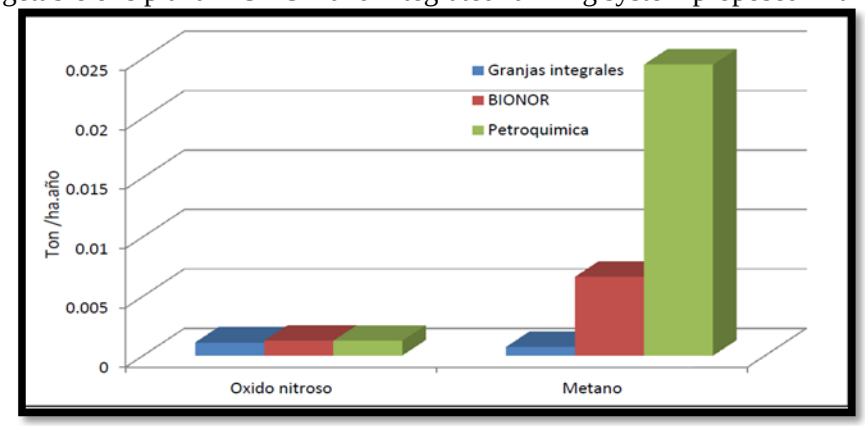

Figure 2. Emissions of methane and nitrous oxide for three different biodiesel production systems.

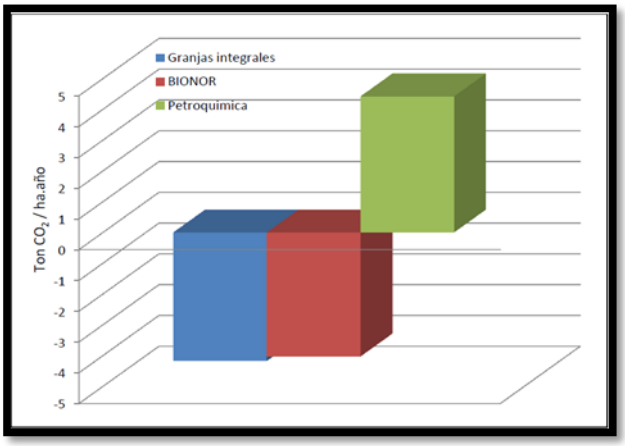

Figure 3. Chart emissions of greenhouse gases in the life cycle of the cases studied (Ton / ha.year).

In the case of integrated farming system were calculated greenhouse gas emissions originated in the production, distribution and use of the mixtures studied. The results are shown in Figure 3, these results are expressed in ton / ha.year. As can be seen in Figure 3 in terms of CO2, production and use of biodiesel prevents the emission of 4.4 tonnes of CO2 per hectare of jatropha planted a year, there is also the absorption capacity of 4.0 and 5.3 tonnes of CO2 per year per hectare of cultivation, the plant for farming and integral BIONOR respectively. In Figure 2 you can see the emissions of methane and nitrous oxide for each case study. In the case of nitrous oxide emission no significant difference in emissions, because these are caused mainly by burning fuel in the transportation and distribution of each system. In the case of methane emissions from the integrated farming system we can see 97% fewer emissions compared to diesel production process from oil, in the case of biodiesel production system made by BIONOR emissions savings amounted to 73%.

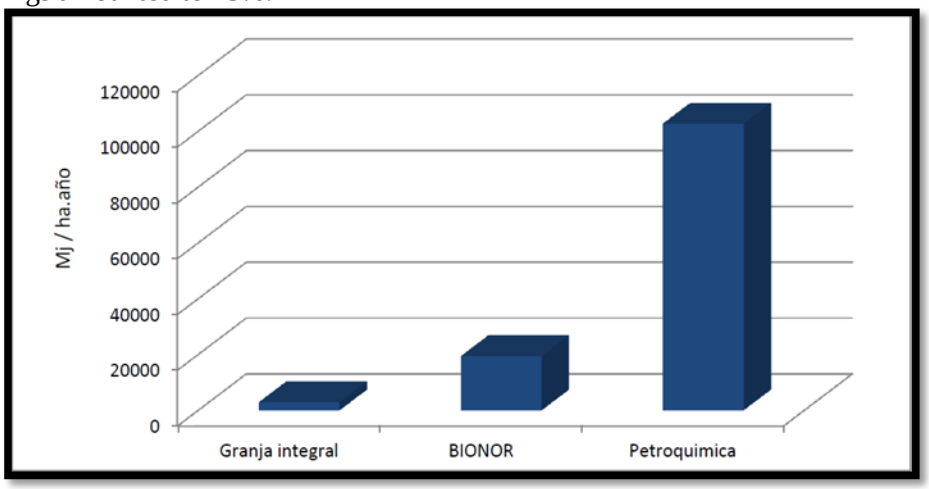

Figure 4. Energy consumption for production of diesel in three different systems. references

Figure 4 shows the energy consumption for each system. In the case of integrated farming system is observed energy savings of 97% compared with the production process from diesel oil, and a 81% savings in the case of biodiesel production system made by BIONOR. One can see that the items with significant energy inputs are the transport and distribution of raw materials and products, representing 18.7% of total energy. Meanwhile the maintenance of the farm, is the most important factor because it represents 75% of total energy required. The latter is essentially determined by the use and disposal of agricultural machinery as well as the necessary displacements thereof during the life of the system.

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Quality Characteristics Of Tortilla From Corn Flour Processed By Continuous Ohmic Heating

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Introduction

Mexico is the principal consumer of tortilla all over the world, it is estimated that 94 % of its population consumes it. Tortilla production is approximately of 12 million tons per year, representing an important percentage of commercialized food products in this country. Tortilla is also an important food in Central American countries.

Tortilla is defined as a flat disc of nixtamalized corn dough, 12 – 18 cm diameter and 1 -2 mm thick, which is cook over a hot iron (260-280 °C). The traditional technology to produce nixtamalized corn tortilla is very old; Aztecs used and transmit this process from generation to generation until these years. Originally, the nixtamalized corn grain was ground in a stone grinder and the diameter of tortillas was approximately 20 cm, which were cooked over hot earthenware. Tortilla was the Spanish name of "tlaxcalli", the Aztec name of this product with special characteristics that appeal to consumers, even now days. In the last decade has been done important effort to look for alternative processes to produce tortilla; the main aim is to reduce processing time and contaminant effluents, among others. An alternative technology studied by Gaytán-Martínez et al. (1) is the ohmic heating, also called resistive heating of electro heating^{RM}, which consists in the application of an alternating electric current to a material, in this case, a food to increase internally its temperature because of dissipation of the electric current. As warming is internal, is distributed homogenously in a short heating time; these are its advantages over the traditional process.

However, when a new technological process is used, it must be especially careful respect to the quality characteristics of the obtained end product demanded by consumers. The main objective of this work is evaluate the quality of tortillas obtained using the traditional nixtamalization process and the ohmic heating.

Materials y methods

Raw corn: White corn was bought in Querétaro, Mexico, with grains 8.64, 11.72 y 4.30 mm width, length and thick, 82 kg/hL hectoliter weight, and 23 flotation indexes. These characteristics qualify the grain as apt to produce tortilla (NMX-FF-034/1-SCFI-2002) and as hard grain (NMX-F-046-S-1980). Corn was processed using the traditional nixtamalization and the continuous ohmic heating system.

Tradiditional nixtamalization: Corn (1 kg) was added to 2 L of water with $Ca(OH)_2$ (1%), cooked to boiling temperature during 30 min, stepping in the same liquid during 19 h, dehydrated in a flash dryer (270 °C income and 40 °C exit temperature), ground in a Pulvex mill, mesh 0.5 and stored in plastic bags at 6$\pm$2 °C.

Continuous ohmic heating system: This equipment was patented by Morales-Sánchez et al. (2) and is divided in two zones: Transport and heating. It consists of a screw conveyor to transport corn flour, coupled to a rectangular ohmic heating cell 25, 5 and 2 cm length, width and height, respectively. At both sides of the cell are two stainless steel electrodes connected to AC voltage. The processed material exit occurs without reduction of area neither exit dye, which avoids the application of pressure over the material. The equipment has a temperature controller Watlow Mod. 981 with a K type temperature sensor, a variable transformer voltage AC at 60 Hz (Variac), a bench multimeter HP to measure AC voltage and current and a speed controller Baldor. The temperature controller allows or avoids the passage of AC current with the appropriate potency to reach the desired temperature (maximum potency is 1500 watts).
Corn grain was milled in a Pulvex mill with mesh 1.3 mm and mixed with 0.3 % of $Ca(OH)_2$ and the amount of water stipulated for each treatment according to the experimental design, and mixed homogenously. The mixture was fed to the ohmic heating equipment, transported to the cooking zone at 10 or 20 rpm and heated until 75 or 85 °C; once processed, it was dried in a Flash type dryer (CINVESTAV development) at an input temperature of 270±5 °C and 40 °C at the exit. Flour was stored in plastic bags at 4 °C.

Corn tortilla obtained by ohmic heating: Dough is prepared adding water to the flour until subjectively it reaches the desire texture (it does not crack and does not adhere to hands). Flat disks (diameter: 12.5 cm, thickness: 1.2 mm) were molded using a manual tortilla machine (Tortilladoras Gónzales, SA de CV. México) and cooked on a hot iron at 270 $\pm$ 10 °C during 17 s on one side, 50 s the opposite and 17 s on the initial side, which inflates (10 replicates for treatment).

Characterization of dough and tortilla: There were determined the chemical composition of corn flour (3, methods 32.1, 934.01, 942.05 and 920.39) dough yield (kg of dough/ kg of flour), hardness and adhesiveness (4) to dough, and tortilla yield (kg of tortilla/ kg of dough), weight loss and resistance force to tension and to cut using a Texture Analyzer TA-XT2 (5)
Experimental design and statistical analysis: It was used a surface response methodology with three independent variables (Table 1) were: feed moisture transport speed and material's exit temperature), second order and five central points. It was also performed a Dunnet's test to evaluate differences between OHFs and TNF. Data were analyzed using a Minitab 14 program.

Table 1. Factors and levels of ohmic heating to produce nixtamalized corn flour.

Variable	Nivel	
	Mínimo	**Máximo**
Feed moisture (%)	50	60
Temperature (°C)	75	85
Transport speed (rpm)	10	20

Results y discusion

Physicochemical and textural properties of dough
The quality characteristics of dough are shown in Table 2. There was not significant difference ($p \leq 0.05$) between ohmic heated flours related to dough moisture and dough yield, as well as between them and traditional nixtamalized flour (Dunnet's test); these characteristics accord with data reported by Figueroa *et al*. (6) stated that they are appropriated to model tortilla and confer desirable texture properties to them. Adhesiveness and hardness were higher ($p \leq 0.05$) in dough elaborated with flours processed by ohmic heating at 85 °C and were different from traditional method (Dunnet's test), while those processed at a lower temperature did not show a different adhesiveness. Adhesiveness and hardness are important quality characteristics related with functional properties of dough; when dough shows a high adhesiveness, it is sticky and it is difficult to model tortilla (7); when dough shows a high hardness, it is related with a low

moisture and low adhesive dough. However, dough from corn flour processed by ohmic heating, in spite of its high adhesiveness and hardness (in some of them), showed high quality textural properties, as can be observed in Table 3 (tensile and cutting force). This behavior may be related to the high water absorption and retention of corn flour processed by ohmic heating.

Table 2. Physicochemical characteristics of dough.

Treatment	Masa Yield*	Moisture (%)	Adhesiveness (g-F)	Hardness (g-F)
46.6[1]/80[2]/15[3]	2.03±0.00	53.43±0.03	22.03±0.83	158.87±15.71
50/75/10	2.05±0.00	52.84±1.12	23.05±2.64	179.70±3.56
50/75/20	2.07±0.00	54.60±0.05	21.83±6.33	207.97±9.97
50/85/10	2.10±0.00	53.66±0.55	25.08±7.04	245.92±2.64
50/85/20	2.07±0.00	53.52±0.07	21.43±4.41	269.98±7.39
55/71.5/15	2.02±0.00	54.25±0.14	25.85±2.90	175.80±8.44
55/80/6.6	2.07±0.00	53.63±0.10	16.33±1.54	225.07±3.50
55/80/15	2.06±0.00	54.62±0.39	17.06±1.63	178.65±11.51
55/80/23	2.07±0.00	54.48±0.34	28.42±2.07	197.62±2.39
55/88/15	2.11±0.00	56.77±0.24	22.07±4.46	154.51±5.56
60/75/10	2.03±0.00	53.97±0.10	20.12±0.51	162.73±8.03
60/75/20	2.00±0.00	54.02±0.52	22.98±0.26	158.82±0.55
60/85/10	2.05±0.00	53.00±0.21	23.28±0.91	208.97±14.88
60/85/29	2.07±0.00	54.08±0.39	30.79±1.24	235.75±16.99
63.5/80/15	2.07±0.00	55.14±0.12	20.75±5.26	178.83±12.57
Tradicional Process	2.04±0.00	53.37±0.19	21.70±2.92	175.94±0.37

[1]Feed Moisture; [2]Temperature; [3]Transport speed *masa kg/flour kg.

Physicochemical, textural and rheological characteristics of tortilla

In Table 3 can be observed that tortilla yielding from ohmic heating flour is slightly higher than that of the traditional nixtamalization. Tortilla yielding is related to the capacity of absorption of water of flour, as well as the period of time that starch is in contact with water; it is also related with the quantity of water evaporated during cooking of tortilla (weight loss); in spite of the long time that corn grain remains in contact with water when the traditional nixtamalization method is used and the short time required to process corn by ohmic heating, the absorption and retention of water is higher when the ohmic process is used. This behavior may be attributed to the heating process as well as the use of the whole grain, which retains the hydrocolloids present in its pericarp, conferring a higher capacity of interaction and retention of water to the flour.

Tensile and cutting force are the way to evaluate the textural quality of tortilla (Table 3), tensile force is related to the resistance force of tortilla when is tearing by hand and cutting force to the resistance force of tortilla to be compressed by molars (8). Dunnet's test did not show significant differences (($p \leq 0.05$) between the ohmic heated flours and the traditional one.

Conclusion

The continuous ohmic heating method proved to be an alternative technology to obtain nixtamalized corn flour, dough and tortilla with similar characteristics to those obtained by the traditional method, so it may be considered as a new industrial technology for this process, which has the advantage of its short processing time, the lack of contaminant alkaline effluents and its efficiency in the energy consumption.

Tabla 3. Physicochemical, textural and rheological characteristics of tortilla.

Treatment	Weight loss (%)	Yield tortilla	Moisture (%)	Tensile strength (g-F)	Cutting force (g-F)
46.6[1]/80[2]/15[3]	15.51±0.98	1.72±0.02	45.09±0.02	86.94±16.46	715.67±12.04
50/75/10	16.29±1.03	1.71±0.02	43.96±0.02	238.53±2.14	954.59±4.69
50/75/20	18.20±1.74	1.70±0.04	44.42±0.03	156.94±2.77	1058.60±68.33
50/85/10	14.87±1.09	1.79±0.02	45.03±0.20	137.58±18.46	724.60±13.08
50/85/20	15.30±1.06	1.75±0.02	44.13±0.18	221.64±49.94	789.70±8.59
55/71.5/15	17.27±1.22	1.67±0.02	43.94±0.09	96.47±17.69	745.47±14.60
55/80/6.6	15.57±0.41	1.74±0.01	44.12±0.10	188.59±7.85	1156.58±71.98
55/80/15	17.02±1.25	1.71±0.03	44.54±1.14	164.31±44.22	865.23±68.12
55/80/23	17.13±0.91	1.72±0.02	44.97±0.29	112.71±13.80	588.04±73.72
55/88/15	17.70±1.26	1.73±0.03	47.20±0.28	142.41±6.78	636.74±29.34
60/75/10	18.50±1.23	1.66±0.03	43.41±0.06	76.59±27.84	835.45±78.92
60/75/20	15.62±0.48	1.69±0.01	44.53±0.16	151.22±13.70	788.27±12.29
60/85/10	18.99±1.64	1.66±0.03	40.92±0.24	168.92±40.19	1066.70±11.25
60/85/29	15.80±0.33	1.75±0.01	45.78±0.10	179.65±22.03	699.78±56.61
63.5/80/15	16.00±0.79	1.74±0.02	46.61±0.06	139.73±19.16	621.07±25.43
Tradicional Process	16.79±1.47	1.69±0.03	42.96±0.05	160.55±16.42	1260.36±22.38

[1]Feed Moistures; [2]Temperature; [3]Transport speed.

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Multiple-pass Ultrahigh Pressure Homogenization of Milk For Unripened Cheese Manufacture

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Abstract

Panela is a non-ripened, rennet-coagulated, high-moisture cheese widely consumed in Mexico. Although traditionally elaborated with raw milk, concerns on microbial safety have prompted the use of pasteurized milk as a substitute. However, changes in cutting time, curd firmness and pH at draining are expected and consequently physicochemical, functional and sensory characteristics may be compromised. Ultra high-pressure homogenization (UHPH) is a novel processing technology with potential applications in cheesemaking and multiple-pass UHPH (MPUHPH) treatments have been proven to enhance microbial and enzyme inactivation, protein denaturation, and fat globule size reduction in milk. Our group has previously reported pasteurization-like MPUHPH conditions to secure microbial safety of whole milk. The aim of this work was evaluating the suitability of these MPUHPH milk treatments to produce *Panela* cheeses with desirable physicochemical attributes after pressing and throughout refrigerated storage. Milk (3% fat) was MPUHPH-processed at 20°C at 250 MPa and 210 MPa (4 and 5 passes respectively) in a Stansted homogenizer model nG7400:350, according to a complete randomized design with two replicates. One control cheese made of pasteurized milk (PM) (72°C, 15 s) was prepared on each cheesemaking day and results were analyzed by ANOVA (α=0.05). *Panela* cheeses were prepared following a standard procedure, vacuum-packaged and stored at 4°C for 21 days. Cheese composition and yield and whey composition were assessed at day 1 while texture profile, pH, color, and SEM microstructure were determined before and after 7, 14 and 21 days of refrigerated storage. Cheeses made with milk UHPH-processed at 250 MPa/4 passes exhibited compositional and microstructural characteristics, pH at day 1 and pH evolution similar to those of PM cheeses. Non-significant differences in color between control and experimental cheeses were observed at all sampling times, while texture parameters (firmness and cohesiveness) decreased more sharply than for PM cheeses during storage. Protein recovery was more efficient in MPUHPH cheeses but syneresis and fat losses in whey were greater. MPUHPH might represent a suitable alternative for producing unripened cheese.

Introduction

Despite its well-established reliability from the food safety standpoint, pasteurization causes chemical changes in milk components, which may be associated with undesirable sensory, functional and nutritive modifications. This in turn may affect the manufacture of dairy products like cheese. In consequence, gentler processing alternatives that may deliver safe milk without such detrimental changes have been explored. Among non-thermal technologies, ultra high pressure homogenization (UHPH) has been reported as an effective approach to preserve liquid food products (1). UHPH is based on the same principles ruling conventional homogenization, but a ceramic valve with a modified mechanical design allows the equipment to reach an operating pressure up to 400 MPa. Pressure, inlet temperature and

number of passes have been identified as the most relevant processing parameters (2). UHPH might reduce both microbial and enzymatic activity of fluid foods such as milk, soy milk, beer, and fruit juices through shear stress, high speed collisions, impingement and cavitation mechanisms. Multiple-pass UHPH (MPUHPH) treatments at lower pressures are considered to have cumulative effects on microbiological milk properties, comparable to those of single-pass UHPH treatments at higher pressures. Considering this, appropriate combinations of pressure and number of passes could be selected according to desirable functional and sensory milk characteristics or milk-end use.

UHPH has been shown to affect casein micelles, whey proteins and fat globules in milk, especially at pressures higher than 250 MPa. These UHPH-induced changes in milk components might be of interest to cheese manufacturers, since protein denaturation and milk fat globule size reduction could increase cheese yield due to a higher water holding capacity while positively improving quality features such as texture and color. Both physicochemical and technological features of acid coagulated (3) and rennet (4) fresh cheeses produced with UHPH-treated whole bovine milk have been recently explored. While yield, texture and moisture and protein contents increased in acid-coagulated fresh cheeses made with milk subjected to a 300-MPa, single-pass UHPH process, cheese-making properties during rennet cheese manufacturing were altered due to poorly fused curd particles which resulted in products with textural defects. MPUHPH treatments at pressures lower than 250 MPa (232 MPa/ four passes; 202 MPa/ five passes) capable of reducing both spoilage and pathogenic counts in an equal or greater amount than 5.0 $\log_{10}$.were recently defined (5). Thus, the objective of this work was evaluating the suitability of these MPUHPH milk treatments to produce *Panela* cheeses with desirable physicochemical attributes after pressing and throughout refrigerated storage.

Methods and materials

Raw whole milk was obtained from a local producer and kept under refrigeration until used; milk was separated (40°C), standardized to 3% fat using a lab-scale milk separator and an ultrasonic milk analyzer (Lactoscan LA; Zagora, Bulgaria), thermized at 20°C and UHPH-processed in a 2-stage high pressure homogeniser (nG7400:350 Hydrive model, Stansted Fluid Power Ltd., Essex, UK). This equipment uses two intensifiers driven by a hydraulic pump and a high-pressure ceramics valve capable of reaching an operating pressure up to 300MPa. A second stage homogenization is applied at ten percent the pressure of the first stage using a conventional pneumatic valve. In order to prevent temperature build-up, both valves were cooled by circulating cool water (4ºC) through an external jacket built around the pipeline after the high pressure valve and a tubular-type heat exchanger located after the second valve. Inlet milk temperature was re-equilibrated at inlet temperature (±1ºC) at each reinsertion, if necessary. The equipment was cleaned after each cycle successively using a phosphate-based detergent, a peracetic acid-based sanitizer and then rinsing it with water thoroughly. Milk was MPUHPH-processed at 250 MPa and 210 MPa (4 and 5 passes respectively) according to a complete randomized design with two replicates. *Panela* cheese was made in duplicate from MPUHPH-processed milk following a standard cheese making procedure. Milk temperature in the vat was adjusted to 32°C; 0.02% calcium chloride and microbial rennet (CHR. Hansen, Mexico, DF, Mexico) were used. One control cheese made of high-temperature, short time (72°C, 15 s) pasteurized milk (PM) was prepared on each cheese making day. All cheeses were vacuum-packaged and stored at 4°C for 21 days. Cheese composition and yield and whey composition were assessed at day 1 while texture profile analysis (hardness, cohesiveness, springiness determined in a TAXTPlus texturometer), pH, color (L,a,b parameters determined in a Hunterlab Colorflex system), and SEM microstructure were determined before and after 7, 14 and 21 days of refrigerated storage. Syneresis was measured as milliliters of whey expelled out of cheese matrix after 21 days. Results were analyzed by ANOVA and means were compared using Tukey's test (α=0.05).

Results and discussion

Cheese yield and composition data are shown in Table 1. Yield of experimental cheeses was significantly higher than that of PM cheeses but no significant differences (α =0.05) were observed in moisture and ashes contents of cheeses made with PM and 250 MPa- MPUHPH milks, while 210 MPa - MPUHPH cheeses exhibited lower percentages. Meanwhile, protein content was significantly higher for experimental cheeses and pH of cheeses made with 250 MPa- MPUHPH did not differ from those of PM cheeses up to 14 days of storage time and changed the least during refrigerated storage (Table 2). Higher protein content in cheeses made with high pressure-treated milk has been associated with denatured whey protein inclusion into the casein matrix (6). However, cheese structure did not allow an appropriate water retention as products with higher initial moisture content also presented the higher syneresis values.

Table 1 Cheese composition, yield and syneresis of MPUHPH and PM cheeses

Cheese	Moisture (%)	Ashes (%)	Protein (%)	Yield (%)	Syneresis at 21 d (ml)
250 MPa, 4 passes	55.29±0.66^{a}	3.42±0.12^{a}	19.37±0.21^{a}	11.77±0.55^{a}	101.37±5.58^{a}
210 MPa, 5 passes	50.56±0.43^{b}	2.71±0.08^{b}	18.96±0.07^{a}	11.20±1.39^{ab}	70.80±3.22^{b}
HTST	53.38±2.41^{ab}	3.34±0.40^{ab}	18.51±0.23^{b}	9.46±0.49^{b}	71.34±2.66^{b}

Different lower letters within columns mean significant difference (α =0.05)

Table 2 pH evolution of MPUHPH and PM cheeses through refrigerated storage

	Storage time (d)			
	1	7	14	21
210 MPa, 5 passes	6.48± 0.05^{aA}	6.52±0.07^{a}	6.18±0.09^{bA}	6.05±0.22^{bA}
250 MPa, 4 passes	6.67± 0.04^{aB}	6.56±0.10^{ab}	6.65±0.06^{aB}	6.40±0.07^{bB}
HTST	6.72± 0.14^{aB}	6.66±0.10^{a}	6.49±0.25^{aAB}	6.17±0.10^{bA}

Different lower letters between columns mean significant difference (α =0.05)
Different capital letters within columns mean significant difference (α =0.05)

Results on texture and color analyses of MPUHPH and PM cheeses are respectively shown in Tables 3 and 4. At day 1, cheeses elaborated with 250 MPa MPUHPH milk were harder, more cohesive and less elastic than all other products indicating a denser structure, which correlates well with their higher protein content and SEM micrographs in Figure 1. Hardness and springiness significantly changed after 21 days of refrigerated storage; firmer PM cheeses were observed, probably caused by syneresis. On the other hand, hardness of experimental cheeses decreased, which might be related to higher proteolytic activity by microbial enzymes released after UHPH-induced cellular lysis. Cohesiveness of experimental cheeses was significantly lower than that of PM cheeses; this crumblier texture could be an indication of a curd not correctly fused during the cheese making process, as described in previous reports (4) and might contribute to fat losses in whey (Table 5). Meanwhile, no color differences were observed between experimental and control cheeses; although luminosity tended to decrease and both *a* and *b* parameters generally increased during storage, these modifications were not significant, excepting for the 210 MPa MPUHPH cheeses and could not be physically observed. MPUHPH treatments did not seem to affect color of *Panela* cheeses when compared to PM cheeses.

Table 3 Texture parameters evolution of MPUHPH and PM cheeses throughout refrigerated storage

	Hardness (kg) at day 1	Hardness (kg) at day 21	Springiness at day 1	Springiness at day 21	Cohesiveness at day 1	Cohesiveness at day 21
210 MPa, 5 passes	816.87± 49.09 ^{aA}	581.98± 199.01 ^{bA}	0.24±0.01 ^{aA}	0.30±0.01 ^{b}	0.83±0.07	0.74±0.03^{A}
250 MPa, 4 passes	1007.15± 84.08 ^{aB}	845.82± 26.31 ^{bB}	0.19±0.01^{aB}	0.17±0.02^{a}	0.87±0.06	0.72±0.06^{A}
HTST	830.33± 131.28 ^{aAB}	1254.32± 129.59 ^{bC}	0.46±0.04 ^{aC}	0.27±0.02 ^{b}	0.85±0.01	0.88±0.02^{B}

Different lower letters between columns mean significant difference (α =0.05)
Different capital letters within columns mean significant difference (α =0.05)

Table 4 *L*, *a*, *b* evolution of MPUHPH and PM cheeses throughout refrigerated storage

	L at day 1	L at day 21	*a* at day 1	*a* at day 21	*b* at day 1	*b* at day 21
210 MPa, 5 passes	89.20±1.24 ^{a}	84.55±1.16 ^{b}	1.38±0.22^{a}	1.93±0.61^{b}	15.93±1.45	15.76±0.79
250 MPa, 4 passes	86.37±1.55	84.98±1.26	1.79±0.22	2.27±0.53	15.76±1.10	15.87±0.87
HTST	87.77±1.06	85.03±0.97	2.03±0.39	2.53±0.52	16.00± 0.94	17.49±1.21

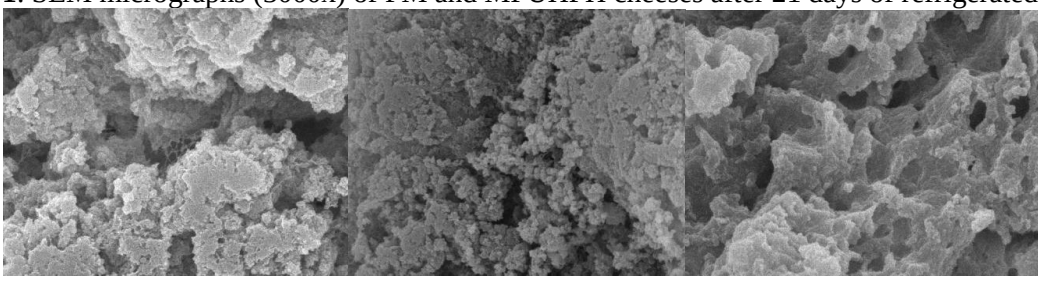
Different lower letters between columns mean significant difference (α =0.05)

Table 5 Whey composition of MPUHPH and PM cheeses

Cheese	Protein (%)	Fat (%)	Ashes (%)	Lactose (%)
250 MPa, 4 passes	0.80±0.04^{a}	1.16±0.11^{a}	0.60±0.05	3.92±0.04 ^{a}
210 MPa, 5 passes	0.85±0.03^{a}	1.23±0.10^{a}	0.67±0.02	4.11±0.07^{b}
HTST	1.12±0.12^{b}	0.06±0.09^{b}	0.69±0.07	3.97±0.11 ^{ab}

Different lower letters within columns mean significant difference (α =0.05)

Figure 1. SEM micrographs (3000x) of PM and MPUHPH cheeses after 21 days of refrigerated storage



210 MPa, 5 passes **250 MPa, 4 passes** **PM**

Even though yield increased of *Panela* cheese produced by MPUHPH of cheesemilk is promising, process need further refinement since fat loss in whey is considerable and both technological and physicochemical parameters of unripened cheeses might show a greater improvement when UHPH-assisted pasteurization is applied (3)

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Effect Of *Pimenta dioica* L. Merril Essential Oil On Physical, Mechanical And Water Vapor Permeability of *Aloe vera*- Gelatin- Glycerol Films

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Over the years, there has been increasing interest in natural polymer based films. In the food industry, natural polymers can be used as protective coatings extending the shelf life of foods. The functional properties of edible films depend on film composition. The ability of hydrophobic substances to retard moisture transfer depends on the final distribution in the hydrophilic matrix. The objective of this study was to evaluate the effect of different concentration of *Pimenta dioica* essential oil (EO) on the physical, mechanical and water vapor permeability of films based on *Aloe vera* - gelatin – glycerol – Tween 80 (1: 0.02: 0.004: 0.001) containing EO at 4 levels (0, 0.05, 0.1 and 0.15 EO w/v). Results indicated that WVP of *Aloe vera: gelatin* films showed similar values than control and there were not influenced of the amount of EO (1.32 E-06 to 1.48 E-06 gm/m^{2}sPa). The incorporation of *Aloe vera* in the composite film gave an opaque appearance, which can be attributed to the content of anthraquinones, which are difficult to be completely eliminated besides that is reactive to air and light. EO addition into the films makes them less flexible and showed higher tensile strength (0.061 to 0.095 KPa) with increasing amount of essential oil. These results are explained by the changes induced by plasticizers and interaction of constituents on the matrix.
Keywords: *Pimenta dioica* oil, edible films, *Aloe vera*, mechanical properties.

Introduction.

Recently, food biotechnology has focused on the study and development of packaging technologies for the preservation of food. Edible films are biodegradable materials used for this purpose. They could be carriers of antimicrobial substances such as essential oils (EO) of plant. Plant extracts are important sources of terpenes and phenolic compound[1]. Eugenol is the major component of *Pimenta dioica*[2] essential oil and it has an antioxidant and antimicrobial effect against *Listeria monocytogenes*[1]. Thus, the objective of this study was investigate the effect of the incorporation of four different concentrations of *Pimenta dioica* L. Merril essential oil (0, 0.05, 0.1 and 0.15% w/v) on the physical, mechanical and water vapor permeability properties of a material composite based on *Aloe vera* gel, gelatin and glycerol.

Materials and methods

Materials. *Aloe vera* gel obtained by the parenquima from fresh *Aloe vera* leaves, glycerol ACS (Productos químicos de Monterrey, S.A de C.V. Fermont Lot: 818333, Monterrey México.), polyoxyethylene sorbitan monooleate (Tween 80) (Hycel de México, S.A de C.V. Lot: 263534, D.F. México), commercial gelatin from cattle skins and *Pimenta dioica* L. Merril, cultured in the Totonac community of Tacuapan located at north of Puebla.

Film production. Films were prepared by grounding *Aloe vera* and centrifuged the gel obtained at 1200 rpm, 0.1% w/v Tween 80 was mixed and centrifuged at 4000 rpm for 40 min. Supernatant was filtered and gelatin (2% w/v) and glycerol (0.4% w/v) were added. The mixture was held at 35 °C during 10 min, and finally the *Pimenta dioica* L. Merril EO was added. EO was extracted by hydro distillation using a water

vapor pressure at 94 °C. 10 mL of suspension were poured into petri dish and allowed to dry for 24 h at 25 ° C.

Characterization of Films.

Thickness. Film thickness was measured with a micrometer (Kafer model F1000/30, Germany), at five random positions around the film. The mean value was used to calculate the water vapor permeability and mechanical properties.

Color properties. Color values of the films were measured with Chroma Meter CR-400 (KONICA MINOLTA). The Chroma meter color scale was used for measuring color values: L*, the lightness variable; a*, from green to blue, b*, from yellow to red, C*, chroma and °h, hue degrees.

Water vapor permeability (WVP). The gravimetric modified cup method based on standard method E96-80 (ASTM, 1989)[3] was used to determine WVP. Test cups holding films were then inserted into the pre-equilibrated 0% RH desiccator. Steady state of water vapor transmission rate was achieved within 5 h. Each cup was weighed 8 times at 0.5 h intervals. Thee replicates of each film were tested. The WVP of the films was calculated by multiplying the steady state water vapor transmission rate by the average film thickness and dividing by the water vapor partial pressure difference across the films.

Mechanical properties. Mechanical properties were determined by a texture analyzer (model TA-XT.plus Stable Micro Systems Ltd., United Kingdom) coupled to a Texture Expert software (Version 2004).
Tensile strength. Standard method D882-97 (ASTM, 1997)[4] was used to measure tensile properties of films. Films were cut into strips with a test dimension of 70 mm by 10 mm and fitted to the tensile grips. The initial distance between the grips was 50 mm, and the crosshead speed of 0.5 mm/s. Tensile properties as tensile strength (KPa), elongation at break (%), and Young´s modulus (KPa) were calculated from the plot of stress (tensile force/initial cross-sectional area) vs. strain (extension as a fraction of original length). Five replicates of each film were tested.
Puncture strength. Is used to determine the puncture or rupture characteristics of a material. Samples were cut in circles of 0.04 m diameter and were placed in cells. In this compressive test, generally the film is compressed by a probe P/2 until the material ruptures or until an elongation limit is achieved [5]. The puncture strength was performed according to Gontard *et al.* (1991)[6]using crosshead speed of 0.5 mm/s.

Statistical analysis. Data were analyzed by one-way analysis of variance (ANOVA) using Statgraphics® computer program (Statgraphics Software, Inc., Centurion XV.I, USA). The comparisons between groups were performed. Tukey test was used to determine the difference at $p<0.05$ significance level.

Results and discussion

Table 1 shows the physical characterization of the material, the film thickness decreases with increasing the concentration of *Pimenta dioica* EO. Furthermore, the weight increases directly in proportion to the concentration of EO. Film containing 0.15% *Pimenta dioica* EO showed the highest density, probably due to intermolecular rearrangement of the compounds of the matrix incorporating the essential oil, as mentioned Rakshit *et al.* 2005[7] and Grossmann *et al.* 2009[8].

Table 1. Physical characterization of *Aloe vera* – gelatin based films incorporated with *Pimenta dioica* L. Merril essential oil (EO)

EO % w/v	Thickness (µm)	Weight (g)	Density (kg/m^3)
0	36 ±1.05	0.0490 ±0.002	1.0673 ±0.001
0.05	37 ±1.4	0.0525 ±0.007	1.1198 ±0.001
0.1	34 ±1.25	0.0570 ±0.008	1.3323 ±0.001
0.15	33 ±1.23	0.0599 ±0.001	1.4141 ±0.001

Color properties of packaging are a matter of prime importance with regard to general appearance and consumer acceptance. Changes of rectangular coordinates (L*, a* and b*) are given in Table 2, where can be seen that these color properties all have the same tendency and increasing the level of *Aloe vera* results in significant increase in L*, a*, and b* of films ($p < 0.05$). There is a color difference between them with increasing concentration *Pimenta dioica* oil used. The brightness is similar for each formula, is within the range of 29.5 - 31.4 and the chromaticity decreasing behaved, the values a* and b* are in the chromatic diagram corresponding to blue-green tones. In general, addittion of *Aloe vera* at higher levels yielded blend films with darker appearance (brown), which can be attributed to the fact that the extracted *Aloe vera*, applied in this study, could contain anthranquinones from the husk, since they are difficult to be completely eliminated and, are air and light sensitive, hence the oxidation of phenol occurred. These observations are consistent with the
study reported by Hossein et al., (2012)[9].

Table 2. Effect of the *Pimenta dioica* L. Merril EO on color evaluation of *Aloe vera*-gelatin based films

% w/v EO	L*	a*	b*	C*	°h
0	31.34 ±0.06	0.82 ±0.001	2.06 ±0.008	2.23	66.58
0.05	30.51 ±0.02	0.10 ±0.004	1.91 ±0.004	1.91	93.03
0.1	29.43 ±0.01	0.18 ±0.007	1.88 ±0.005	1.89	95.48
0.15	29.73 ±0.02	0.57 ±0.003	1.70 ±0.004	1.80	72.92

One of the main functions of packaging is to protect foods by avoiding or reducing the transfer of moisture, forming a barrier to prevent or reduce the contact of foods with the external environment. Table 3 shows the WPV of the films in the incorporation of the essential oil at four different concentrations, 0, 0.05, 0.1 and 0.15% w/v. The WVP increases as the concentration of essential oil is greater, because the intermolecular structures are open and exposes more OH groups, with the hydrophilic material causing the barrier properties to water vapor are weaker. Rakshit *et al*., (2005)[7] show WVP increased with increasing concentration of garlic oil from 0.1% to 0.4% alginate-based edible films. In the same year, Rakshit *et al*., (2005)[10] reported the WVP in chitosan-based edible films and the effect it has to incorporate three different antimicrobial agents (garlic oil, potassium sorbate and nisin) in four different concentrations, and found that as the concentration of agent increases the WVP, same behavior presented in this research.

An important property of the materials refers to the ability to retard moisture loss from the food, which directly affects the preservation of food. Water vapor transmission generally occurs through the hydrophilic portion of the film and depends on the hydrophilic– hydrophobic ratio of the material. WVP of the films containing *Pimenta dioica* EO, increased slightly from 1.27×10^{-6} to 1.48×10^{-6} g m/m^2 s Pa,

possibly due to EO addition and could contribute to enlarge the intermolecular interactions in the matrix structure.

Table 3. Effect of the *Pimenta dioica* L. Merril EO on the water vapor permeability of *Aloe vera* – gelatin based films.

Film (EO % w/v)	Water Vapor permeability (WVP) (g m/m^2 Pa s) (E-06)
0	1.32 ±0.098
0.05	1.27 ±0.098
0.10	1.29 ±0.106
0.15	1.48 ±0.165

Also is attributed mainly to the presence of terpene-like compounds, not lipids. So, the barrier and mechanical behavior of the material depends on the nature of their structure, composition, method of transformation between others[11].

Table 4. Effect of the concentration of *Pimenta dioica* L. Merril EO on the mechanical properties of *Aloe vera* – gelatin based films.

EO % w/v	Puncture strenght (KPa)	Tensile strength (KPa)	% Elongation at break	Young´s modulus (KPa)
0	6.10 ±0.001	0.061 ±0.001	56.6 ±0.003	0.10 ±0.009
0.05	5.30 ±0.008	0.070 ±0.001	45.1 ±0.001	0.75 ±0.006
0.1	4.44 ±0.002	0.080 ±0.003	45.5 ±0.001	0.55 ±0.002
0.15	3.80 ±0.007	0.095 ±0.003	56.0 ±0.001	0.40 ±0.002

The concentration of essential oil of *Pimenta dioica* L. Merril has an effect on the film characteristics. The mechanical test results are shown in Table 4. Tensile strength, elongation, and elastic modulus are parameters that relate mechanical properties of films to their chemical structures. Incorporation of EOs caused a significant reduction ($p < 0.05$) in puncture strength of the films. Tensile strength increased in all films containing EO. Significant differences were observed in the elastic modulus between films with and without EO. On the contrary Grossmann *et al*., (2009)[8] observed an increase of Young's modulus with increasing concentration of the essential oil of oregano in film based on cassava starch and chitosan. The percent elongation of control was 56.6% and increased in all films containing EO, reaching a maximum value of 56%, same behavior reported Zivanovic *et al*., 2005[12] with edible films based on chitosan and incorporating essential oils. (Table 4). The formulation with *Aloe vera* without oil reporting more rigid. In general, addition of the essential oil of *Pimenta dioica* L. Merril a composite material based on *Aloe vera* makes the material less dense, facilitating movement inside the polymer matrix and improves flexibility of the film.

Conclusions

The results suggested that the incorporation of *Pimenta dioica* L. Merril into film-forming *Aloe vera*-gelatin solution can have a considerable influence on the properties of the obtained *Aloe vera* gelatin composite films.

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Evaluation Of Shelf Life Quality Attributes Of Apple Juice Treated By High Pressure Technology

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Abstract

The shelf life of Golden delicious apple juice was evaluated in terms of color, total polyphenol content, antioxidant capacity and ascorbic acid content. In addition, pH, titratable acidity, viscosity, polyphenol oxidase (PPO) activity and pectin methylesterase (PME) activity were also measured to observe the juice changes for six weeks. Fresh Golden delicious apple juice was added with 325 ppm and 800 ppm of citric acid and ascorbic acid, respectively, to prevent the oxidation reactions. The juice was treated in an ultrahigh hydrostatic pressure (HPP) unit at 430 MPa for seven minutes, and stored at room temperature (20°C) and in refrigeration (4°C) for the above mentioned period. No significant changes ($p<0.05$) in physicochemical properties or sensory attributes were detected in the treated apple juice, but the PPO started recovering its activity during the storage, without causing significant difference ($p<0.05$) in the color of the stored juice. These processing conditions seemed to preserve the physicochemical and sensory quality of apple juice, while keeping polyphenols content and antioxidant capacity. Indigenous micro biota of the raw juice was also eliminated by the HPP treatment, confirming the feasibility of such technology for real industrial applications.

Introduction

The consumers are demanding natural food products, or minimal processed, with high nutritional value, prolonged shelf life and excellent taste and flavor. A technology that may be used by food processors to face these challenges is HPP. One of the most resistant enzymes to HPP processing is PPO that causes browning in fruits and vegetables. The principal color change is due to polymerization of ortho-quinones (enzymatic browning), produced during catechol enzymatic oxidation. Some antioxidants such as ascorbic acid and polyphenols can prevent and reverse this oxidation in the initial stages. Contradictories results have been founded on the literature, were some researches have found an increment of the activity after treatments at pressures from 450 to 700 MPa (1, 2), while others (3, 4) observed inactivation at about the same conditions. The aim of the present study was to minimize the pressure used to inactivate PPO and evaluate the effect of storage temperature in nutritional and quality changes after treatment for 34 days.

Materials and methods

Golden delicious apples were obtained from the local supermarket, and processed in a domestic juice extractor. Ascorbic and citric acid (800 mg/L and 325 mg/L respectively) were added to the juice to prevent oxidation and color change during enzyme clarification: Zymapect and Glucozyme (ENMEX S.A. de C.V., Mexico) for 12 hours at 4°C. The juice was filtered with cheesecloth before HPP treatment.
Samples of clarified juice (90 mL) were packed in low-density polyethylene bags (Whril-Pak, USA) minimizing as possible the air into the bags before sealing. Samples were transferred to the 5 L pressure treatment chamber (ACIP 6500/5/12VB; ACB pressure systems, Nantes, France) equipped with temperature and pressure regulators. Water was used as the pressure transmitting medium.

The DPPH (1,1-diphenyl-2-picryl-hydrazyl; Sigma Aldrich) assay was done according to the method of Brand-Williams et al. (5) with some modifications. The stock solution was prepared at 0.1 mM with methanol and stored at -20°C until needed. 100 µL of filtered juice in Whatman #1 paper was mixed with 2.9 mL. After 1 hour in the dark, the absorbance was taken at 515 nm (Visible spectrophotometer, Novaspec II, Biochrom Ltd., Cambridge, England). The standard curve was linear between 0.08 and 1.25 mM trolox (Acros organics) solution. Results are expressed in trolox equivalent antioxidant capacity (TEAC) in mM. Additional dilutions were needed if the DPPH value measured was over the linear range of the standard curve.

PPO activity was assayed using aliquots of 0.1 mL of the clarified juice and 2.9 mL of a solution of 0.15 M cathecol (Sigma) in 0.05 M sodium phosphate buffer (pH 6.5). The reaction was measured at 420 nm and 25°C with the spectrophotometer (6). PME activity was assayed using 0.2 mL of clarified juice adjusted to pH 7.5, and mixed with 9 mL of 0.5% (w/v) citrus pectin (Sigma) and 0.8 mL of 0.01% (w/v) bromothymol blue, both prepared with 0.003 M potassium phosphate buffer (pH 7.5). The reaction was spectrophotometrically measured at 620 nm and 25°C (7). The enzymes activity were determined measuring the slope of the reaction, and was defined as the change in absorbance per minute per gram of the sample. Both readings were done in a visible spectrophotometer, Novaspec II (Biochrom Ltd., Cambridge, England).

Ascorbic acid was determined using the 2,6-dichloroindophenol (DIP; Sigma) method with a modification (8). 0.1 mL of clarified juice was filtered with Whatman No. 1 filter paper and mixed with 0.9 mL of 1% (w/v) metaphosphoric acid (Fisher). Nine milliliters of 50 µM DIP was added to the acidified sample and measured immediately at 515 nm and 20°C (Visible spectrophotometer, Novaspec II, Biochrom Ltd., Cambridge, England). The concentration of the ascorbic acid was determined through comparison with the absorbance of standard L-ascorbic acid (Sigma) at different concentrations.

Total phenolics concentration was determined mixing 1 mL of clarified juiced pre-filtered with Whatman No. 1 filter paper with 5 mL of 0.2 N Folin-Ciocalteau solution (MP Biomedicals). After 5 minutes, 4 mL of 7.5% (w/v) Na_2CO_3 (Sigma) solution was added and the mixture was allowed to stand 2 hours in the dark. The absorbance of the mixture was measured on a spectrophotometer (Visible spectrophotometer, Novaspec II, Biochrom Ltd., Cambridge, England) at 765 nm, and correlated with a standard curve using different gallic acid concentrations (8).

Juice color analysis was evaluated in terms of tone (Hue angle) and Saturation (Chroma) using a hand-hold tristimulus colorimeter (Minolta CM-508d spectrophotometer, Japan).

Sensory evaluation was conducted by the same four panelists during all the shelf life analysis who where experienced in sensory evaluation of foods, but without training. General perception was evaluated by using a 5-point hedonic scale test (1=extremely dislike, 9=extremely like).

A response surface methodology (central composite rotatable design) was used to select the higher inactivation parameters: Pressure (430-570 MPa) and time (1-8 min) of PPO, and response can be modeled with:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad (1)$$

where Y is the response calculated by the model; X_1 and X_2 are the coded pressure and time respectively, and b_1 and b_2 are linear, b_{12} is interaction and b_{11} and b_{22} are quadratics coefficients respectively. Minitab 16 (Minitab Inc, USA) was used to analyze and optimize this design. Repeated measures design was carried in IBM SPSS v20 (IBM, USA) and used to analyze the effect of the storage time (34 days) in clarified apple juice processed with the optimum HPP value, over quality parameters on juice stored at 4°C and 20°C. Minitab 16 was also used to perform a one way ANOVA analysis of these quality parameters during shelf life.

Results and discussion

High pressure and time effects over the PPO activity are shown in Figure 1. Short time treatments at any pressure tested increased the PPO activity, up to 1.6 times the native enzyme activity, whereas at highest pressures, it was more difficult to inactivate the PPO. The best conditions to inactivate this enzyme were at 430 MPa for 7 min.

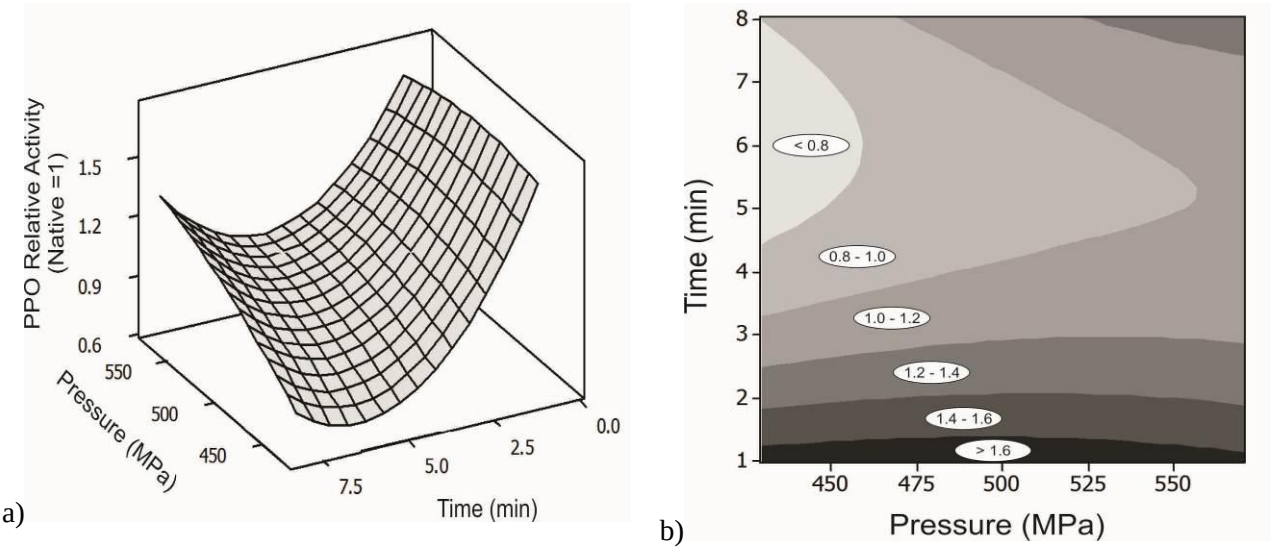

Figure 1. Effect of HPP at differents holding times in the PPO activity. showed as a surface (a) and contour (b) plot.

Ramaswamy et al. (3) reported a 0.12 log cycles PPO activity reduction in apple juice with one pulse of 400 MPa. On the other hand, PPO activation increase by 20% was reported by Terefe et al. (1) in strawberry HPP treated at 690 MPa. Del Pozo-Insfran et al. (2) reported a 3-fold activation increase of grape PPO treated at 400 MPa and 550 MPa respectively. These activity changes are due to different denaturing pathways of the enzyme. Secondary structure is mainly stabilized by hydrogen bonds and tertiary structure is maintained by hydrophobic and ionic interactions, which are disrupted at high pressures. Hence, the activity achieved after HPP treatment is due to active site modification, which can be exposed increasing their activity or can be enclosed or denatured resulting in a decrease of the activity. From these studies, some useful parameters can be done: at pressures under 450 MPa, PPO can be inactivated with long holding times, pressures from 450 to 700 activate it, and pressures above 700 can reduce its activity. PPO was partially denatured and completely inactivated during HPP treatments, but after 13 days an increase in its activity was noted in both storage temperatures (Figure 2b), recovering 20% of its capacity to oxidize chatecol, but not significant changes ($p>0.05$) in total poliphenols was observed during the storage time. The PPO active site was partially recovered after two weeks, but was unable to react with the poliphenols, probably due to its tertiary structure modification.

A microbiological study was done after the HPP treatment. No bacteria, fungi or yeast was detected after that. Similar results were obtained by Varela-Santos et al. (9), where microbial shelf life was extended for more than 35 days. Moreover PME assays did not detect any activity of this enzyme after the HPP treatment and shelf life analysis.

Minimal, but significant changes in pH and color (Hue angle) were observed during the storage time. On the other hand, titratable acidity expressed as malic acid equivalents and °Brix did not change significantly ($p>0.05$) in the same time period, as showed in Table 1. Ascorbic acid oxidation was due to oxygen permeation across the polyethylene bag affecting directly the TEAC (Figure 2a) independently of storage temperature, which decrease at same rate as vitamin C concentration.

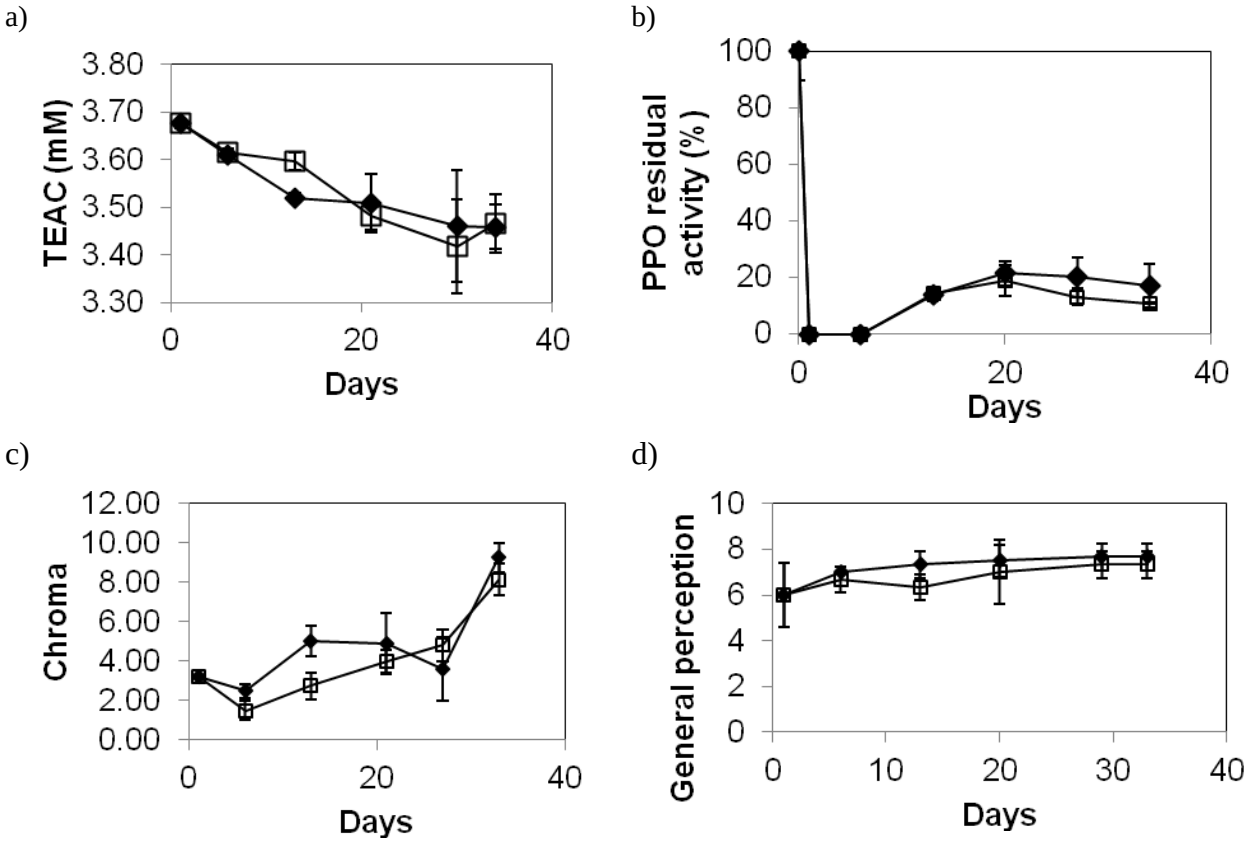

Figure 2. Changes in trolox equivalent antioxidant capacity (a), polyphenol oxidase residual activity (b), color as Chroma (c) and general perception (d; sensory evaluation) during 34 days of storage at 4°C (♦) and 20°C (□).

Table 1. Variation of pH, Color (Hue), malic acid, °Brix and ascorbic acid during 34 storage days at 4°C and 20°C

Day	Storage temperature	pH	Malic acid equivalent	Brix	Hue	Ascorbic acid content
1	4°C	3.425 ± 0.007 a	3.79 ± 0.05 ab	12.05 ± 0.07 a	111.46 ± 0.18 a	211.75 ± 4.95 a
6	4°C	3.355 ± 0.021 ab	3.82 ± 0.00 ab	11.65 ± 0.07 b	78.63 ± 4.89 d	47.13 ± 6.12 b
13	4°C	3.335 ± 0.047 b	3.72 ± 0.05 b	12.05 ± 0.07 a	89.17 ± 9.32 c	39.88 ± 0.11 b
20	4°C	3.295 ± 0.021 b	3.92 ± 0.05 a	12.10 ± 0.00 a	100.37 ± 3.74 ab	39.75 ± 0.08 b
27	4°C	3.360 ± 0.014 ab	3.92 ± 0.05 a	12.00 ± 0.07 a	94.69 ± 2.32 bc	´0.81 ± 3.19 c
34	4°C	3.355 ± 0.007 ab	3.92 ± 0.05 a	11.98 ± 0.04 a	98.05 ± 0.84 bc	1.83 ± 5.85 c
1	20°C	3.425 ± 0.007 a	3.79 ± 0.05 a	12.05 ± 0.07 a	111.46 ± 0.18 a	211.75 ± 4.95 a
6	20°C	3.425 ± 0.035 a	3.86 ± 0.05 a	11.80 ± 0.28 a	90.62 ± 13.46 b	38.49 ± 3.46 b
13	20°C	3.378 ± 0.124 b	3.92 ± 0.05 a	12.05 ± 0.07 a	86.645 ± 5.26 b	39.90 ± 0.19 b
20	20°C	3.240 ± 0.028 b	3.92 ± 0.05 a	12.10 ± 0.00 a	90.02 ± 4.04 b	5.96 ± 5.32 c
27	20°C	3.255 ± 0.007 b	4.02 ± 0.19 a	11.98 ± 0.04 a	89.41 ± 4.30 b	1.83 ± 1.06 c
34	20°C	3.310 ± 0.014 b	3.92 ± 0.05 a	11.98 ± 0.04 a	91.55 ± 0.94 b	´-0.99 ± 5.58 c

Values that not share a letter in the same column of each storage temperature are significantly different ($p<0.05$)

Changes in color as Chroma (Figure 2c) were significant ($p<0.05$), but the predominant color was light yellow until the end of storage period and no was affected by the storage temperature ($p>0.05$). Varela-Santos et al. (9) and Zhang et al. (10) also observed significant color changes during HPP treatments.
In sensory testing, the general perception (Figure 2d) was that the juice was not affected during shelf life analysis, being always preferred the juice stored at 4°C, likely owing to better taste, flavor and aroma retention. Moreover, storage temperature had significant effect ($p<0.05$) during the storage time in pH, PPO activity, ascorbic acid content and color expressed as Chroma.

Conclusions

In this study, HPP processing was able to decrease PPO activity to not detectable levels, but a HPP resistant fraction was also suggested, which recover its activity during long storage periods. However HPP treatment (430 MPa, 7 min) preserved the clarified apple juice with minimal flavor and taste changes if is compared with fresh juice, becoming an excellent alternative to produce fruit juices with high antioxidant capacity and poliphenols content..

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Alternative Sources Of Microorganisms Capable Of Producing Biodegrable Polymers: Polyhydroxyalkanoates

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Polyhydroxyalkanoates known as PHAs are the environmentally friendly polyesters which can be accumulated to as much as 90% of cellular dry weight. The PHA are stored intracellularly as reserve material in over 300 different microorganism in form of inclusion bodies under conditions of nutrient imbalance and/or limitation called stress conditions (7,9,11). The monomeric unit of these polymers is a hydroxyacid, in linear form, called (R)-3-Hydroxy-acid. These monomers have a base structure (Fig. 1) with the difference that in the C3 alkyl group varies. (14,18). On the basis of their monomer composition, the biopolyesters are classified into short chain length (scl-; chain length of the monomers ranging from C3 to C5), medium chain length (mcl-; C6 and longer), and large chain length (lcl- with more than 14 carbon atoms and is rare) (2,8,17, 14,). These polymers have similar properties to the plastics of petrochemical origin, the properties ranging from rigid to be elastic, with the advantage of being totally biodegradable and biocompatible, and therefore it can be produced to renewable resources (carbohydrates, oils, alcohols, organic acids, hydrocarbons, etc.) (6,14,19) can be used for food packaging. The type and amount of the biopolymer synthesized depend on the bacteria strain and culture conditions (2), being carbon source one of the most important variable (1,14,18). Many gram positive and gram-negative bacteria are known to be able to synthesize PHAs (3) Among the main species with high production capacity are *Cupriavidus necator, Pseudomonas spp., Alcaligenes latus, Azobacter vinelandii and Bacillus megaterium* (3,7,9). The biosynthesis of PHAs can be given mainly by three metabolic pathways, which dependent on the kind of nutrients and producing microorganism; that is by synthesis from carbohydrates, by the β-oxidation of fatty acids and by the novo biosynthesis of fatty acids (2). The key enzymes of PHA biosynthesis are the polyester synthases (also designated PHA synthase), which catalyze the enantio-selective conversion of (*R*)-3- hydroxyacyl-CoA substrates to PHAs with the concomitant release of CoA (13). PHA synthases comprise a new family of enzymes with unique features, particularly considering the functional role in biogenesis of the water-insoluble subcellular structures called PHA granules, and the association with a phospholipid monolayer.

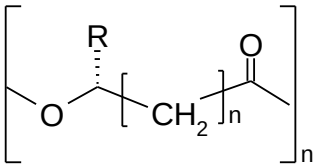

Fig 1. General structure of PHA´s.

These enzymes can be divided into four classes on the basis of the organization of the gene locus and the structure-function properties of the PHA synthase (polymerase) enzymes (17), where the class II *pha* opero, found in pseudomonas, comprises two PHA synthase genes (*phaC1* and *phaC2*) flanking a PHA depolymerase gene (*phaZ*) (17). Solaiman et al. developed the first PCR procedure that specifically detects class II *pha* genes. The primers were designed on the basis of the highly conserved nucleic acid sequences in regions of the class II *pha* loci of varios *Pseudomonas*. Because of the unusually high consensus in the regions, the design of nondegenerate PCR primers (I-179L and I-179R) was possible.

Then, it is possible to amplify a specific segment of 0.54kb from *phaC1* and *phaC2* isogenes of the class II *pha* locus (Figure 2).

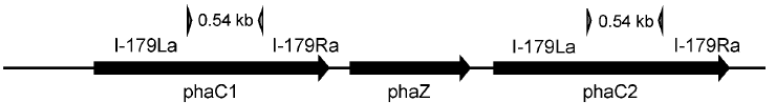

Fig. 2. PCR of class II phaC1 and phaC2 genes. With I-179L(a)/I-179R(a) primer pair then resulted in a specific 0.54 kb *phaC1* and *phaC12* fragment .

In this work, the microorganisms were isolated from a stressful environment through the use of microbiological techniques using culture media nutritive and some selective culture media. Many strains were recovered and once they were isolated in pure culture the strains were identified by molecular biology techniques and biochemical tests.

Methods and materials

Isolation PHA producing bacteria

Water samples were taken from the waste water treatment plant of Colima-Villa de Alvarez at nine different points of the process. Each samples were treated under two different methods in order to recover, as many as possible, potentially useful microorganisms. In the first method, the samples were diluted with peptone water, and aliquots of 0.1 ml were plated in three different culture media: nutritive agar, Minimal Medium modified (MM) and Pseudomonas Agar and incubated for 24-48h at 37°C. On the other hand aliquots of 5 ml were inoculated in Minimal Medium modified (12.8 g $Na_2HPO_4 \cdot 7H_2O$, 3g KH_2PO_4, 0.5g NaCl, 1g NH_4Cl, 10mL glucose 20% as carbon source, supplemented with 1.3 ml of Micronutrient element solution per liter (1mL $MgSO_4$ 1M, 0.1mL $CaCl_2$ 1M, 0.01mL H_3BO_4 0.4M, 1µL $CoCl_2 \cdot 6H_2O$ 0.03M, 1µL $CuSO_4 \cdot 5H_2O$ 0.01M, 1µL $MnSO_4$ 0.01M, 1µL $ZnCl_2$ 0.01M and 0.2mL $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ 0.05M). The final pH was adjusted to 7.4 with NaOH 0.1M. The seed cultures were incubated at 37°C for 24 h at 150 rpm on a rotary shaker and then were diluted and spread onto selective media. The colonies were differentiated by color, elevation, form, and edge appearance and then were isolates and purified at the same conditions.

Genomic DNA extraction

The genomic DNA was isolated, using a physical breakdown method with glass beans as follow: Bacteria cells were grown overnight in 45mL of Nutritive broth. Cell were washed with distilled water, centrifuged and resupended in 0.2mL of extraction mixture(2% triton, 1% SDS, 10mM NaCl, 10mM Tris-HCl pH 8) EDTA 1mM, adding 0.2 ml of 25:24:1 phenolchloroform:isoamilic alcohol, 0.06g of 0.5 mm glass beads and 0.5mL sterile distilled water. Finally, the cells were homogenized by vortex during 1 min at high speed for 8 times. The tubes were chilled on ice for 1 min between runs. The upper layer was recovered and transferred to a clean tube and 1 volume of pure chloroform was added, the tube was mixed carefully and centrifuged at 11 000 rpm for 5 min. the aqueous phase was saved in a clean tube and 20µg/ml RNAase was added and incubated for 1h at 37°C. After 1 volume of 25:24:1 phenolchloroform:isoamilic alcohol was added and centrifuged again. The aqueous phase was transferred to a clean tube with 500µL of isopropanol and 50µL of amonium acetate (4M) and was carefully mixed and incubated for 15 min at room temperature. Supernatan precipitated was centrifugated for 10 min and the DNA was washed with 70%v v^{-1} ethanol, vacuum-dried and dissolved in 30µL of TE (10mM Tris-HCl, 1mM EDTA pH 7.5)

Amplifying the *phaC* gene fragment

To screen the capability of PHA production, a PCR based method developed by Solaiman et al. (2000) was applied using 179R/L primers; (1-179L 5´-ACA GAT CAA CAA GTT CTA CAT CTT CGA C-3´)

and (179R; 5´-GGT GTT GTC GTT GTT CCA GTA GAG GAT GTC-3´). PCR was optimized using the following components: 0.3µL Taq DNA polymerase (250U), 1 µL (100pM) of each primer, 2µL of genomic DNA, 5µL of 10X PCR buffer, 5µL of $MgCl_2$ solution (25mM) 1µL 10mM dNTP´s mix(10mM) at a final volume of 50µL. Amplification reactions were carried out in an thermocycler (C-100, Biorad) using the following programme: 1 cycle of 94°C for 10 min; 40 cycles of 94°C for 20s, 60 °C for 45s, 72°C for 1min; and a final incubation step at 72 °C for 5 min and 4°C forever. Electrophoresis on 1.5% (w/v) agarose gels (1X TAE) was used for detecting PCR amplification products. The gel was stained with ethidium bromide solution and amplified DNA fragments were visualized under UV light and recorded with Gel Doc™ XR+ image digitaliser (Biorad).

Amplifying the 16S rDNA

The 16S rDNA regions of the PHA-positive bacteria were amplified from genomic DNA using the forward primer UBF 5´-AGA GTT TGA TCC TGG CTG AG-3´ and the reversed primer 1492R 5´-GGT TAC CTT GTT ACG ACT T-3´, 1500 bp (10). Following the same conditions used to amplify the phaC gene fragment. Amplification reactions were carried out in an thermocycler (C-100, Biorad) using the following programme: 94°C for 12 min; 35 cycles of 94°C for 1min, 50 °C for 45s, 72°C for 2 min; and a final incubation step at 72 °C for 12 min and 4°C forever. PCR products were analyzed by electrophoresis with 1.5% agarose gel in TAE buffer, following ethidium bromide staining and Molecular Imager Gel Doc™ XR+ System with Image Lab™ Software.

Identification bacterial

The PCR products for each PHA-positive bacterium was purified by the GenElute PCR Clean-Up Kit (Sigma) and quantified and sent for sequencing to Potossiannnnn institute. Microbiologically, the PHA-positive bacteria were identified by oxidase, catalase, staining, and biochemical tests

Results and discussion

A total of 150 strains were isolated and purified, from the 9 sampling points, with base on its morphology. In order to perform the molecular identification, each strain was processed for genomic DNA extraction. Then, the search of the phaC gene was performed and only those microorganism that amplify a band with a weigh about 540 bp were considered as positive (17), as it is show in microorganism is a potential producer of polyhydroxyalkanoates and it has PHA synthase of class II Fig. 3.

The *phaC* + native microorganism, was obtained the 16S rDNA gene partial sequence, from DNA genomic, where we expect to obtained by splicing the 1500 bp (amplified with UBF y 1492R) as show in the Fig. 4 and then it was purified and quantified.

The quantified was determined in a The Thermo Scientific NanoDrop 2000, obtained concentrations about 38.6 ng/µl, 39.2 ng/µl and 84.4 ng/µl respectively necessary for the sequencing and have the security of bacterial genus belongs. Succeeded in identifying *Pseudomonas spp*.

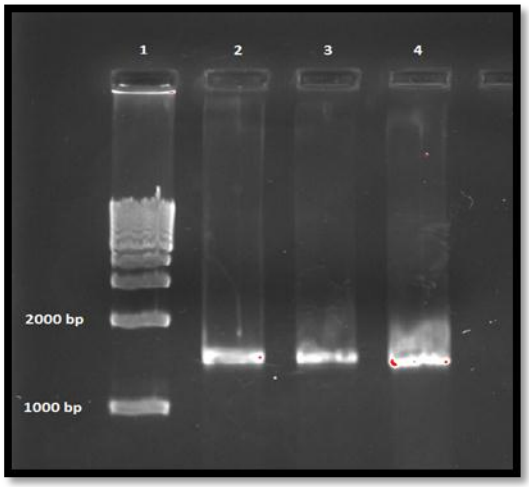

Fig. 4. PCR amplified the 16S rDNA. In the first line, molecular weight marker (EZ Load™ 1 kb, Bio-rad); 2-4, it the same native bacterial that had phaC +.

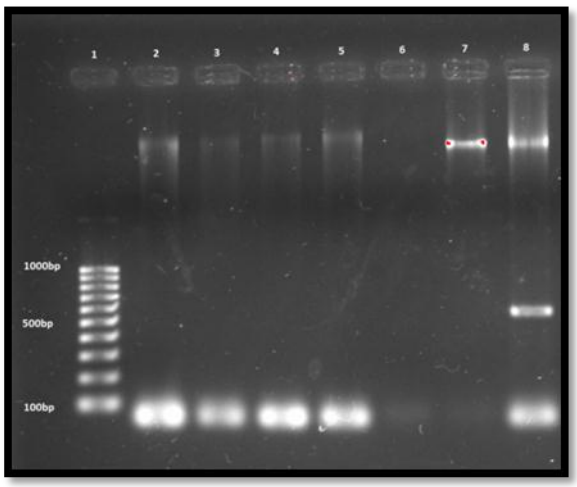

Fig. 3. PCR amplification of a *phaC* gene fragment using 179L and 179R primers. Lane 1, Molecular weight marker (EZ Load™ 100 bp, Bio-rad); 2-8, different native bacteria isolated.

Additionally was done determination microbiological in order to identify the presumptive producer bacterium of polyester, due to possible false-positive and false-negative results (contamination, low specificity of the amplification reaction and polymerase inhibitors) was plated onto with nutritive agar medium and pseudomona agar; due to the results of testing obtained and as a particular they produced a fluorescent green pigment in both medium (incubated 37°C/24h), shown in fig 5.

			TSI		
Catlase	Oxidase	Gram staining	Glc	Lac	H_2S
Positive	Positive	Negative	Negative	Negative	Negative

Fig. 5. Microbiological identification. Biochemical test and bacterium produced a fluorescent green pigment in the medium.

With all information both microbiological and molecular we can suggest that microorganism belongs to the genus *Pseudomonas spp*. that has already reported as a product of this polyesters called polyhydroxyalkanoates. (15)

Detecting *phaC* gene in native bacteria combined with the preliminary characterization by 16s rDNA has proved to be an option for the precise selection of native bacteria having potential from accumulating PHA´s.

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Relative Viscosity Of Corn Flour Cooked By Ohmic Heating

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Introduction

Ohmic heating is the passage of an alternating electrical current through a food sample that serves as an electrical resistance (1). The sample responds by internally generating heat supporting a rapid heating rate, and the current from the voltage gradient and the electrical conductivity is associated with the amount of heat generated (2). Ohmic heating has the capability to heat multiphase mixtures uniformly (3). The temperature of a food is known to have a linear relationship with the electrical conductivity as electric field strength increases (4). Higher electric field strengths and lower frequencies resulted in greater electrical conductivity with shorter times to reach target temperatures (5). However, in non-homogeneous foods, the electrical conductivity (s) of the particles and its relation to the fluid conductivity is pointed as a critical parameter to the understanding of the particles' heating rate under OH. Proper electric conductance management is essential to successfully apply OH (6).

Wang and Sastry (16) found that electrical conductivity can influence the heating rate of a food sample, and for starch it increased with temperature, but decreased with degree of starch gelatinization. Li et al. (8) found a linear relationship between the electrical conductivity and temperature of starch suspension before and after gelatinization. However for integrally processed cereals has not been reported behavior of electrical conductivity when processed ohmically.

The relative viscosity has been used to measure the degree of gelatinization of corn starch, principal carbohydrate, when subjected to a thermal process. The evaluation of the rheological properties of foods is essential for the design of operations related to processing and to predict their quality. The aim of this study was to determine the changes in viscosity of corn flour ohmically processed and assess changes in the electrical conductivity.

Materials y methods

Corn: It was used comercial white dent corn (harvest 2011) obtained in the food supply market of Queretaro City. All samples were thoroughly threshed and cleaned to remove broken and foreign material, milled in a hammer mill (Pulvex-200, Pulvex, S.A. de C.V., México, D. F.), using a 0.8 mm mesh and stored in plastic bags.

Ohmic heating equipment: It was used a nylamide cell (capacity, 100 g) appropriate for high viscosity foods (9) connected to a voltage source by two stainless steel electrodes (Figure 1). Temperature was measured with a Watlow temperature controller model 981 (Watlow Electric Manufacturing Co., St Louis, MO) with a type T sensor.

Ohmic heating processing: It was thoroughly mixed milled corn (100 g) with food grade calcium hydroxide (El Topo, Monterrey, N.L, México) and water, placed in the ohmic heating cell and processed until the mix reached 85 °C.

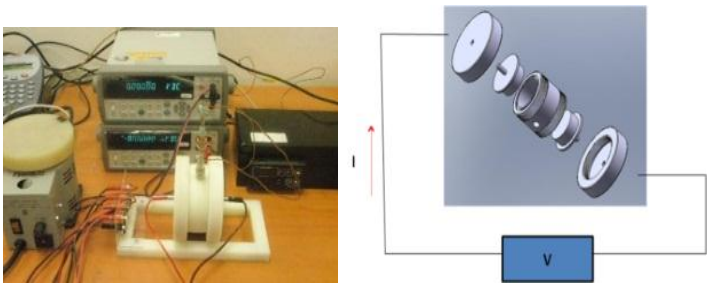

Figure 1. Ohmic heating equipment and diagram.

Relative viscosity: It was measured to a starch (3 g): water (24 mL) suspension, using an Anton Paar rheometer (Anton Paar, modelo Physica NCR 101) recording pasting temperature (°C), initial and maximum viscosity (cP), as well as ΔT (final viscosity – minimum viscosity).

Experimental design and statistical analysis: It was used a completely randomized design with factorial arrange 3x3x3; independent variables were raw corn moisture (50, 55, 60%), calcium hydroxide concentration (0, 0.3, 0.6%) and voltage (70, 80, 90 V). Each sample was processed by duplicate. Statistical analysis consisted of ANOVA and Tukey tests ($p \leq 0.05$).

Results y discussion

Relative viscosity

According Figure 2, corn flour cooked with ohmic heating did not show a significant effect on pasting temperature; however, calcium hydroxide concentration decreased it indicating that it was required less energy to swell it. Biliaderis et al. (10) defined gelatinization as a group of three non-equilibrium thermodynamic processes, water diffusion within the starch granules, melting promoted by hydration, and crystal structure disintegration due to swelling of the starch granule. Pasting temperature indicates the beginning of the swelling of the starch granule and it changes just when there is not any starch granule to gelatinize.

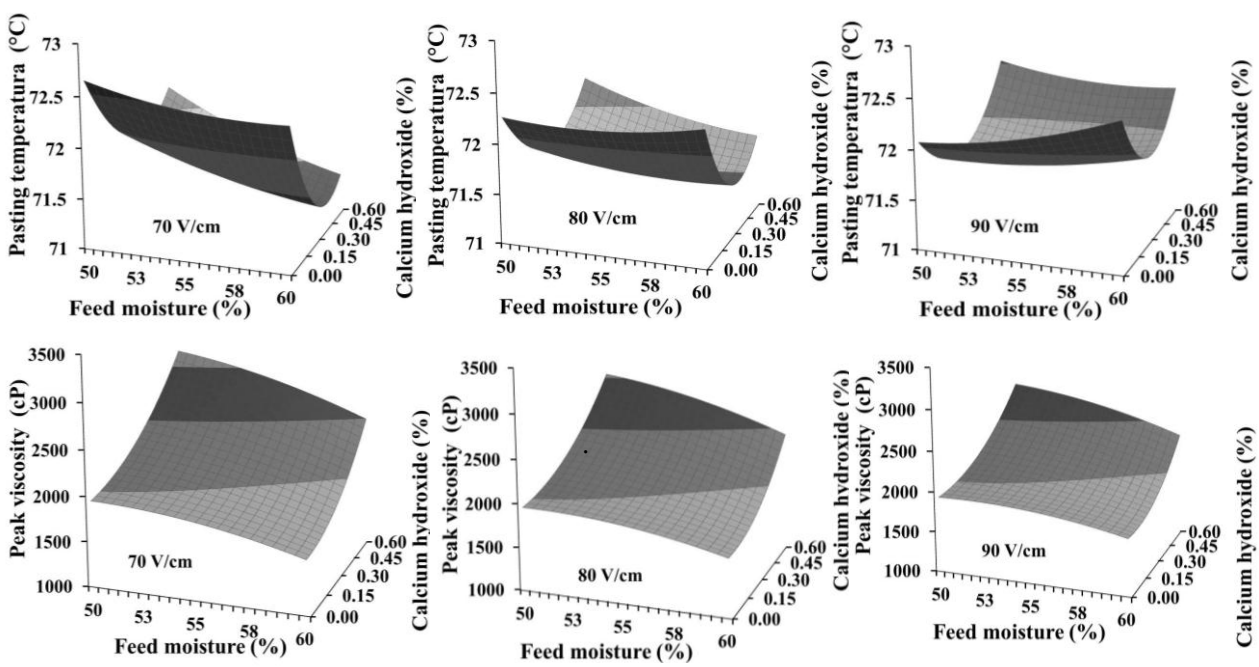

Figure 2. Relative viscosity of corn processed by ohmic heating

Peak, minimum and final viscosity diminished when feed moisture increased, as well as retrogradation; these effects occur at both processing temperatures. Steeneken (11) has reported that, in a dilute regime, the viscosity is governed by the volume fraction of swollen granules; however, in a high starch concentration regimen, there are some other factors to be considered, such as concentration of starch, temperature, deformability of the granules, and degree of molecular entanglement (12), some of which agree with data found in this work.

Calcium hydroxide increased viscosity (directly) in corn; voltage did not influence viscosity. In general, amylose acts as an inhibitor of swelling but amylopectin is likely to swell due to the weakness of the intra- and inter-molecular coherence in starch (13).

Electrical conductivity

Electrical conductivity of ohmically cooked corn flour is shown in Figure 3; this variable was significantly increased by calcium hydroxide concentration and decreased by raw corn moisture, whereas voltage did not affect it, but the interaction voltage – moisture did.

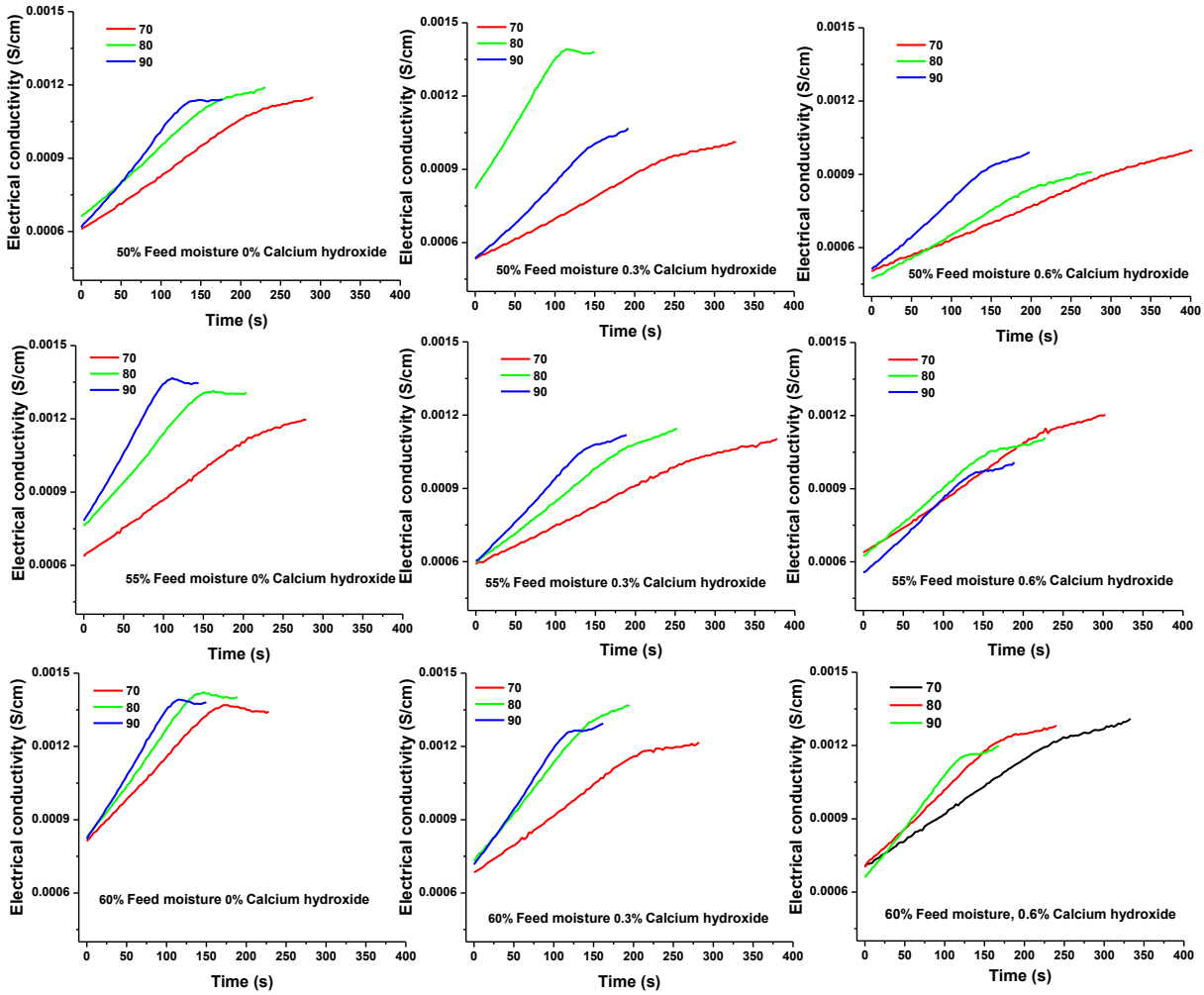

Figure 3. Electrical conductivity of corn processed by ohmic heating.

Electrical conductivity is the capacity of a material to allow passage of the electric current through it. In foods containing starch, such as corn, the electrical conductivity varies with the degree of starch

gelatinization. When the swelling of starch granules increases, electrical conductivity decreases because of the diminished movement of ions in the solution (14).

The interaction between voltage and humidity showed a linear relation. At low moisture, low conductivity; if moisture increase, conductivity increase.

The heating rate of particles in a fluid depends on: (i) the relative conductivities of the system's phases and (ii) the relative volume of those phases. Low conductivity solid particles, comparatively to the fluid conductivity, tend to lag behind the fluid at low concentrations related to the volume of the fluid. However, in conditions where the concentration of the particles is high, those same low conductivity particles may heat faster than the surrounding fluid. So, the phenomenon of particle-lagging or particle-leading depends on the significance of particle resistance to the overall circuit resistance (4). This phenomenon occurs because, with the increase of the particles' concentration, the electric current path through the fluid becomes more tortuous, forcing a greater percentage of the current to flow through the particles. This can result in higher energy generation rates within the particles and consequently in a greater relative particle heating rate (4). This fact indicates that it may be possible to adjust the heating pattern of solid fluid systems by adjusting the overall influence of particles' resistance in the system through setting the particles concentration in the fluid.

The electric conductivity of some systems may also be altered to achieve the ideal OH situation, when the conductivity of the particles is equal to the surrounding fluid (16). However, as pointed by Halden et al. (17) it is unlikely that the exact same heating rate can be achieved throughout the process, even though the thermal difference, in practical terms, may be too small to be significant. In fact, the electric conductivity is a function of the structure of the material and often changes by heating (cooking); however in some foods, the overall heating effect might be too little to alter the pattern of electrical conductivity (18).

Conclusions

The amount of water added to the raw corn and the calcium hydroxide concentration were the independent variables responsible for the changes in viscosity of the flours processed by ohmic heating. Electrical conductivity increased as the amount of water added to the raw corn increased. Voltage did not have any effect on both, relative viscosity and electrical conductivity.

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Corn Flour Processed By A Low Shear Stress System

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Introduction

SIACON-SIAP (1) reported 20,202,600 ton of corn harvested in 2009, one third of it is used to prepare tortilla (2). Actually, two thirds of the Mexican corn tortilla production is from dough (masa); however, its production from flour has become increasingly popular in the tortilla industry. Nixtamalized corn flour is produced mostly by a nixtamalization process modified for its industrial application (3,4) and by extrusion process at lesser degree. Extrusion is an excellent technology to be applied for snack production; extruded corn flour presents some disadvantages: as a result of the shear stress and the temperature applied during this process, starch dextrinization occurs its quality diminishes. Consequently, the industrial use of extrusion for producing instant corn tortillas flour is not widespread (5). The main objective of this research was to evaluate the quality characteristics of corn flour processed by a low shear transport system related to those of traditionally nixtamalized corn flour.

Materials and methods

Raw corn: A commercial corn Sinaloa brand harvest 2010 was bought at the wholesale food market of the city of Querétaro. The properties of the corn were: a flotation index: 8.3 floating grains; weight of 1000 grains: 390 g; density: 0.84 g/mL; length: 12.3 mm; width: 7.4 mm; thickness: 2.7 mm; protein 7.5%; fat: 5%; ash: 1.3%; moisture: 10.75%.

Mechatronic system of low shear transport (LSTS): A low shear transport system (LSTS) was used, one that is based on extrusion but which separates the transport stage from the cooking stage (6). The system consists of three sections: feeding, transport and cooking (Figure 1). The feeding section consists of a hopper (A) that is fed manually, the transport section, which is carried out by a patented vane pump (B), and the cooking section, which consists of a laminar cooker without a die (C). The low shear function is due to two actions: a) the transport of the material is carried out by a laminar fluid transporter, which transports the material with the fluid vectors aligned, thus eliminating turbulence and diminishing the shear force; b) the laminar cooker is open (without a die), minimizing the pressure difference between entrance and exit and thus without shear at exit.

The LSTS is coupled with a motor that permits the control of velocity (rpm) from 1 rpm up to 60 rpm, as well as the control of temperature from room temperature up to 150 ºC.

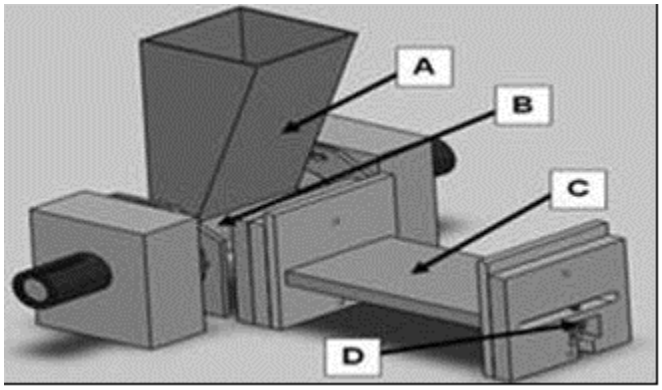

Figure 1. Low shear stress transport system
A: feeding stage. B: transport stage. C: laminar cooking stage. D: temperature sensor

Preparation of nixtamal corn flour by low shear transport system: Three kilograms of corn was milled in a hammer mill with a 1.3 mm sieve (Pulvex, model 200, MaquinariaparaMoliendas y Mezclas, México, D.F.). The raw corn meal was conditioned with a 55 % feed moisture and 0.3 % calcium hydroxide (w/w) (El Nevado, Liquid Química, Mexicana, S.A. de C.V.). After conditioning, raw corn meal - mix (raw corn meal, calcium hydroxide and water) was processed in the LSTS at different feed velocities and temperatures. Once processed, the samples were dehydrated in a homemade flash dryer (temperature, 250 °C for 5 s, residence time) (Flash dryer, CINVESTAV-Querétaro). The nixtamal corn flour was then stored at 4 °C for later analysis.

Nixtamal corn flour by the traditional method: Three kilograms of corn was placed in nine liters of water at 92 °C that contained 1% calcium hydroxide, for 25 min. It was left in the solution at room temperature for 11 hours. The cooked corn, now nixtamal, was washed and milled in a stone mill (Fumasa, Fundición y Maquinaria Sánchez S.A. de C.V.). The nixtamal corn flour was dehydrated in a homemade flash dryer (temperature, 250 °C for 5 s, residence time) (Flash dryer, CINVESTAV-Querétaro). The nixtamal corn flour was then stored at 4 °C for later analysis.

Characterization of nixtamal corn flour: Water absorption indexes (WAI) and water solubility (WSI) were determined following the method described by Anderson (7). To perform Dunnet's test, ISA values for ohmic heating and traditional process samples were adjusted by adding to the traditional process samples the solid remains left in the decanted water, in accordance to results reported by Campechano(8). Dynamic viscosity was determined using a Physica MCR 1001 rheometer, following the method described by Gaytán(9).

Characterization of dough and tortilla: Dough (masa) adhesiveness and cohesiveness were determined by a texturometer (Texture Analyzer XT-2), according to the method of Martínez(10). The yield of tortillas was calculated according to the methodology of Mauricio (11).The surface color of the nixtamal corn flour produced by a low shear transport system was measured using a color spectrophotometer (model XS2725, Gretag Macbeth LLC, Switzerland).

Experiment design and data analysis: A completely randomized experimental design was used. Independent variables weretransport speed (5, 7.5, 10, 12.5 and 15 rpm) and temperature (80 and 90 °C). The experimental data were analyzed using Minitab® Statistical Software version 14. The means of the response variables were compared using Tuckey with a difference significance level of α=0.05 in the treatments.Dunnet's test was carried out to assess differences between ohmic heating treatments and treatments obtained with the traditional process.

Results and discusion

Table 1 shows that temperature is the process variable that most affected the quality characteristics evaluated in corn flour, dough and tortilla. Variables affected by temperature were WSI, WAI, dough

yield and tortilla yield directly proportional, and Viscosity, “a” and “b” (color) indirectly. Transport speed affected viscosity and cohesiveness directly, and WAI and “a” indirectly.

Table 1. ANOVA

FACTOR	VARIABLE	MS	F	P	R^2
Transportspeed	WSI	0.5182	3.05	0.384	0.7795
Temperature		11.1384	65.59	<0.0001	
Transportspeed	WAI	0.0192	7.24	0.0007	0.8837
Temperature		0.3657	138.16	<0.0001	
Transportspeed	Viscosity	145232	6.27	0.0016	0.9069
Temperature		4384514	189.24	<0.0001	
Transportspeed	Doughyield	0.0040	4.33	0.0098	0.6662
Temperature		0.0248	26.58	<0.0001	
Transportspeed	Tortilla yield	0.0024	2.10	0.1147	0.7554
Temperature		0.0134	11.54	0.0026	
Transportspeed	Cohesiveness	0.1484	5.18	0.0043	0.5052
Temperature		0.0495	1.73	0.2019	
Transportspeed	Adhesiveness	0.0010	1.74	0.1771	0.2722
Temperature		0.0007	1.27	0.2724	
Transportspeed	L	4.7028	4.55	0.0079	0.4530
Temperature		0.0185	0.02	0.8947	
Transportspeed	a	0.2952	6.67	0.0011	0.6901
Temperature		0.9881	22.32	0.0001	
Transportspeed	b	0.6789	1.74	0.1769	0.4526
Temperature		4.3766	11.22	0.0029	

The temperature increase results in a higher water absorption capacity and as a consequence, higher dough and tortilla yield, a higher leaching of solutes from starch to water, a lower viscosity and color of tortilla slightly more yellow and red, as can be observed in Table 2. The transport speed increase results in a less water absorption capacity, a higher viscous flour suspension and more cohesive dough (Table 3); it is considered that this change is due to a longer residence time in the equipment at 80 or 90 °C. It is also observed that tortilla is slightly less red and luminous, due to the same reason.

Table 2. Response variables related to temperature.

Variable	Temperature (°C)			
	80		90	
WSI (%)	8.56	b	9.82	a
WAI (%)	2.80	b	3.03	a
Viscosity (cP)	2125.64	a	1334.21	b
Dough yield (kg flour/kg dough)	2.02	b	2.08	a
Tortilla yield (kg dough/kg tortilla)	1.59	b	1.63	a
a	1.31	a	0.94	b
b	17.45	a	16.66	b

Means with different letter in the same line are significantly different ($P < 0.05$).

Table 3. Response variables related to transport speed.

VARIABLE	TRANSPORT SPEED (rpm)				
	5	7.5	10	12.5	15
WSI (%)	9.09 b	8.87ab	9.16ab	9.11ab	9.82 a
WAI (%)	3.00 a	2.91a	2.94 a	2.87ab	2.83 b
Viscosity (cP)	1518.3 c	1799.8 abc	1648.8 bc	1893.8 ab	1951.3 a
Dough yield(kg flour/kg dough)	2.09 a	2.02 b	2.06 a	2.02 b	2.07 a
Tortilla yield(kg dough/kg tortilla)	1.65 a	1.59 a	1.60 a	1.61 a	1.61 a
Cohesiveness (N)	1.60 a	1.63 a	1.39ab	1.53 a	1.17 b
a	1.11 a	1.24 a	1.20 a	1.30 a	0.64 b
b	17.30 a	17.31a	17.13 a	17.04 a	16.32 a

Means with different letter in the same line are significantly different ($P < 0.05$).

Dunnet's test showed no statistical differences ($p \leq 0.05$) between traditionally nixtamalized corn flour characteristics and those of processed by the low shear transport system, except in viscosity; however, there must be considered that corn processed by LSTS is milled before processing and the traditional method uses the whole grain. Textural characteristics of dough and tortilla from corn processed by LSTS are similar to those prepare from traditionally nixtamalized corn flour indicating that starch does not change during this process more than during the traditional one.

Conclusions

The characteristics of dough and tortilla from corn processed by LSTS are similar to those prepare from traditionally nixtamalized corn flour indicating that starch does not change.

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Use Of Cultivated Axihuitl Extracts As Bioinsumo In Treatment Of Phytopathogens

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Abstract

Axihuitl (*Eupatorium aschembornianum* Sch.) has been widely used in traditional medicine in Mexico. Other strains of *Eupatorium* have been successfully appling for biological control of phytopathogenic fungi and bacteria. The goal of this project is to evaluate and compare the *in vitro* efficacy of extracts of wild and cultivated Axihuitl against phytopathogenic bacteria. The evaluation was made applying 100 µl of an *Erwinia chrysanthemi* 3937 suspension with an optical density of 0.6 at 600 nm uniformly distributed on LB agar plates and allowed to dry for 10 min. Filter paper discs of 0.6 cm of diameter were then placed in agar plates and 5 µl of different concentrations of each extract at (250, 500, 1000 and 1500 $mgml^{-1}$) were added to them. Hidrolsis halos were measured every 8 h for 48 h. Ampicillin 250 μgmL^{-1} and Tween 80 1% were used as positive and negative controls, respectively. Results showed higher antimicrobial efficacy of the wild extract in smaller concentration (from 500 $mgml^{-1}$) and necessary incubation time (from 12 h.), so this extract was selected to be applied as treatment for other bacterial strains (*Pseudomonas syringae, Pseudomonas aeruginosa*) where antimicrobial activity was also showed.

KEY WORDS: Biological control, *Eupatorium aschembornianum Sch., Erwinia chrysanthemi*.

Introduction

Most of the plant illnesses are caused by fungi, insects, bacteria and virus, who colonize different parts of the plant, resulting from the decrease in product quality, until the total plant loss [1].

Ornamental plants are mainly affected by phytopathogenic fungi and bacteria including opportunistic species that are even soil saprophytes. The susceptibility of crops to these diseases primarily affect the aesthetic qualities of the plant causing great economic losses. Pest and disease control depends in part of the application of chemicals: For example, in Morelos, cultivation of tuberose (*Polyanthes tuberosa*), is a major ornamental crop in the region and the main pest of tuberose, the weevil (*Scyphophorus acupunctatus*), represents 60% of the production cost per cycle (1.5 to 2.0 years) on chemical insecticides. Normally, this insect is associated with pathogenic bacteria causing soft rot of plant tissue (bulb rot).
Production of these ornamental plants under greenhouse conditions suffer recurrent diseases of bacterial type (*Erwinia* sp., *Pseudomonas* sp., etc.). The use of agrochemicals has led to substantial increases in production, however, its adverse effects are significantly impacting the sustainability of agriculture. The indiscriminate use of these, has caused serious environmental pollution problems [1]. Another important aspect is that pathogenic microorganisms have developed resistance to the active ingredient of some synthetic fungicides [2]. The practice of monoculture and contamination by the indiscriminate use of agrochemicals have reduced biodiversity in agroecosystems, causing instability of the same which is manifested among other harmful effects, in an increased incidence of pests and diseases in crops. This and public health and safety problems inherent in the production and use of chemicals has led to the search and development of alternative pest. Thus arises the interest in the ecological control [3].

One such alternative is the biological control by the use of plant antagonists, if it exists in nature, and a wide range of plants that produce a variety of toxic secondary metabolites. One way to exploit this antagonism is by preparing extracts or infusions from the leaves, seeds and flowers.

The Axihuitl (*Eupatorium aschembornianum* Sch.) is a bushy plant from Astereceae family, widely used in the town of Tepoztlan, Morelos, Mexico, as a healing plant, easy to acquire, because their production is abundant in the rainy season from November to February. There has not currently obtained large scientific data about this plant, as the healing properties thereof are not known through scientific clinical research, but is believed to possess some antimicrobial activity. For that above, this research was aimed at evaluating the antimicrobial activity of extracts of wild and cultivated Axihuitl against phytopathogenic bacteria.

Methodology.

Preparation of extracts of cultured and wild Axihuitl.

The lyophilized Axihuitl extracts, cultivated and wild, were provided by the Center for Research and Assistance in Technology and Design of the Jalisco State, AC (CIATEJ). Extracts were prepared by diluting the freeze-dried in 10% Tween 80 for improved dispensing. Concentrations used for evaluating the antimicrobial capacity were 1500 mg mL^{-1}, 1000 mg mL^{-1}, 500 mg mL^{-1} and 250 mg mL^{-1}.

Growth inhibition assay in plant pathogenic bacteria.

Different plant pathogenic bacteria used were grown in different culture medium (Table 1).

Table 1. Bacteria strains used in antimicrobial test and culture médium where they were grown.

Bacteria	Culture medium
Erwinia Chrysanthemi	LB
Pseudomona syringae	*KB*
Pseudomona aeuroginosa	*Nutritive*

Each bacteria was inoculated into the corresponding liquid medium (Table 1) and tracked their cell growth to reach its exponential growth phase (0.6 a $D.O._{600nm}$).

After reaching an optical density of 0.6, 100 µL of each bacterial suspension were inoculated on appropriate agar plates, extending the suspension uniformly on the agar with the aid of a Digalsky rod and allowed to dry for 10 min. Filter paper discs of 0.6 cm of diameter were then put in plates and 5 µl of diffetent concentrations of extracts were added on these.

250 µg/mL ampicillin and 10% Tween 80 were used as positive and negative controls, respectively, for inhibition test. All plates were incubated at 30 °C for 48 h and inhibition zones were measured according Cavalieri [4] with the aid of a vernier.

Results and discussions

Growth inhibition assay in plant pathogenic bacteria.

Cultivated and wild Axihuith extracts were analyzed by the logarithmic method [4], using a strain of *Erwinia chrysanthemi* as a model organism to select the best antibiotic treatment. Inhibition zones were

monitored and measured at 12, 18, 24 and 36 h (Fig. 1). Results showed marked differences between treatments (Fig. 2) and according to the analysis of variance showed a significant difference between the two treatments according to Fisher method ($\alpha = 0.05$) at 95% confidence.

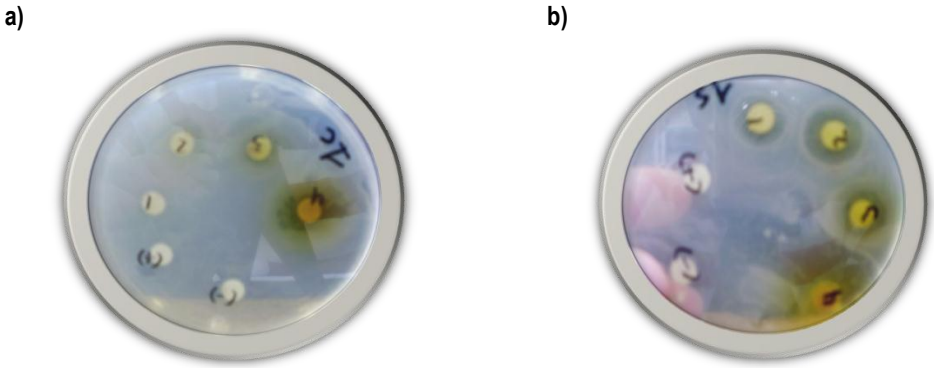

Figure 1. Inhibition test of the growth of *Erwinia chrysanthemi* incubated at 30 °C for 24 hours: a) cultivated extract b) wild extract.

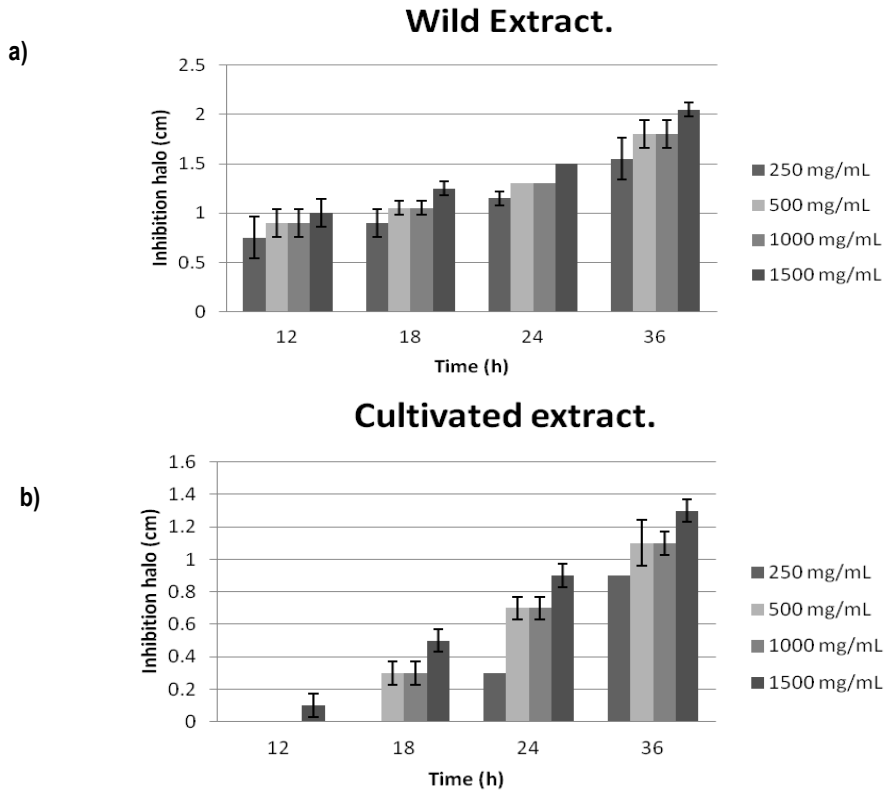

Figure 2. Evaluation of radial growth inhibition of *E. chrysanthemi* at different times of incubation with different concentrations of extracts a) wild extract b) cultivated extract.

As shown in Figure 2, wild Axihuitl extracts showed a growth inhibitory effect of higher than cultured ones, in different concentrations and different incubation times, so that treatment was selected for subsequent assays.

Although there are no reports of the effectiveness of Axihuitl to control *Erwinia chrysanthemi*, Sanabria et al. [5] demonstrated that *Chomolaena orodata*, a shrub belonging to the same family Astereceae, is able to inhibit the growth of some Gram negative, consistent with the obtained results.

Having chosen the wild Axihuitl, its ability to control *Pseudomonas aeruginosa* and *Pseudomonas syringae* was probed. In general, no remarkable difference was observed in the inhibitory effect between bacterial strains, showing in all cases a proportional increase in the growth inhibition relation with the administered concentration of extract by means of culture as shown in Figure 3.

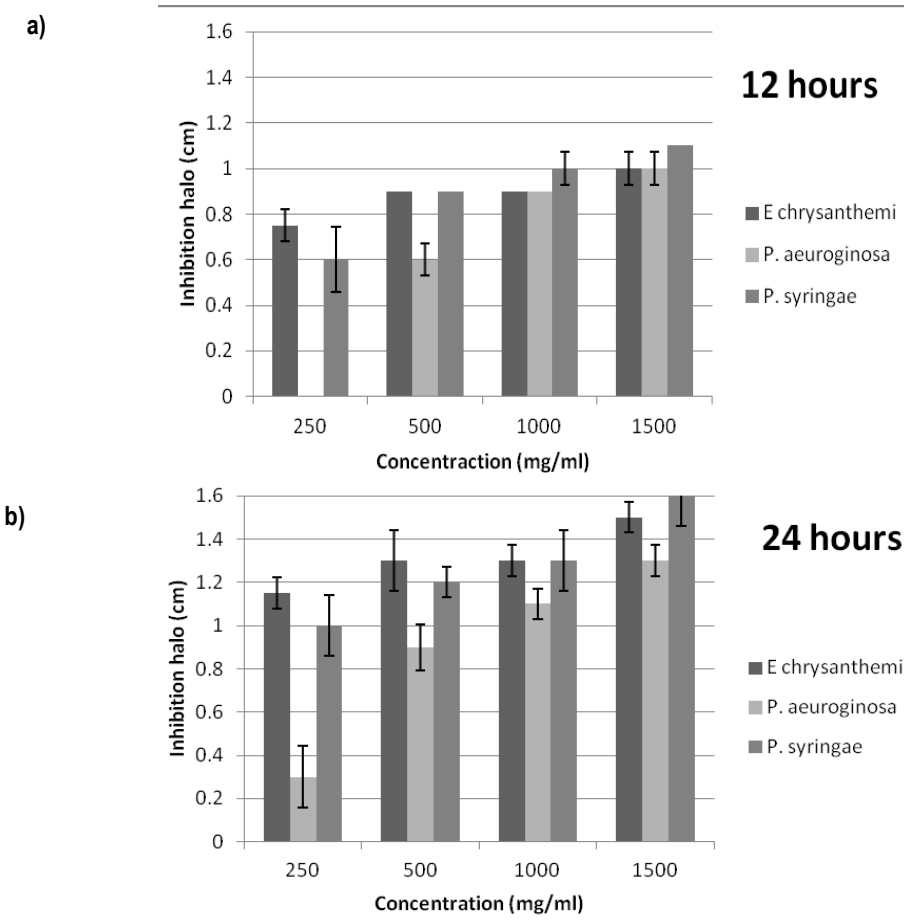

Figure 3. Growth Inhibition of different bacteria strains by wild Axihuitl extracts at different incubation times: a) 12 h, b) 24 h.

Some other authors [6,7,8] state that there are a variety of plants belonging to this family that produce antimicrobial substances, which explains the effectiveness of the Axihuitl extract to inhibit these bacteria strains.

Conclusions

Wild Axihuitl extract showed higher antimicrobial activity against *Erwinia chrysanthemi*, *P. aeruginosa* and *P. syringae* at lower concentration and incubation time compared with cultivated Axihuitl extracts.

Awards

The authors wish to thank the CIATEJ, especially to Dr. Gabriel Rincón Enriquez, for their support to carry out this work.

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Behavior Of Pectinesterase In Sweet Granadilla *(Passiflora Ligularis)*

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Enzymes are responsible of many sensorial and quality changes in fruits and vegetables. The pectinmethylsterase (PE) it's the responsible of texture deterioration in fruits. The thermal treatments are the more employed method in food preservation inhibiting the enzymatic activity. The objective of this work was to identify the behavior of pectinmethylsterase (pectin-pectin-hidrolase) in Sweet Granilla (Passiflora ligularis) pure after thermal treatments. The thermal inhibition of enzymes with hot water and with microwaving at 1200 Watts and 2450 MHz were the treatments applied for 5, 10, 30 and 45 s. The enzymatic activity was measured by titration in terms of equivalent milligrams of hydrolyzed ester per minute per gram of enzyme (PE U/g): the PE was extracted from fruit pulp with saline and slowly added a solution of sodium chloride, sodium hydroxide, purified pectin and Hinton indicator at 7.5 pH. The inhibition resulting from each treatment was the differential between the enzyme quantification in natural (0.0155 Pe U/g) and treated samples. The lowest activity of pectinmethylsterase (0.0024 Pe U/g) was achieved with hot water treatment for 30 s; but treatment for 45 s increased considerably its activity (0.0074 Pe U/g) and samples reached 40 oC. Microwave treatments lowered the enzyme activity but not greatly (0.0118 Pe U/g with 30 s, 0.0068 with 45 s); longer treatments yielded lower pectinesterase activity.

(Keywords: granadilla, pectinmethylsterase, thermal, microwaves)

Introduction

Pectins contribute to texture composition of fruits and vegetables. Viscosity and turbidity of fruit juices are the result of pectins present, released during preparation. Some manufacturing processes inhibit enzymes with thermal treatments, up to 80 and 90 ^{o}C and relaying on pH changes. Also, concentration and ^{o}Brix act as protecting barriers for many enzymes [1]. There is evidence that enzymes in vegetables and juices subject to thermal treatments might reactivate as a result of poor processing. Residual activity of pectinmethylsterases is proof of effectiveness in thermal treatments; it is defined as the number of methoxyls released per gram of soluble solids. The enzyme's inhibition by thermal treatment is a process aimed to increase shell life for products which undergo further procedures. The microwave thermal treatment is a technique used for pasteurization of juices, meat defrosting and fruit blanching, avoiding elution of its components [2].

The produce selected for this work was Sweet Granilla (Passiflora ligularis), also known as Granada China in México, Granadilla in El Salvador and Parcha in Venezuela. The fruit is orange in color with tiny spots when ripe. In contrast with the Passiflora edulis variety, the Granadilla shell is very hard around the peduncle making it difficult to cut with a knife. The fruit's pulp is white and tasty, very sweet and not acid. Its smell is very sweet, evidence of readiness to eat. The objective was to establish conditions in which the activity of pecthynmethilsterase is at its lowest: compare hot water immersion and microwave thermal treatments, and evaluate the enzyme's behavior for different treatment times.

Methodology

The entire sweet Granadilla with shell was cooled in icy water, for at least an hour. Then, the pulp was extracted, discarding seeds; only the pulp was subject to thermal treatments. Samples of 40g± 5g were stored in individual plastic bags. Each treatment was executed three-fold.

The traditional treatment was immersion in hot water at 85 °C during 5, 10, 30 or 45 s [3]. The microwave treatment involved the use of a 1200 watts and 2450 MHz oven for the same amount of time [4]. After the treatment, the samples were cooled in icy water, for half an hour. The pectinmethylsterase (PE) was extracted with saline 0.25 M with a pH lower than 8, the solution rested for an hour [1].

Equivalent milligrams of hydrolyzed ester per minute per gram of enzyme were measured by titration, following the technique described next. We added 2 mL of NaCl 1.5M to 10 mL of pectin solution; then added a few drops of Hinton indicator and titrate at a pH of 7.5 with NaOH 0.02 N. The resulting solution was placed in a water bath at 30 °C. When the solution reached that temperature, the previously extracted enzyme was added along with water to reach 20 mL. Slowly added the alkali to the solution until it reached a pH of 7.5. The time and volume of the 0.02 N alkali used to reach the pH were registered. It took almost 10 min and between 1 and 3 mL of NaOH to yield 7.5 pH, in average for all samples.

The results were expressed in units of pectin-methyl-esterase (PE U/g), comparable to equivalent milligrams of hydrolyzed ester per minute per gram of enzyme [5, 6].

$$PEU / g = \frac{VmL \text{ of } 0.02\text{N NaOH consumed} \times 3.1 \times 1\text{min}}{VmL \text{ enzymesolution} \times \text{Total titration time (min)}}$$

Results and discussion

The lowest pectinmethylsterase activity was achieved at the 30s of hot water immersion treatment. However, less and more time with this traditional treatment yielded higher activity of the enzyme. At 45s of hot water treatment, the enzyme's activity increased greatly, as shown in Figure 1.

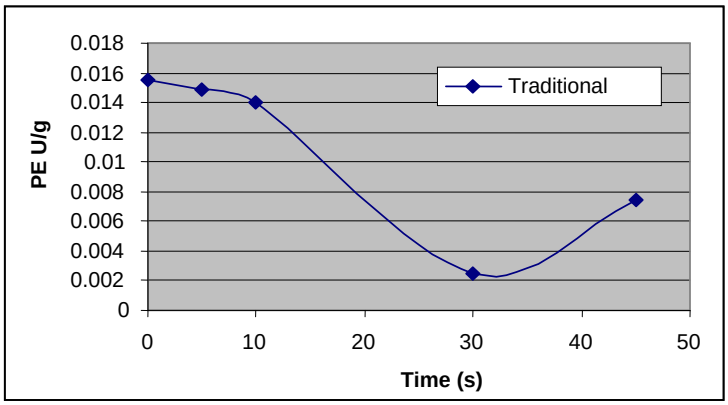

Figure 1. Traditional Treatment for Sweet Granadilla

These results showed improvement compared with the enzyme activity at room temperature without treatment. The inhibition was as high as 84% with 30 s in hot water treatment; and 52% better at 45 s with that same treatment although it was the treatment's worst result.

In terms of the microwave thermal treatment, the results were not as good. Unfortunately, the treatment for all time intervals damaged the consistency of the samples. The high content of water in the sweet granadilla was consumed by the heat produced with microwaves.

Still, the pectinmethylsterase activity was steadily reduced thru time. The highest inhibition took place at 45 s of microwave treatment, as shown in Figure 2. Comparably, it was a 56% better compared with the PE activity at room temperature. At 50 s, the sample was destroyed.

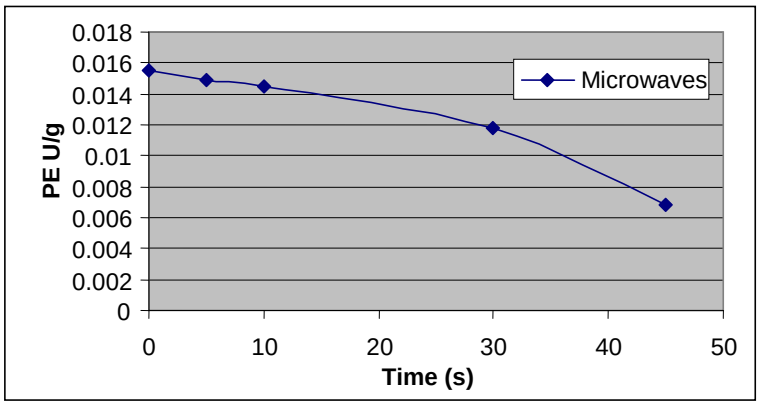

Figure 2. Microwave treatment for sweet granadilla

The highest inhibition was achieved at 30 s of hot water treatment. The microwave treatment is not recommended due to the fruit's high content of water and subsequent destruction.

Conclusions

Although the pectinmethylsterase activity steadily diminished with greater microwave treatment time, the sweet granadilla texture drastically changed. Besides, the highest inhibition of PE was obtained with hot water treatment. Both treatments showed better results for the enzyme compared with fruit without any treatment.

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Carica Papaya Biocatalysts: An Alternative For (*R,S)*-Ketorolac Esters Synthesis.

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Abstract

In the pharmaceutical industry, the lipophilization allows to change the hydrophilic/lypophilic balance of certain molecules, as the Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), facilitating its vectorization to different target organs. A typical example is the esterification of the ketorolac acid function, which allows the cutaneous application of the drug, avoiding gastrointestinal lesions caused by the oral administration. Such reaction might be carried out by *Carica papaya* auto-immobilized biocatalysts with esterase/lipase activity, obtained from latex and petioles, by a simple washing treatment. The obtained biocatalysts: Lyophilized Latex (LL), Treated Lyophilized Latex (TLL), Treated Lyophilized Petioles (TLP) and Bagasse from Petioles (BP), were used for the (*R,S)*-butyl ketorolac (BK) synthesis in different reaction solvents at 50°C whose conversion of the reaction was determined by HPLC. Higher BK conversions were reached with the latex biocatalysts (26-86% conversion after 48h) in comparison to the petiole ones (14-24% conversion after 96h), using isooctane as reaction solvent. Afterwards, (*R,S)*-octyl ketorolac (OK) synthesis was assayed at the best reaction conditions and solely LL (60%) and BP (22%) were able to perform the esterification reaction. Interestingly, LL synthesized preferentially short alkyl chains esters (BK) over the long ones (OK), meanwhile BP synthesized both esters at similar rates. This fact suggests that *Carica papaya* biocatalysts may have different esterase/lipase activities with different selectivities. This work demonstrates that the CP biocatalysts are a potential alternative for supersede the chemical catalysts employed currently in the (*R,S)*-ketorolac esters synthesis.

Keywords: *Carica papaya*, biocatalyst, esterase/lipase, esters, ketorolac.

Introduction

Carica papaya is a tropical plant from de *Caricaceae* family, its unripe fruits are rich in latex-producing cells called laticifers. This cells are composed of a water miscible and a non-water miscible phase, the first contains a wide rank of hydrolytic enzymes (*e.g*. proteases, chitinases, etc.) and several hydrophilic compounds (*e.g*. sugars, minerals, etc.), while the second one contains highly lipophilic enzymes (mainly esterases and lipases) which are strongly attached to a polyisoprenoid matrix [1, 2]. In particular, the hydrolytic activity of *Carica papaya* latex on medium-long chains triglycerides, as well as its properties as an auto-immobilized biocatalyst (*sn*-1,3 selectivity, enantioselectivity, among others) in organic synthesis, were formerly attributed to the lipase activity (commonly named as *Carica papaya* Lipase; CPL) [1, 3-5]. Nowadays, it is well known that those properties are due to at least one esterase and one lipase [1, 6]. On the other hand, prior studies showed that some hydrolytic activities are distributed at different proportions in the plant; for example the protease activity was found mostly in leaves, petioles and stem [7], while the lipase activity has been reported in seeds, fruit peels, meristems, leaves and petioles [8, 9]. These results strongly suggest that the esterase/lipase activity may also be an alternative source of new biocatalysts for the synthesis of esters of pharmaceutical interest.

In the pharmaceutical industry, the lipophilization of certain molecules such as anti-inflammatory drugs (NSAIDs) is very useful. It is documented that changing the hydrophilic/lipophilic balance of NSAIDs may help to vectorize the drug into the desired organ. A typical example is the esterification and amidation of the acid function of NSAIDs such as ketorolac, which allows the transcutaneous application of the drug avoiding the typical gastrointestinal damage caused by this kind of drugs when they are orally administered [10]. The synthesis of NSAID esters is generally accomplished by chemical catalysis under severe reaction conditions (*i.e.* high temperatures and pressures, use of strong acids or bases). Such reactions may be catalyzed by esterases or lipases in milder reaction conditions, thereby replacing the conventional chemical catalysts that may be toxic and unfriendly to the environment. Under the premise that *Carica papaya* latex has been used successfully for hydrolysis and synthesis of some NSAIDs esters with similar structure, such as naproxen [4, 11, 12], this work focused in the obtainment of new biocatalysts with esterase/lipase activity from different residues of *Carica papaya*, useful for the synthesis of ketorolac esters.

Methods and materials

1. Obtainment of Carica papaya biocatalysts.

Latex was collected by making four longitudinal incisions along the unripe fruits and lyophilized to obtain the "Lyophilized Latex" (LL) biocatalyst. The "Treated Lyophilized Latex" (TLL) biocatalyst was obtained after washing raw latex with distilled water at 4°C in a relation 1:7 (v/v), shaken vigorously, centrifuged at 8000 RPM at 4°C for 20 min and finally lyophilized. For the case of petioles, they were separated from *C. papaya* plants and milled with an industrial disks mill (EXMEX) to obtain a liquid extract and humid bagasse. The liquid extract from petioles was treated as TLL to obtain the "Treated Lyophilized Petioles" (TLP) biocatalyst. The remaining humid bagasse was dried in trays at room temperature, milled and sieved (mesh #30) to obtain the "Bagasse from Petioles" (BP) biocatalyst. The washing treatment was repeated until no significant protease activity was detected in the supernatant. All latex biocatalysts and TLP were delipidated with an anhydrous mix of *t*-butanol:hexane 50:50 (v/v) at 4°C in a relation 1:150 (w/v).

2. Determination of esterase/lipase activity by pH-stat method

The esterase/lipase activity was determined by titrating during enzymatic hydrolysis the released fatty acids from tributyrin (TC4) and trioctanoin (TC8) emulsions with NaOH 0.1N. Assays were performed in a pH-stat GP Titrino (Metrohm®) at a constant pH value of 7.0 at 25°C, using a Tris–HCl 2.5mM and NaCl 150mM buffer. The spontaneous hydrolysis rate was taken into account for all substrates and biocatalyst used in the study. The activity was expressed in international units (U) by milligram of biocatalyst. One unit corresponds to 1µmol of fatty acid released by minute at the assay conditions.

3. Chemical synthesis and purification of the ketorolac standards.

The acid ketorolac was obtained after precipitation of ketorolac tromethamine (55mM) with HCl 1N. Butyl ketorolac (BK) and octyl ketorolac (OK) standards were synthesized by chemical means as follows: 10mM acid ketorolac were mixed with 1.1 equivalent of 1,3-dicyclohexylcarbodiimide (22.6 mg, DCC) and 40mM of *n*-butanol or octanol in anhydrous dichloromethane. 4-dimethylaminopyridine (DMAP) at 10% mol was used as catalyst. The reaction was maintained at constant magnetic stirring at 25°C for 24h. At the end of the reaction, the mixture of reaction was filtered using a 0.45µm nylon filter, washed with brine and finally with distilled water. The organic phase was concentrated, and the ester purified by column chromatography using hexane:ethyl acetate (90:10, v/v) as mobile phase.

4. Synthesis of ketorolac esters by Carica papaya biocatalysts.

Acid ketorolac (10mM) and alcohol (40mM) were placed in 1.5mL vials in the presence of 25mg of biocatalyst (only for BP 37.5 mg/mL) at 50°C in a final reaction volume of 1mL (using different solvents). For BK the solvents used were: iso-octane, hexane, toluene, di-isopropyl ether, *t*-butanol and

dichloromethane. For OK only the best reaction solvent was used. *Candida antarctica* and *Candida rugosa* lipases were used as positive controls of the reaction. Reactants and products were extracted from each vial using ethyl acetate:heptane (1:3, v/v), then evaporated and resuspended in the (HPLC) mobile phase for further analysis. Reactions were monitored each 12h. All assays were performed by triplicate.

5. Determination of synthesis conversion of ketorolac esters.
All samples were filtered through a 0.45µm nylon membrane prior to injection. 30µL of each sample were injected into a Chiralpak ® AD-H (250 x 4.6mm) column from Chiral Technologies Europe (Strasbourg, France). A HPLC (UV detector VARIAN brand, operating system Galaxie ®) was used for the analysis, which was carried at 25°C and at a flow rate of 1mL/min. The mobile phase was hexane:2-propanol: trifluoroacetic acid (90:10:0.1, v/v/v). The detection of ketorolac and their esters was performed at 254nm. Conversions were estimated with their respective calibration curves (0.2-2.0mM).

Results and discussion

Esterase-lipase activity of Carica papaya biocatalysts.
In a former work, it was demonstrated that latex and petioles form *Carica papaya* contained the highest esterase-lipase activity [9], hence in this study we focused only on this residues to generate four biocatalysts: Lyophilized Latex (LL), Treated Lyophilized Latex (TLL), Treated Lyophilized Petioles (TLP) and Baggase from Petioles (BP). The esterase-lipase activity of the four biocatalyst using tributyrin (TC4) and trioctanoin (TC8) as substrates is shown in **Table 1**. In most cases, the activity on both substrates did not present significant differences in comparison to a previous report [9], only the activity using TLL biocatalyst was 4 to 5 fold lower.
Interestingly, TLL and TLP biocatalysts were enriched in TC4 hydrolytic activity and LL and BP in TC8 hydrolytic activity ; remarking that LL and BP showed 6 and 10 fold higher hydrolysis towards TC8 than TC4 when compared with TLL and TLP. This result may suggest that different hydrolytic enzymes are present in the obtained biocatalyst (probably different lipases and esterases).

Table 1. Esterase-lipase activity in Carica papaya biocatalysts.

Biocatalysts	***TC4 activity***	***TC8 activity***	***TC8/TC4^{x100}***
LL	*4112*	*330*	***8***
TLL	*12130*	*196*	*1.6*
TLP	*1177*	*20*	*1.7*
BP	*43*	*8.5*	***20***

Activity is expressed in U/g. TC4: Tributyrin; TC8: Trioctanoin; TC8/TC4: Relation between TC4/TC8 activity by 100.

Ketorolac esters synthesis with Carica papaya biocatalysts.
Butyl ketorolac (BK) synthesis was realized in different organic solvents and the maximum conversions reached are summarized in **Table 2**. The most of the biocatalysts reached the maximum conversions when iso-octane was used as reaction solvent, similar to other reports for the synthesis of NSAIDs esters [13]. Only TLL and *Candida rugosa* lipase (CRL) reached the highest conversion in hexane (33 and 93%, respectively). In the case of *Carica papaya* biocatalysts, the highest conversion was reached with LL (86%) at 48h, this result is promissory because is only 7% smaller than the conversion obtained with *Candida antarctica* lipase B (CALB). Besides, the four biocatalysts were able to perform the synthesis in at least one hydrophilic solvent like di-isopropyl ether, dichloromethane and *t*-butanol, where many enzymatic reactions are complex [12]. It is worth noting that this important treat (activity in hydrophilic solvents) represents a technological advantage that allows the implementation of a continuous synthesis reactor, because ketorolac acid is completely soluble for example in dichloromethane. On the other hand, the yield between PL and BP were similar (conversion difference not superior to 10%), this probably suggests that in both biocatalysts (PL and BP) the same hydrolase performed the synthesis reaction,

nevertheless, further studies are needed in order to confirm this hypothesis. Under the studied conditions, *Carica papaya* biocatalysts yields were similar or higher than those reported for ibuprofen and ketoprofen esters synthesis using microbial lipases (15 and 36% conversion) [13].

Finally, the (*R*, *S*) octyl ketorolac (OK) synthesis was assayed with all the *Carica papaya* biocatalysts in iso-octane. Surprisingly, only LL (60%, 48h) and BP (22%, 96h) were able to perform the OK synthesis. It is worth noting that these biocatalysts showed a significantly major TC8 hydrolytic activity proportion (**Table 1**). Therefore, these results could suggest that *Carica papaya* biocatalysts may have different esterase/lipase activities with different selectivities. Hence, in this work we demonstrated that *Carica papaya* biocatalysts are a very interesting and economical alternative for the implementation of clean processes for ketorolac esters synthesis.

Table 2. *Maximal conversion reached for the enzymatic synthesis of (R, S) butyl ketorolac.*

	Conversion (Mole %)					
Solvent	***LL(48h)***	***TLL(12h)***	***TLP(72h)***	***BP(96h)***	***CALB(4h)***	***CRL(24h)***
Iso-octane	**85.7**	26.3	**14.1**	**23.9**	**93**	50
Hexane	47.7	**32.8**	11.0	19.2	83	**93**
Toluene	58.2	**32.9**	7.1	5.9	81	48
Di-isopropyl ether	32.8	23.3	6.3	---	66	---
Dichloromethane	4.5	7.8	---	4.2	52	---
t- butanol	5.9	7.8	---	---	89	---

---: Conversion was not detected

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Dietary Fiber- Invertase Supplement For Sucrose Intolerance Regulation

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Introduction

Sucrose or table sugar is obtained from sugar cane (1). It is a disaccharide formed from glucose and fructose, in order to be adsorbed in the small intestine, is needed sucrose hydrolysis. Sucrase is the name given to enzymes that catalyze sucrose hydrolysis; among these enzymes are sucrase α-glucosidase, sucrase-isomaltase and β-fructofuranosidase (invertase). These enzymes are located in the brush border small intestine. When these enzymes are not secreted sucrose intolerance is produced (2). Sucrase deficiency produces an increase of disaccharides in intestine, causing diarrhea, nausea, abdominal distention, flatulence, malabsorption syndromes, malnutrition, and also can cause infantile hypercalcemia (1, 3). Medical recommendations are commonly related to eliminate sucrose intake of the diet (1) or oral administration of these enzymes. Invertase, as most of the enzymes, is a fragile molecule sensible to changes in ionic strength, temperature and pH (4). Considering pH variations on digestive system, invertase stability is needed, which can be achieved through immobilization technology (5). Many investigations have been focused on the support for invertase immobilization; among them are PVA, polyacrilamide, chitosan and cellulose (6). In general, immobilization matrices such as cellulose improve stability of the bound enzyme (5). In the present study prickly pear peel fiber, which is an agricultural waste rich in cellulose (7), and *Plantago* fiber used normally as a dietary fiber supplement (8), were chosen as a carrier of invertase in order to develop an alimentary supplement for sucrose intolerance regulation. Properties of enzyme immobilized by adsorption and covalent procedures were compared, among them knetic constants, number of enzyme recycle, thermal and storage stability, and influence of pH on enzyme activity.

Materials and Methods

Plantago psyllium and prickly pear peel where used as supports for invertase immobilization. Commercial invertase (E 3.2.1.26, Sigma Co.) was applied. For covalent immobilization, 0.01 g of both fibers (with particle size of 300 µm were oxidized with 0.15 M periodate at 30°C under agitation for 1 h in dark conditions (9). Treated fibers were washed 5 times with 2 ml of water and then incubated at 4°C with 2 ml of invertase solution prepared in acetate buffer, pH 4.5, at 20 µg ml^{-1} during different incubation times (0, 0.6, 1, 2, and 3 h). After incubation, the fibers were washed 2 times with 22 ml of Tris-HCl buffer (50 mM, pH 7). These washing wastes and residual enzyme solution were collected to be used for protein detection. Enzyme preparations were stored in acetate buffer (50 mM, pH 4.5) at 4°C prior to use. For adsorption immobilization, oxidation step was not carried out. The amount of immobilized protein was estimated as the difference between the amount of protein applied to the support and the amount of protein recovered in the supernatants and washings. The content of protein in solution was determined by Bradford method (10) using BSA as standard.

Invertase activity was measured by dinitrosalicylic acid (DNS) method applied to determine the amount of reducing sugars produced in sucrose hydrolysis (11). One international unit (IU) of activity was defined as the amount of enzyme required to hydrolyze 1 µmol of sucrose per min at 37°C and pH 4.5. Free and immobilized invertase activity evaluation was performed at different pH (1-8) and sucrose concentration (0.05-0.35 M), after their pre-treatment at various temperatures (37-70°C), after repeated use of the same

preparation, as well as during their storage at 4 °C. The highest value of enzyme activity in each assay was considered as 100%.

Results and discussions

Invertase immobilization. Results of monitoring of protein immobilized by different techniques on both carriers are presented in the Figure 1. The higher immobilization percentage at 88% on *Plantago* fiber and 68% on prickly pear fiber was detected at 1 h and 2 h, respectively, in the case of adsorption process. This can be attributed to the capacity of fiber to retain molecules due free hydroxyl groups. As time passes, immobilization percentages decreased due to enzyme desorption (12). Mahmoud (13) reported invertase immobilization yields around 80% using materials with high cellulose content. Covalently immobilized invertase achieved the 85 % of bound protein at 3 h and 1 h for *Plantago* and prickly pear peel fiber, respectively (Fig 1).

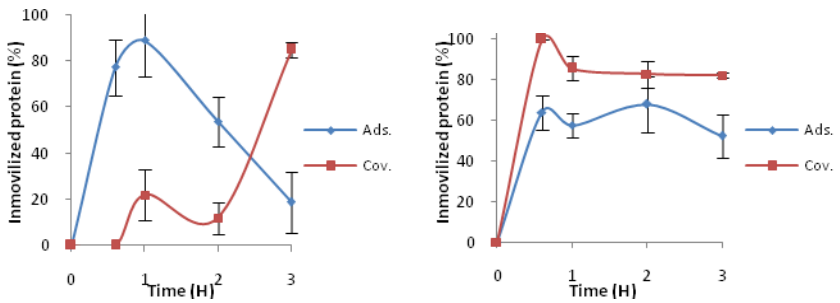

Fig. 1. Immobilization invertase kinetics on *Plantago* (left) and prickly pear peel (right) fibers through adsorption (Ads) and covalent (Cov) binding.

In *Plantago*, higher enzymatic activities (3.88 IU/(g of support) and 4.1 IU/(g of support)) were detected at 3 h and 1 h, respectively for covalent and adsorption immobilizations. These results are correlated with immobilization percentages (Fig 1). On the other hand, in prickly pear peel, higher enzymatic activities were detected at 1 h: 3.3 IU/(g of support) for adsorption immobilization and 12.4 IU/(g of support) in covalent immobilization.

Kinetic constants. To determine kinetic parameters (K_m and V_{max}) of enzymatic process, the effect of sucrose concentration of free and immobilized invertase activities were studied. Kinetic parameters were estimated from Lineweaver-Burk plots (Fig. 2). The apparent parameters K_m and V_{max} for free invertase were 1.002 M y 0.04 M min^{-1}, respectively; for invertase immobilized by adsorption on *Plantago* fiber, the kinetic constants were 0.93 M y 0.032 M min^{-1}, and 0.45 M y 0.017 M min^{-1} in prickly pear peel fiber, respectively. Decrease of K_m may be signified an increase of enzyme affinity to substrate in the case of immobilization on prickly pear peel fiber. Processes catalyzed by invertase immobilized covalently in *Plantago* fiber and in prickly pear peel fiber were characterized by kinetic parameters: 17.14 M and 0.41 M min^{-1}, as well as 1.99 M and 0.058 M min^{-1}, respectively. K_m values of covalently bound enzyme were higher than for free invertase: structural changes produced by the immobilization led to decrease of enzyme affinity to substrate (4).

Effect of pH on invertase activity. The pH effect on the activity of free and immobilized invertase preparations was studied in pH between 1- 8. In both supports, for invertase immobilization by adsorption, the pH range with greater activity was extended from 2 to 7 (Fig 3). Thus, the invertase immobilized by adsorption displayed a wider range of optimum pH than free and covalently immobilized forms. Mahmoud (13) presented similar results for invertase immobilized by adsorption on sawdust, extending

higher enzymatic activity in a range of 3.6 – 5.6. Covalent immobilization of invertase and invertase in free form showed greater activity at pH 4.5 and 5, respectively.

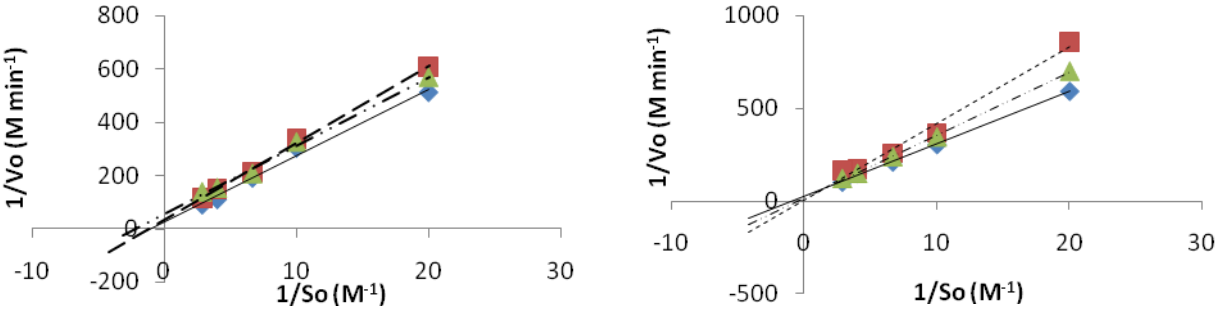

Fig. 2. Lineweaver – Burk plots for free enzyme (◊),invertase immobilized on *Plantago* (□) and on prickly pear peel (Δ) fibers. Left, - immobilization by adsorption; right,- covalent immobilization.

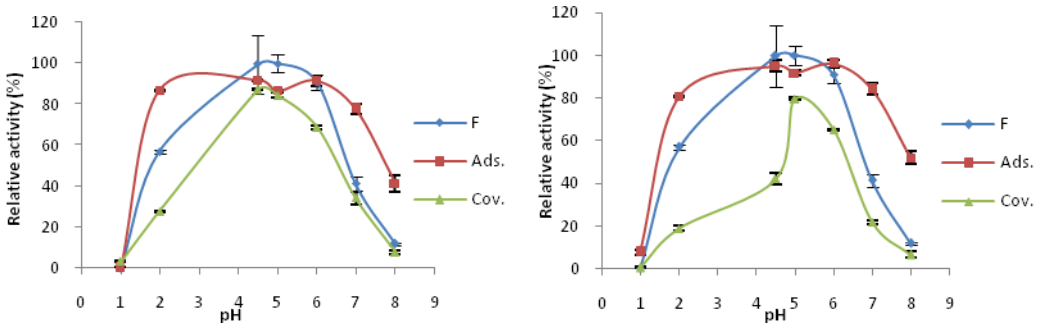

Fig. 3. Effect of pH on relative activity of free (F) and immobilized by adsorption (Ads) and covalently (Cov) on prickly pear peel (left) and *Plantago* (right) fibers.

Thermal stability of free and immobilized invertase. Thermal stability of free and immobilized invertase were determined by measuring the residual activity of the enzyme exposed to different temperatures (37, 50 and 70°C) in acetate buffer (50 mM, pH 4.5) for 180 min. The behavior of free and immobilized enzyme was similar in all studied cases (Fig. 4 and Fig. 5): invertase preserved its activity between 90 and 100 % at 37 and 50°C, whereas at 70°C, invertase lost their initial activity after 30 min. The obtained results indicated that invertase thermal stability is not affected during immobilization process.

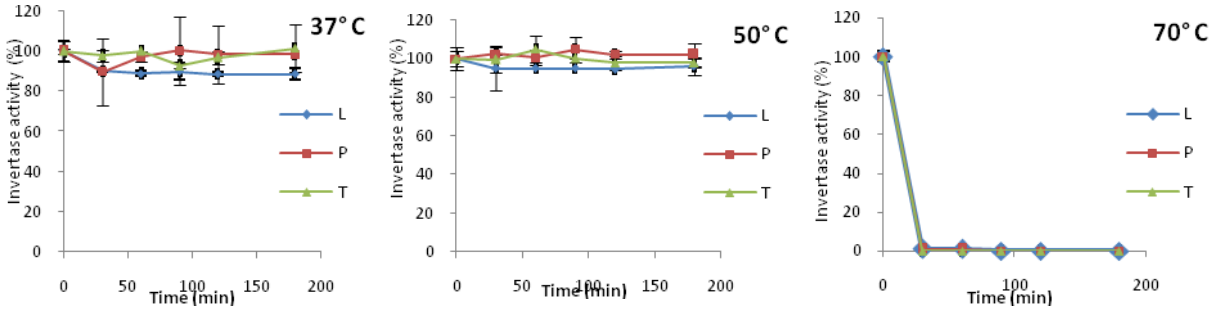

Fig. 4. Thermostability of free invertase (L), and invertase immobilized covalently in *Plantago* (P) and prickly pear peel (T) fibers.

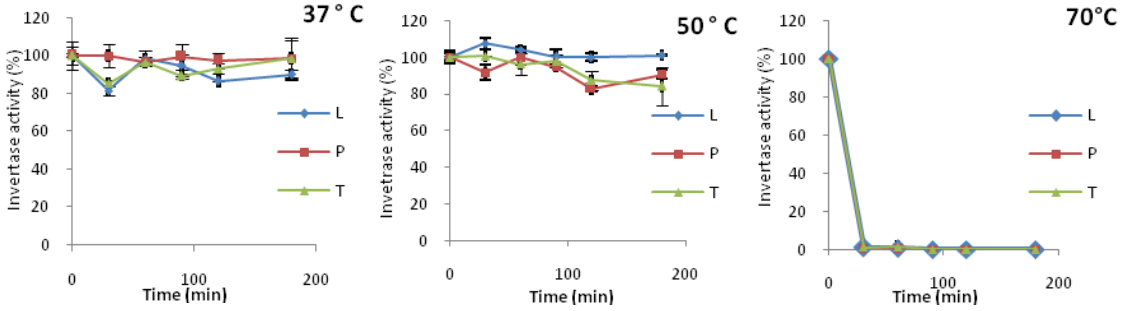

Fig. 5. Thermostability of free invertase (L), and invertase immobilized by adsorption in *Plantago* (P) and prickly pear peel (T) fibers.

Re-using of immobilized invertase. The activity of immobilized invertase was tested by studying of its ability to hydrolyze sucrose in 15 cycles of reuse (Fig 6). Enzyme immobilized by adsorption on prickly pear peel fiber lost its activity since the second cycle, whereas invertase immobilized on *Plantago* fiber decreased its activity to 5% in the 15th cycle. Covalently immobilized enzyme maintained 30% and 50% of its activity on prickly pear peel fiber and *Plantago* fiber, respectively, after 15 cycles of its application.

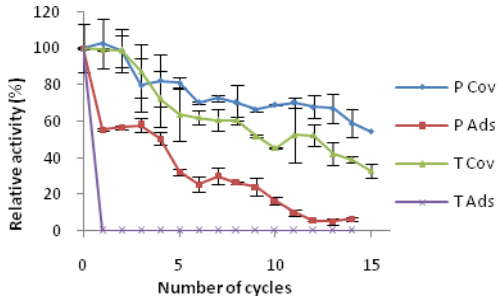

Fig 6. Activity of invertase immobilized in *Plantago* (P) and prickly pear peel (T) fibers by covalent binding (Cov) and adsorption (Ads) at various cycles of its application.

Storage stability. Most of the enzymes are not stable during storage in solution, therefore their activity reduced through the time. The storage stabilities of free enzyme and invertase immobilized on *Plantago* and prickly pear peel fiber are showed in Fig 7. Free and immobilized invertase preparations were kept in acetate buffer (50 mM, pH 4.5) at 4°C. Free enzyme lost 52% of activity within 30 days. On the other hand, invertase immobilized by adsorption lost its activity similarly to the free enzyme (Fig. 7, left). Covalently immobilized enzyme lost 6% during the same storage period (Fig. 7, right). Covalent immobilization increases storage stability of the enzyme probably due to fixing of its conformation (6).

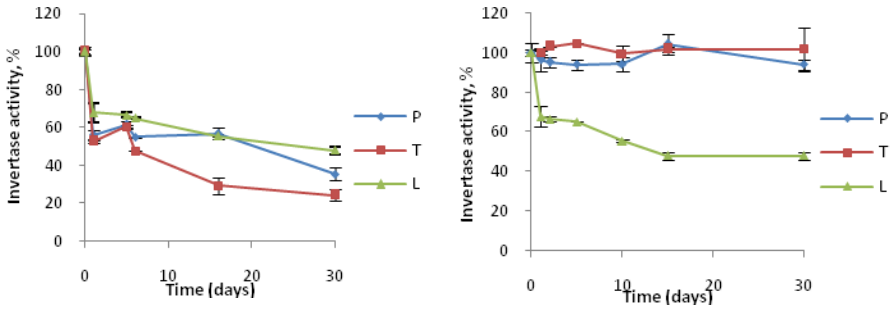

Figure 7. Storage stability of free (L) enzyme and invertase immobilized on *Plantago* (P) and prickly pear peel (T) fibers. Right, - covalent immobilization and left,- immobilization by adsorption.

Conclusion

The obtained results demonstrate various advantage of invertase immobilization on prickly pear peel and *Plantago* fibers, among them higher storage stability, activity in repeated cycles, in some case higher affinity to substrate, as well as wider range of optimum pH. Thus, studied natural fibers are good carriers for invertase immobilization that may be applied to design a sucrase deficiency treatment. Moreover, the use of prickly pear peel fiber as support is reported for the first time in the present study.

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Phytase Production By *Aspergillus niger* And *Aspergillus terreus* In Solid State Fermentation Using Wheat Bran

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Abstract

Phytases have been one of the focal enzymes for animal nutrition during the past two decades. In this work, the use of wheat bran as substrate for phytase production in Solid State Fermentation (SSF) was studied. In order to find the best fermentation conditions for phytase production using a laboratory strain indentified as *Aspergillus niger*, three different pretreatments (1: wheat bran without wash and autoclaved, 2: wheat bran washed and autoclaved, and 3: wheat bran without wash and UV exposed for 2h); and two different impregnation media (A (g/L): sucrose 5, $MnSO_4$ 0.1, $MgSO_4$ 0.5, $Fe_2(SO_4)_2$ 0.1, KCl 0.5, yeast extract 2; and B (g/L): glucose 5, starch 5, $MnSO_4$ 0.1, $MgSO_4$ 0.5, $Fe_2(SO_4)_2$ 0.1, KCl 0.5, $CaCl_2$ 0.5, NH_4NO_3 2) were tested. Fermentations (pH 6.5, 60% moisture at 30°C) were carried out for 12 days in 15 mL tubes containing 2.5 g of solid culture media (SCM). Subsequently, the best pretreatment and impregnation media were used to investigate the phytase production in column reactors containing 25 g of the formulated SCM, using two strains identified as *Aspergillus niger* and *Aspergillus terreus*. The fermentations (pH 6.5, 60% moisture at 30°C) were monitored for 8 days. The best fermentation conditions found using 15 mL tubes as reactor were: wheat bran without wash and UV exposed for 2h and culture media A; reaching a maximum phytase activity of 1.91±0.25 U/gdm (gram of dry matter) at 192h of fermentation. The other wheat bran pretreatments as well as culture media B do not present significant phytase activity. Maximum phytase activity reached using column reactors were: 4.2±0.71 U/gdm at 96h of culture for *Aspergillus niger* and 0.49±0.005 U/gdm at 96h for *Aspergillus terreus*. The use of 15 mL tubes fermentation system; show to be an easy and practical way to assay different fermentation conditions at the same time, obtaining rapid and reliable results. UV sterilization is an excellent process for using wheat bran as substrate for phytase production. Moreover the column reactors fermentation increased significantly the phytase productivity. The fermentation conditions obtained in this research can be used to find the best phytase producing strain for future experiments.

(Keywords: Phytase, Solid State Fermentation, Wheat Bran.)

Introduction

Phytic acid (myo-inositolhexaphosphate) is a polyanionic chelating factor that form complexes with divalent cations of great nutritional importance (Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+} y Mn^{2+}) [1-3], as well as protein and amino acids [4]. Phytic acid is an energy source and phosphorus store commonly found in cereals, legumes and oilseeds [5-7], these raw materials are used for animal feed production. Monogastric animals have a low content of phytate degrading enzymes [3-6], therefore their nutrition is altered due to the decrease of available nutrients in food.

Enzymes that hydrolyze phytic acid are called phytases, which are a type of hydrolases from the phosphatases family, which posses phosphomonoesterase activity [6], they are capable of hydrolyze phytic acid producing orthophosphate releasing myo-inositol and a minor concentration of partial hydrolyzed phosphate esters [1, 2, 4, 7-9]. Phytases can be classified in order to their regioselectivity in 3 (from microbial) and 6 (from plants) phytases, pH optima (alkaline or acid phytases) and catalytic mechanisms (histidine acid phosphatases, ß-propeller phytase, cysteine phosphatases or purple acid phosphatases) [10, 11].

The industrial demand for phytases with high specific activity and stability under high temperature conditions for feed pellet production and under acidic conditions in the stomach of monogastric animals continues to stimulate the search for new enzyme sources [12]. Phytases are found naturally in plants and microorganisms, particularly fungi. Most of scientific work has been focused on *Aspergillus* spp. phytases due to their biochemical properties found. *Aspergillus niger* PhyA was the first well characterized and commercialized phytase, with two optimal pH at 2.5 and 5.0–5.5, an optimal temperature at 55–60°C, and high affinity for phytic acid [13]. *Aspergillus fumigatus* phytase shares a 66% sequence similarity with *A. niger* PhyA phytase, but displays better thermotolerance [14].

Filamentous fungi require phosphorus to carry out oxidative phosphorylation, where the energy is obtained in NADH, $FADH_2$ and ATP form, which is used for metabolic regulation and signal transduction pathways [15]. Thus, the use of a culture media containing phytic acid as sole phosphorus source may induce phytase production [16].

An alternative for phytase production is solid state fermentation (SSF), it offers several advantages over submerged fermentation (SmF), as hiperglycosylated proteins, making them more stable, higher yields and productivities [16-18].

Wheat bran is one of the agroindustrial residues most used in phytase production by SSF, due to their high phytic acid concentration (1-2%) compared to other residues [16]. It is important to give an adequate pretreatment to the residue in order to be used for phytase production avoiding contamination and preventing phytic acid hydrolysis [17].

In the present study, Filamentous fungi (164) belonging to CIATEJ collection, isolated from different agroindustrial wastes and unconventional environments (mescal, cocoa fermentation, coffee pulp, etc.), were grown in a media using phytic acid as sole source of carbon and phosphorus in order to find phytase producing candidates. A screening method using 15 mL tubes fermentation system was performed to assay different fermentation conditions at the same time, obtaining rapid and reliable results. Three different pretreatments for wheat bran and two different impregnation media where studied. Phyatse production was studied by solid state fermentation using two laboratory strains *Aspergillus niger* and *Aspergillus terreus*.

Methods and materials

Microorganisms and Inoculum Preparation

Aspergillus niger and *Aspergillus terreus* selected from the CIATEJ collection, were identified by molecular techniques. These strains were cultivated in a medium using wheat bran as substrate, for 5 days at 30°C. Spores were collected with 50 mL of sterile distilled water containing 0.01% Tween-80.

Inductor Substrate-Support Preparation

Wheat bran was used as substrate-support for solid fermentation, with three different pretreatments: 1; wheat bran without wash and autoclaved, 2; wheat bran washed and autoclaved, and 3; wheat bran without wash and UV exposed for 2h, in order to study the pretreatment that favored phytase production.

Impregnation Media

Two different impregnation media were used; A (g/L): sucrose 5, $MnSO_4$ 0.1, $MgSO_4$ 0.5, $Fe_2(SO_4)_2$ 0.1, KCl 0.5, yeast extract 2; and B (g/L): glucose 5, starch 5, $MnSO_4$ 0.1, $MgSO_4$ 0.5, $Fe_2(SO_4)_2$ 0.1, KCl 0.5, $CaCl_2$ 0.5, NH_4NO_3 2), these media are commonly used for phytase production.

Solid State Fermentation

Fermentations were carried out with 2.5 g (15 ml tubes) and 12 g (column reactors) of wheat bran moistened with the corresponding impregnation media, cultures were incubated for 12 days at pH 6.5, 60% moisture and 30°C, an inoculum of $3X10^7$ spores/gdm was used.

Enzyme Extraction

Enzyme extraction was carried out adding 5 mL (for 15 mL tubes) or 50 mL (for column reactors) of distilled water containing 0.1% (v/v) Tween-80 at the fermented solids. The mixture was shaken at 200 rpm for 1 h at room temperature. Solid fermented was centrifuged at 5,000rpm for 20 min at 4°C. The supernatant was collected and used for enzyme assay.

Enzyme Assay

Phytase activity was assayed according to Harland and Harland. One international unit of phytase was defined as the amount of enzyme required for releasing 1 μmol of inorganic phosphorus per minute at 37°C and pH 5. Enzyme yield was expressed as U/gdm.

Results

Effect of wheat bran pretreatments and impregnation media on phytase production

Three different pretreatments for wheat bran and two impregnation media were tested in order to improve phytase production, it was observed that pretreatment 3 (wheat bran without wash and UV exposed for 2 h) and impregnation media A favored phytase production (Figure 1-A). In these conditions *Aspergillus niger*, known as a phytase producer, reached a maximum phytase activity of 1.91±0.25 U/gdm at 192 h of fermentation. The other wheat bran pretreatments as well as culture media B do not present significant phytase activity. Although these results were lower than the results found by Gunashree (2008) [2], reporting 66 U/gdm in SFF with a collection strain *Aspergillus niger* CFR335. This might be caused by different fermentation conditions, however, the study conducted in 15 mL tubes helped to find factors that favor phytase production in a shorter time.

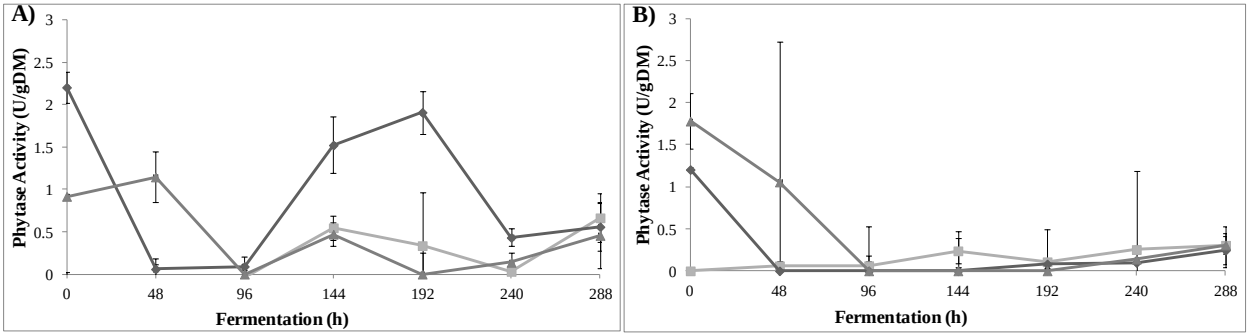

Figure 1. *Aspergillus niger* fermentation using the three different pretreatments: 1 ( ); wheat bran without wash and autoclaved, 2 ( ); wheat bran washed and autoclaved, and 3 ( ); wheat bran without wash and UV exposed for 2h; and the two impregnation media: A) medium A and B) medium B.

Phytase production in column Reactors

Solid state fermentation in column reactors is known to be a good fermentative system in order to perform studies at laboratory scale [18]. Maximum phytase activity reached using column reactors were: 4.2±0.71 U/gdm at 96 h of culture for *Aspergillus niger* and 0.49±0.005 U/gdm at 96 h for *Aspergillus terreus* (Figure 2). Compared to phytase activity obtained in 15 mL tubes, for *Aspergillus niger* activity was 2-fold higher and was reached in a shorter time.

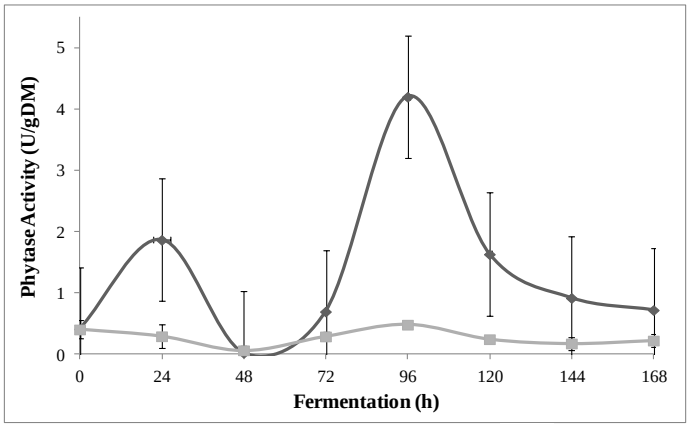

Figure 2. Phytse production in column reactors by *Aspergillus niger* ( ) and *Aspergillus terreus* ( ) using wheat bran as substrate-support and impregnation media A.

Conclusion

The use of 15 mL tubes fermentation system; show to be an easy and practical way to assay different fermentation conditions at the same time, obtaining rapid and reliable results. UV sterilization is an excellent process for using wheat bran as substrate for phytase production. Moreover the column reactors fermentation increased significantly the phytase productivity. The fermentation conditions obtained in this research can be used to find the best phytase producing strain for future experiments.

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Partial Purification And Recovery Of Vegetable Protease By Means Of Ultrafiltration

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Introduction

Enzyme use is growing-up in different areas (food, pharmaceutical, etc.), specifically, proteases are one of the most important groups, having around of 25 % of total sales in enzyme business. In 1998 proteases represented 60% of world market with near of 1.5 billion dollars (Briones y Cortés, 2008). Beef meat and fish processing are one of the most important areas of use for proteases, being papain and bromelain the most useful in this field. *Karatasina* is a proteolytic enzyme contained in *Bromelia karatas* juice, and was discovered, described and studied for the first time ever by enzymology group of the Biotic Products Center (CEPROBI-IPN). This protease is an enzymatic complex formed by 8 isozymes with proteolytic activity, and with isoelectric points in the ranging from 3.4 to 10.4 (Briones *et al*., 2001). *Karatasina* recovery from *B. karatas* juice has been reported. In those works used separation strategies like: ammonium sulfate precipitation, two-phase extraction, chromatography, etc., being a common factor the low values of purification factor achieved (Zuñiga, 2010).

Bruno et al (2003), obtained *hieronimy I*, protease extracted from fruits of *Bromelia hieronymi.* Purification consisted of fractionating with acetone and cation exchange chromatography on CM-sepharose. The homogeneity of the enzyme was confirmed by isoelectric focusing and SDS-PAGE, resulting in a pI > 9.3 and molecular weight of 24 kDa, with a proteolytic activity of 90 % over that obtained initially before purify. Bruno et al (2003), using a similar strategy, obtained a different protease (MW = 23.4kDa) from the juice of *B. hieronymi.* They used anion exchange chromatography on Q-sepharose, followed by cation exchange chromatography on SP-sepharose but with anion exchange chromatography on SP-sepharose, they called *hieronimy II*.

Zuñiga (2010) apply the ammonium sulfate precipitation at different concentrations in combination with biphasic aqueous extraction (PEG-ammonium sulfate at different concentrations ratios) to purify *karatasina* from *B. katarasa* juice, obtaining a purification factor of 2.54 for better separation conditions.

Materials and methods

Experiment design

Experiment design was a factorial 3^2, two factors (pH and Ionic strength) and three levels for each factor, being a total of 9 combinations and a total of 18 experiments with repetitions.

Extract conditioning

Extracted juice from B. hemisphaerica was diluted with deionized water (ratio 1:6). Aliquots (100 mL) were taken and adjusted to proposed conditions pH (3.5, 5.5 and 9) and ionic strength (without NaCl, 200 and 50 mM of NaCl).

Total protein quantification

Total protein was determined by Bradford method. 0.5 mL of sample were taken and placed in a test tube with 1.5 mL of Bradford reagent. Mixture was stirred during one minute and allowed to incubate at room temperature for 15 minutes. Absorbances reading were performed at a 595 nm and data were correlated with a BSA standard curve (concentration between 0.1 to 1.0 mg-mL^{-1}).

Proteolytic activity quantification

Protease production was quantified by assessing proteolytic activity of the enzyme using Kunitz-Lowry method and a mathematical model that related optical density as a function of tyrosine concentration. Mathematical model used was: $Y = 0.009X - 0.026$ ($R^2 = 0.995$).

Ultrafiltration Experiments

Ultrafiltration test were conducted in a dead end cell (Amicon 8200) with volume of 180 mL. Regenerated cellulose membrane (31.67 cm^2) was used. 70 mL of juice conditioned to the pH and ionic strength evaluated were placed into the cell and ultrafiltration was performed at a transmembrane pressure of 12 psi for a time of 3 hours. After ultrafiltration, volumes of retentate and permeate were measured and taken a sample of 5 mL of each one to quantify selectivity (**Ψ**) and purification factor (**P**).

Permeate flux determination

As show in Figure 1, flux measurements were made using an analytical balance with SW32 data acquisition board and Win Wedge 32 program. This program export weight data to an Excel spreadsheet where data are converted to permeate flux. Experiments were carried out without stirring, at 25 °C and transmembrane pressure of 12 psi applied with compressed nitrogen.

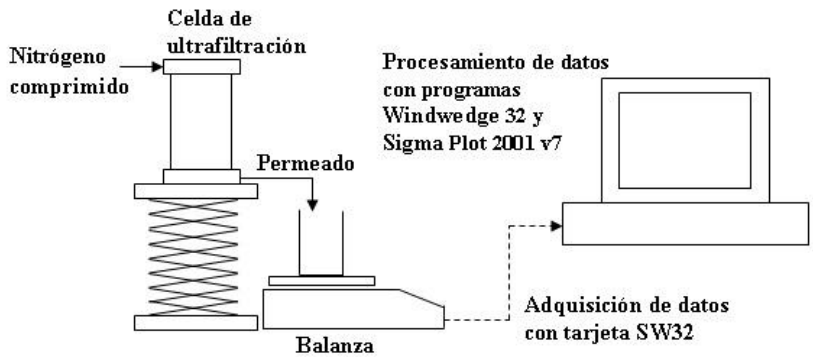

Figure 1.- Dead end ultrafiltration system

Membrane characterization

In order to evaluate losses of flux permeate due to clogging, as well as if membrane is able to recover its original flux to reuse it, was necessary characterizer the membranes. Once armed ultrafiltration system, cell was filled with deionized water and flux permeate was evaluated at 8 psi of pressure membrane during 5 minutes. Water flux permeate measurements were made at 12, 16 and 20 psi.

Loss permeate flux determination

Once ultrafiltration finalize, membrane was removed from cell and putted into a crystallizing dish with 50 mL of deionized water and stirred during 10 minutes. After this the membrane was drained, placed back in the ultrafiltration cell and filled with deionized water. Flux permeates was measured at membrane pressures of: 8, 12, 16 and 20 psi and compared with those obtained in the membrane characterization.

Membrane enzymatic washes

After determine loss permeate flux, membrane was placed in crystallizer dish with 400 mL of 10 % of Tergazyme (enzymatic soap) and stirred with heating (45 °C) during 10 minutes. After this membrane was placed back into ultrafiltration cell and passed the 400 mL of the enzymatic soap at 8 psi of membrane pressure, repeating this step three times (always at 43 °C ± 2).Finally passed 500 mL of deionized water at 8 psi to remove residues of enzyme solution.

Data analyses

Ultrafiltration process analysis can be assessed using parameters such as selectivity and purification factor (Van Reis and Zydney, 2007). Selectivity (Ψ) is a dimensionless value defined by:

$$\Psi = \frac{S_1}{S_2} \qquad \text{and} \qquad S_X = \left(\frac{C_f}{C_S}\right)$$

where S_1 and S_2 are separation coefficients observed for contaminant proteins and hemispherycin respectively, and C_S and C_f are specific concentration in filtrate and feed respectively. Purification factor (P) for hemispherycin in retentate is defined as:

$$P = \frac{\frac{(VC_2)_F}{(VC_2)_i}}{\frac{(VC_1)_F}{(VC_1)_i}}$$

where V is work volume, C_1 and C_2 are concentration of contaminant proteins and hemispherycin respectively, and susxcripts I and F correspond to beginning and ending respectively.

Results and discussion

Selectivity (Ψ), purification factor (P) and permeate flux at steady state (J_{SS}) obtained at evaluated conditions. In these we can see that under the conditions tested, there is no significant effect of pH and ionic strength on the Ψ values, that is, separation capacity according to the proteolytic activity remains constant. This could be because hemispherycin is an enzymatic complex, composed of five proteolytic isoenzymes, whose isoelectric points are within a range from 3 to 9, being evaluated conditions favorable to any of the isoenzymes, keeping the proteolytic capacity ratio between the retentate and the permeate with the amount of contaminant solutes (mainly sugars and other proteins).

Otherwise, it can be observed a significant effect from pH and ionic strength over purification factor (P) and the steady-state flux (J_{SS}). With respect to P values, presence of NaCl generally has a negative effect, obtaining the best values of P in absence of NaCl for all pH values tested. On the other hand, increase of ionic strength generally affects positively the J_{SS} values.

Table 1.- Selectivity values (Ψ) Purification factor (P) and permeate flux (J_{SS}) obtained in the ultrafiltration of the extract of *B. hemisphaerica* to partially purify the hemispherycin at different conditions of pH and ionic strength.

pH		Ionic strength [mM NaCl]		
		0	50	200
3.5	Ψ[--]	0.981	0.998	1.001
	P[times]	1.560	1.024	0.948
	Jss[L-m^{-2}-hr^{-1}]	3.123	5.131	5.482
5.45	Ψ[--]	0.989	0.994	1.000
	P[times]	1.356	1.227	0.975
	Jss[L-m^{-2}-hr^{-1}]	5.002	4.194	6.779
9	Ψ[--]	0.998	1.003	0.998
	P[times]	1.092	0.851	1.140
	Jss[L-m^{-2}-hr^{-1}]	6.318	6.563	6.866

Table 2 shows results of process resistances: Mechanics (R_M), Total (R_T) of polarization layer (R_P) and adsorbed protein (R_A). As can be observe, amount of adsorbed protein is minimal because R_A and R_M values have a same magnitude order, indicating a very low affinity between membrane and proteins, which brings benefits to the process, because protein lost is minimum. In general there is no significant effect of pH and ionic strength in behavior of the resistances.

Table 2. - Values of process resistance: Mechanical (R_M), Total (R_T), polarization (R_P) and adsorption (R_A) obtained in the ultrafiltration of the extract of *B. hemisphaerica* to partially purify of hemispherycin at different conditions of pH and ionic strength.

		Ionic Strength [mM NaCl]		
pH		0	50	200
3.5	R_M	1.35E+08	2.41E+08	2.41E+08
	R_T	2.78E+10	3.20E+10	1.03E+11
	R_A	1.47E+08	2.48E+08	2.60E+08
	R_P	2.75E+10	3.15E+10	1.03E+11
5.45	R_M	2.81E+08	2.41E+08	2.41E+08
	R_T	5.30E+10	2.33E+10	3.12E+10
	R_A	3.46E+08	2.64E+08	2.72E+08
	R_P	5.24E+10	2.28E+10	3.07E+10
9	R_M	2.81E+08	2.41E+08	2.41E+08
	R_T	1.71E+10	1.60E+10	2.17E+10
	R_A	3.01E+08	2.49E+08	2.60E+08
	R_P	1.65E+10	1.55E+10	2.12E+10

Conclusions

Changes in pH and ionic strength within the range evaluated do not have a significant effect on selectivity and resistances calculated, however, it has a significant effect on the purification factor. Otherwise, purification factor values increase with decreasing concentration of NaCl, while the permeate flux increases with increasing concentration of NaCl.

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Phytic Acid Quantification In Agroindustrial Residues And Their Potential As Inducers For Phytase Production In Solid State Fermentation

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Abstract

Phytases have been one of the focal enzymes for animal nutrition and human health during the past two decades. In order to study typical agroindustrial residues used in animal feed as potential inducers for phytase production in Solid State Fermentation (SSF), a methodology to quantify phytic acid in solids was implemented. The agroindustrial residues used for this study were: agave bagasse, sugarcane bagasse, coffe husk, corn husk, cottonseed meal, wheat straw and wheat bran. Bartlett (1959) technique for phytic acid extraction and Haug-Lantzsch (1983) for phytic acid quantification were implemented. Three different acids were assayed for phytic acid extraction (3% HCl, 5% H_2SO_4 and 10% trichloroacetic acid) using 1:20 (w/v) ratio for 1 h at 30°C. The acid showing the best extraction results was used for further studies to improve the phytic acid extraction conditions. Three acid concentrations (0.2M, 0.6M and 1M); temperatures (25°C, 50°C, and 100°C) and weigh/volume ratios (1:5, 1:10 and 1:20) were tested. Solid state fermentations were carried out for 8 days, pH 6.5, 60% moisture and 30°C, using polyurethane as inert support and a laboratory strain indentified as *Aspergillus niger*. Only, three agroindustrial residues (corn husk, cottonseed meal and wheat bran) showed a considerable phytic acid content (approximately 0.5%) using 3% HCl for extraction. The best conditions for extracting phytic acid were: 0.2M of HCl, 25°C and 1:20 ratio for the three agroindustrial residues, finding 0.57 %, 0.45 % and 0.67 % of phytic acid for corn husk, cottonseed meal and wheat bran, respectively. The three extracts obtained were assayed as inducers for phytase production in SSF. The maximum phytase activity reached was 0.39±0.09 U/gDM (grams of dry matter) at 24 h of fermentation using a wheat bran extract as inducer, followed by corn husk extract with 0.35±0.01 U/gDM at 24 h of fermentation; cottonseed meal extract used as SSF inducer, does not present significant phytase activity. It was possible to implement a quantification methodology for phytic acid in agroindustrial residues and the extracts obtained proved their potential as inducers in phytase production.

(Keywords: Phytic Acid, Agroindustrial Residues, Phytase)

Introduction

Phytic acid (PA) (myo-inositol hexa-phosphoric acid, IP6) is the most abundant phosphorus storage form in seeds, legumes and cereals, it may account for more than 60-90% of the total phosphorus (P) [1]. In these plants, phytate is approximately between 1% and 2% of the total plant weight. In terms of location, phytic acid in legumes and seeds is uniformly distributed in the cotyledon and associated to protein structures [2]. In cereals is found predominantly in the external aleurone layer.

Phytate chelates mineral cations as well as proteins and amino acids, is therefore considered to be an antinutritional factor [3]. Animal food is elaborated using cereals, seeds and legumes as row material, therefore monogastric or single stomach species cannot utilize P in phytic acid because they do not possess sufficient levels of phytic acid-degrading enzymes in their digestive tract. Traditionally, the limited bioavailability of plant phosphate was compensated by adding inorganic feed phosphates in animal

rations. This led to increasing phosphate contamination known as eutrophication. To solve this problem phytic acid-degrading enzymes are added, known as phytases.
Phytases are hydrolases from phosphatases family with phosphomonoesterase activity [4], capable of hydrolyze phytic acid producing orthophosphate, partial hydrolyzed phosphate esters and releasing myo-inositol [5-10]. Phytases can be classified in order to their regioselectivity in 3 (from microbial) and 6 (from plants) phytases [10]. Microbial phytases are used for commercial applications, obtained generally from fungus due to their biochemical properties. Fungal phytases can be produced by solid state fermentation (SSF) due to its potential compared to submerged fermentation (SmF), obtaining less contamination, cheaper process, using agroindustrial residues as substrate-support [11].
Researches has been focused in phytase production from agroindustrial residues rich in phytic acid, but phytic acid extracts from row material have not been used as inducers.
Analytical determination of phytate levels in feed raw materials is considered "primitive", since most methods for photometrical determination of phytate are based on indirect measurements (inositol phosphate) or establishment of stoichiometric ratio between phytate and some cations easy to determine with a direct tool. Several methods have been published for phytate quantification. Most of them (Bartlett, 1959) with acidic extraction carried out with HCl, H_2SO_4, or trichloroacetic acid with different concentrations and extraction times, followed by precipitation with Fe^{3+} [12]. Unprecipitated ferric ions are determined by spectrophotometry. The difference between initial and remaining ferric ion concentration is then used to calculate phytate concentration. Acid extraction is preferable, because raw materials such as wheat might contain high levels of endogenous phytase, which could degrade phytate if allowed. A well-known photometrical phytate method is the protocol developed by Haug and Lantzsch that uses 2,2′-bipyridine as a complexing chromogenic agent to quantify ferric ions [13].
In the present study phytic acid extracts were obtained from agroindustrial residues: agave bagasse (AB), sugarcane bagasse (SB), coffe husk (CFH), corn husk (CH), cottonseed meal (CM), wheat straw (WS) and wheat bran (WB). The Haug-Lantzsch modified method was used for quantification. Subsequently phytic acid extracts were tested as inducers for phytase production by solid state fermentation.

Methods and materials

Phytic acid extraction
Agave bagasse, sugarcane bagasse, coffe husk, corn husk, cottonseed meal, wheat straw and wheat bran, were ground and sieved on 40 net. Phytic acid extraction was performed according to Bartlett (1959) [12]: 20 mL of diluted acid (3% HCl, 5% H_2SO_4 and 10% trichloroacetic acid) were added to one gram of raw material (dry) and incubated for 1 h at 30°C and 200 rpm. Supernatant was recovered by centrifugation (4000 rpm, 15 min) and used for phytic acid quantification.
The acid showing the best extraction results was used for further studies to improve the phytic acid extraction conditions. Three concentrations (0.2M, 0.6M and 1M); temperatures (25°C, 50°C, and 100°C) and weigh/volume ratios (1:5, 1:10 and 1:20) were tested.
Phytic acid quantification
Phytic acid quantification was implemented according to Haug-Lantzsch (1983) with modifications [13]. 0.5 mL of sample (supernatant for the extraction process) were added to 1 mL of ferric solution (0.2 g of ferric ammonium sulfate dissolved in 100 mL of concentrated HCL graduated with water to 1000 mL). The mixture was heated to boiling for 30 min and then cooled with ice for 10 min. 0.5 mL of the solution were added to 1 mL 2,2'-bipyridine solution (1g 2,2-bipyridine plus 1 mL of thioglycolic acid and graduated with water to 100 mL). Absorbance of the mixture was read at 519 nm. The same procedure was applied to construct a calibration curve using phytic acid (0-1 g/L) as standard.
Solid State Fermentation
Aspergillus niger, a strain indentified by molecular techniques in our laboratory, was cultivated in a medium using wheat bran as substrate for 5 days at 30°C. Spores were collected with 50 mL of sterile distilled water containing 0.01% Tween-80. Polyurethane was used as inert support, 0.8 g were moistened with 0.12 mL of impregnation media: phytic acid extract, mineral salt solution ((g/L): sucrose 5, $MnSO_4$

0.1, $MgSO_4$ 0.5, $Fe_2(SO_4)_2$ 0.1, KCl 0.5, yeast extract 2) and inoculum ($3X10^7$ spores/gDM). Fermentation was carried out in column reactors for 8 days, pH 6.5, 60% moisture and 30°C.

Enzyme extraction

Enzyme extraction from 1.4 g of fermented solids was carried out by adding 3.6 mL of distilled water, containing 0.1% (v/v) Tween-80, and shaken at 200 rpm for 1 h at room temperature. Fermented solid was then centrifuged at 5,000rpm for 20 min at 4°C. The supernatant was collected and used for enzyme assay.

Enzyme Assay

Phytase activity was assayed according to Harland and Harland. One international unit of phytase was defined as the amount of enzyme required for releasing 1 μmol of inorganic phosphorus per minute at 37°C temperature and pH 5. Enzyme yield was expressed as U/gDM (gram dry matter).

Results

Phytic acid content

Corn husk, cottonseed meal and wheat bran showed a considerable phytic acid content (approximately 0.5%), while in the rest of agroindustrial residues tested, phytic acid was undetectable. The best phytic acid extraction was reached with 3% of HCl. In the other hand, when H_2SO_4 and trichloroacetic acid were used, low phytic acid extraction was achieved. This result differs from those of Hatzack (2008) [1], that found thrichloroacetic acid as the best acid for phytic acid extraction. Further studies to improve phytic acid extraction were carried out. The best conditions for extracting phytic acid were: 0.2M of HCl, 25°C and 1:20 ratio (residue:acid) for the three agroindustrial residues tested, finding 0.57% (567 mg PA/gM), 0.45% (447 mg PA/gM) and 0.67% (665 mg PA/gM) of phytic acid for corn husk, cottonseed meal and wheat bran, respectively. Phytic acid extraction reached in wheat bran was 10-fold greater than that reported previously (58.39 mg PA/gM) by Hatzack (2008) [1]. Proposed modifications to Bartlett (1959) [12] technique were promising for phytic acid extraction in solid agroindustrial residues, finding higher phytic acid extraction.

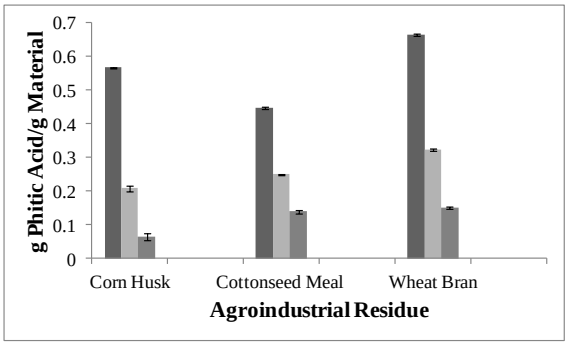

Figure 1. Ratio (residue/acid) (w/v) for phytic acid extraction in agroindustrial residues. Extractions were carried out with 0.2M of HCl at 25°C, for 1 hour at 200rpm. Where ■ is 1:20 ratio; ■ is 1:10 ratio; ■ is 1:5 ratio.

Phytase production in SSF

Three phytic acid extracts (corn husk, cottonseed meal and wheat bran) were assayed as inducers for phytase production in SSF using polyurethane as inert support. The maximum phytase activity reached was 0.39±0.09 U/gDM at 24 h of fermentation using a wheat bran extract as inducer, followed by corn husk extract with 0.35±0.01 U/gDM at 24 h of fermentation. Cottonseed meal extract used as SSF inducer, does not induce a significant phytase activity. Phytase production reached was 6-fold higher than the obtained for Ríos (2008) [14] (0.06 U/gDM) in SSF with a collection strain of *Aspergillus ficuum* DSM 932, this might be due to fermentation conditions (mineral media) used by the author, 20 g of glucose as carbon source, which is reported to repress phytase production [15]. However the phytase activity in this study was lower compared to phytase activity reported by Gunashree (2008), for a collection strain of *Aspergillus niger* CFR335 (66 U/gDM) [6]. The higher activity is probably due to the different phytase activity technique used for quantification, besides the authors improved the conditions for phytase production. Phytic acid extracts proved their potential as inducers for phytase production in SSF.

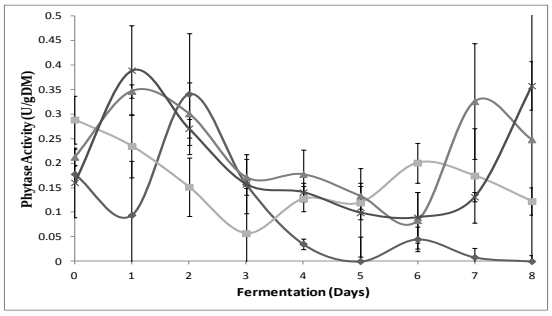

Figure 2. Effect of phytic acid extracts on phytase production by *Aspergillus niger* in SSF. Where ◆, phytic acid; ■, cottonseed meal; ▲, corn husk; and ✕, wheat bran.

Conclusion

It was possible to improve a phytic acid extraction and quantification methodology to be used in agroindustrial residues. The extracts obtained proved their potential as inducers in phytase production by SSF using polyurethane as inert support.

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Production And Partial Characterization Of Lipase From*Aspergillus niger* Obtained By Submerged Fermentation in Conventional Media

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Introduction

Lipases (EC 3.1.1.3) catalyze the hydrolysis of triglycerides to fatty acids and glycerol, and under certain conditionscatalyze other chemical reactions different than hydrolysis, as esterification, interesterification, alcoholysis, acidolysis and aminolysis [1]. These enzymes are widely distributed in nature, and can be obtained from microorganisms, plants and animals [2]. In recent years, the interest in these enzymes has been increasing due to its numerous catalytic properties. This has caused to become them to essential catalysts for different applications, such as in the food industry, for textile, detersive, trash, medical, cosmetic, environmental treatments and design of biosensors [3]. Discovery of new microorganisms producing lipases and the search for the precise conditions for their production is very important for any of the above applications. This paper aims to select, characterize and produce lipase from *Aspergillusniger* by submerged fermentation.

Materials and methods

The microorganisms used for this study were 12', HSF 14', 7A1 and 17.1 of *Aspergillusniger* strainsfrom a microbial strain collection (U. A. deC.).

Determination of lipase activity in plate

To select fungi strains, the method of triglyceride hydrolysis in presence of Rhodamine B dye was employed[4]. Plates were preparedwithrhodamine B (0.01% w/v), potatodextrose agar (15 g/L), gum arabic (1g/L)and olive oil (3% w/v). The plates were inoculated and incubated at 25°C for 7 days. The lipase production was evidenced by the formation of a fluorescent orange halo when the plates were irradiated with UV light at 350 nm.

Lipase production by submerged fermentation

A medium used for submerged fermentation contained (g/L): 12 NaH_2PO_4, KH_2PO_4 2, $MgSO_4.7\ H_2O$ 0.3, $CaCl_2$ 0.25,sucrose (2%) as carbon source, and2 % of olive oil (in suspension with Span 80), as inductor and carbon source. The initial pH was adjusted to 6. The inoculum was applied at 1 x 10^6 spores/ml,and the culture was grown at 25 °C and 120 rpm for 60 hours.

Collection and concentration of the crude enzyme

Fungal cultures were vacuum filtered and then centrifuged at 12 000 rpm for 5 min. The obtained supernatants were considered as crude lipaseextracts and were stored at 4 °C. Forenzyme concentration Amicon ® Ultra tubes-4 with 50 and 30 kDa membranes were used, considering thatthe enzyme molecular weight is approximately 35 kDa. The tubes were centrifuged at 3000 rpm for 15 min in a refrigerated centrifuge at 4ºC. Enzymatic activity was evaluated in all obtained fractions.

Characterization of lipase obtained by *Aspergillusniger*submerged fermentation

Lipase activity was determined using the method of hydrolysis of *p*-nitrophenyl propionate applied as enzyme substrate [1]. To define pH effect on enzyme activity, the reaction was carried out in different buffers: 0.05 M sodium acetate for pH 5, 0.05 M sodium phosphate for 6 and7, as well as 0.05 M sodium carbonate for pH 8, 9 and 10. The effect of temperature on lipase activity was studied by performing the enzymatic reaction at different temperatures from 20 to 70 ° C.

The influence of pH on the stability of the enzyme was studied by exposure of the lipase to the previously obtained optimum pH at 37 ° C for 4 h, while the influence of temperature on the stability of the lipase was performed by incubating the enzyme at the optimum temperature for 4 h. Every hour lipase activity was measured and the residual activity was determined.

Results and discussion

Figure 1 presents evidence of results obtained for lipase-producing capacity evaluation by four *Aspergillusniger*strains. Fluorescence was observed in all strains after 48 h of incubation. This is a qualitative quick method to demonstrate enzyme production. Some authors suggest that the fluorescence is due to the formation of a complex between the cationic rhodaminedye and uranyl ion of fatty acids [5]. The fluorescent halos were more evident for the strains identified as 12 'and HSF 14'.

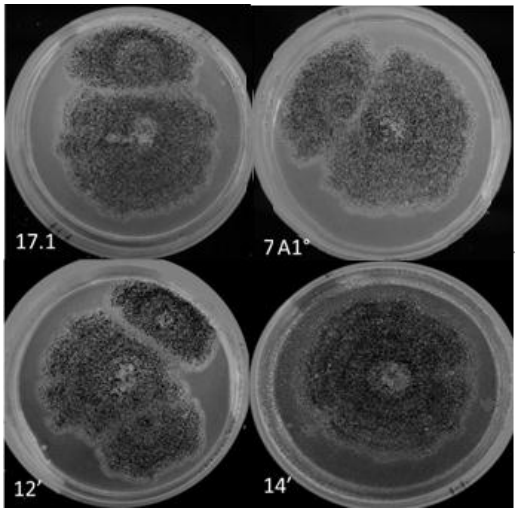

Figure 1.Fungal growth on potato dextrose agar/rhodamine Bmedium,supplemented witholive oil as inductor and carbon source. Orange fluorescent halos revealed by UV light irradiation were detected in all strains and were more intense in the case of strains identified as HSF 14 'and 12'.

Lipase production by the *Aspergillus niger* strain for submerged fermentation

Four strains of *Aspergillusniger* (12´, HSF 14´, 7A1, 17.1)were evaluated on their ability to produce the enzyme lipase for submerged fermentation.The highest levels of lipase activity were observed at different times for different strains: for strain 17.1 after 24 h of incubation had 14.54 IU/ml, for strains 12' and HSF 14' at after 48 h obtained 16.26 IU/ml and 16.83 IU / ml, respectively, andfor strain 7A1 reached 14.83 IU/ml after 36 h of growth (Figure 2).

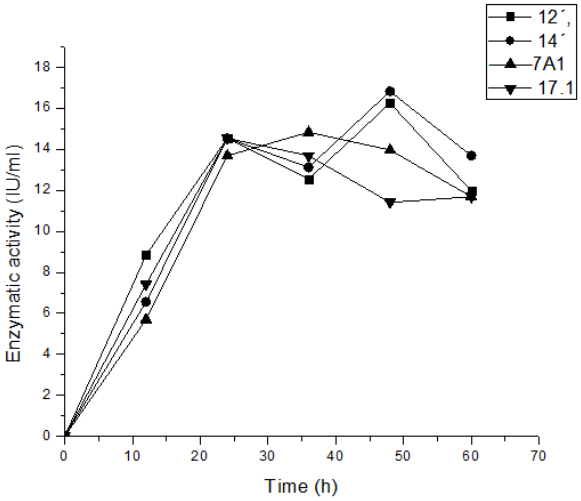

Figure 2. Production of lipase from four *A.niger* strains in submerged fermentation using sucrose as source carbon and olive oil as inducer at 25 °C and 120 rpm for 60 hours

Lipase activity levels detected in submerged fermentation assay on present study were significantly superior than reported by other authors for several strains of *A. niger*[2, 6, 7]. The higher level of enzyme activity could be explained by considering the simple modifications performed with medium composition: an extra source of carbon (sucrose) was applied, as well as the inductor (olive oil) was introduced in suspension with Span 80, which allowed a better homogeneity of the system.
Therefore, it can be concluded that the 4 strains of *A. niger* can be considered as excellent lipases producersunder conditions of submerged fermentation. However, for the purposes of this study only the strain with highest level of enzymatic activity (strainHSF 14') was selected for its followed characterization.

Lipase characterization

Partial purification of enzyme was carried out by means of ultrafiltration assisted by centrifugation. The enzymatic activity was detected only in fraction corresponded to protein with molecular weight higher than 30kDa, but less than 50 kDa. It is according with reports indicating that molecular weight of lipase from different *Aspergillus* strains is near 35 kDa [1, 3].
A summary of the purification procedure was shown in Table 1. The detected lipase in the supernatant fraction of the 50 kDamembrane,was purified 1.61-fold with 63.79% recovery; and the detected lipase in the precipitate fraction of the 30 kDa membrane, was purified 2.09-fold and 76.73% recovery.

Table 1.Purification of a lipase from *Aspergillusniger*

Step	Total protein (mg/ml)	Total activity (UI/ml)	Specificactivity (UI/mg)	Purification factor (fold)	Yield(%)
Crudeenzyme	0.02888	33.09	0.21	1	100
Supernatant of 50 kDa	0.01613	21.11	0.34	1.619047619	63.79570867
Precipitate of 30 kDa	0.00995	25.39	0.44	2.095238095	76.73012995

Figure 3 (a) presents the results of pH effect on lipase activity. The highest enzyme activity was detected at pH 7-8. Thus, the optimum pH for lipase of studied *A. niger* is similar to the reported for lipase from *Aspergillusfumigatus* [8,9], *Aeromonas*and *Bacillus*sp [10].
Figure 3 (b) demonstrates that the greatest lipase activity is in a range between 50 and 70 ° C. This means that the enzyme is thermostable since it supports high temperatures. The optimum temperature (60 ° C) was similar to reported for lipase from *A.niger*[11, 12].

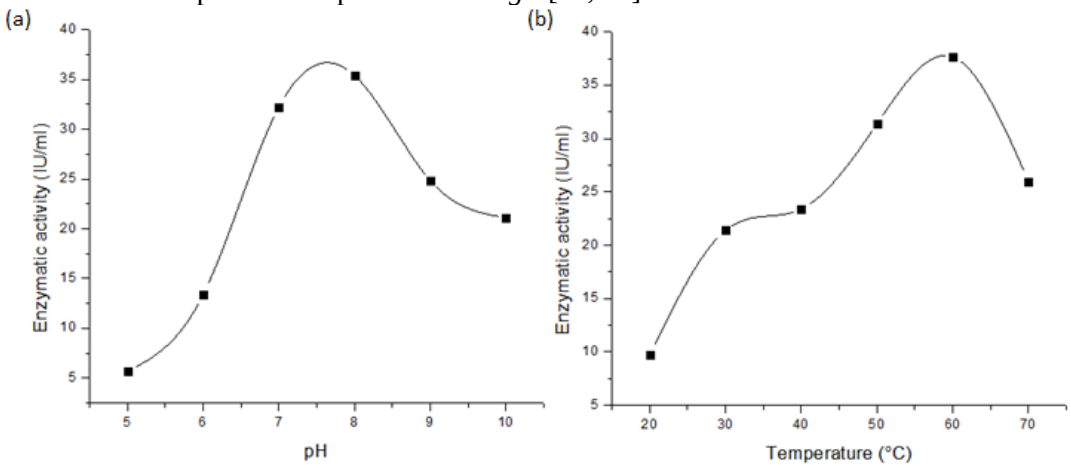

Figure 3. Influence of the pH (a) and temperature (b) on the activity of lipase produced by *Aspergillusniger*HSF 14'.

Figure 4 (a), shows the profile for stability of the lipase in function of optimal pH. We appreciate that the activity decreases to 50% approximately in 4 hours. The profile for stability in function of optimum temperature is observed in Figure 4 (b). We can see that the activity decreases to 58% approximately in 4 hours. Both the profile of stability against the pH of lipase of *A. niger* as the temperature were very similar to those reported for other lipases of fungal origin[12].

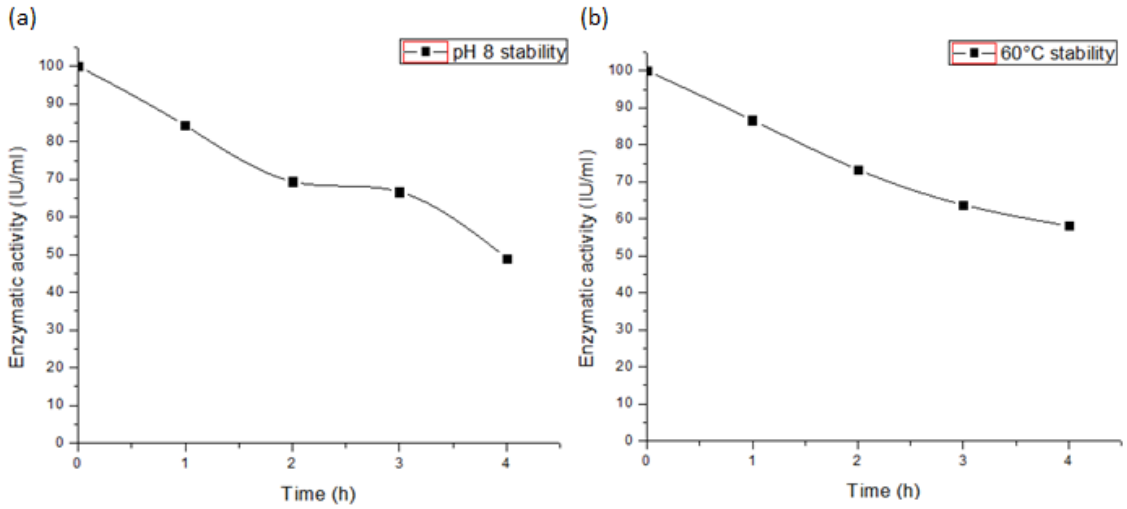

Figure 4. Stability of lipase of *A. niger*HSF 14' (a) at optimum pH and (b) at optimum temperature.

Conclusions

All *Aspergillusniger*strains previously isolated from the semi-desert of Coahuila, Mexico, were able to produce lipase in plate with medium, as well as in submerged fermentation. Due to highest enzyme activity, the strain of*Aspergillusniger*, HSF 14, was selected as good lipase producer. The molecular weight of enzyme is in the range from 30 to 50 kDa, the optimum pH is in the range from 7 to 8, and the optimum temperature is 60°C approximately. The optimum pH and temperature doesn´t affect the enzyme stability.The strain *Aspergillusniger* HSF 14 may be consider as new source of thermostable enzyme.

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Study On The Interaction Site Of B-Lactoglobulin Dimer And Electrophilic Molecules

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Introduction

Bovine β-lactoglobulin (β-lg) is the major whey protein, composing up to 50% of whey proteins and 12% of whole cow milk proteins (1,2). Each β-lg monomer consists of 162 amino acids with a mass of approximately 18.3 kDa. The secondary structure of β-lg is composed of eight up and down antiparallel β-strands that form a calyx (3) followed by a three-turn α-helix and ending with a β-strand (4). Although its biological function is uncertain (5-9), β-lg has been the subject of numerous studies because of its abundance, ease of purification and ability to bind small hydrophobic ligands, such as fatty acids, retinol and peptides (7,10-12).

Many studies have attempted to establish the ligand-binding site of β-lg (7), and despite remaining uncertainty, two potential binding sites have been postulated for small hydrophobic molecules: one inside the calyx and the other at the outer surface of the protein between the α-helix and the β-barrel (3,13). Recent studies have shown that in addition to binding small polar and non-polar ligands, β-lactoglobulin can also bind to *Kluyveromyceslactis*β-galactosidase (KLβ-gal), increasing its activity (14,15). It has recently been reported that the interaction between these proteins is likely to occur through lysine ε-amino groups of β-lg, and studies using molecular docking of the monomer and succinic anhydride as a model ligand for the protein showed that the interactions between β-lg and KLβ-gal may occur specifically through Lys^{138} (15).

The oligomeric association and dissociation behavior of β-lg has been widely studied through different experimental techniques. Bovine β-lgoligomeric states change as a function of pH, a phenomenon known as Tandford transitions (16). At room temperature and pH values below 4.0 and above 5.2, the protein consists predominantly of monomers and dimers. At approximately pH 4.7, larger oligomeric structures, such as octamers, are formed (1). At approximately pH 6.8, which is the pH of cow's milk, β-lg exists as a dimer (16).

It has been determined that the interactions of β-lg with hydrophobic molecules occurs through the internal calyx of the protein (17,18); however, this mechanism cannot explain interactions with a large protein, such as KLβ-gal, suggesting that the interactions should occur through an exposed region of the protein. Few studies have reported this type of interaction, and there is limited information regarding the possible interaction sites or mechanisms. Under most experimental conditions, bovine β-lg exists as a dimer; therefore, this work aimed to compare the probable interaction sites of the dimer with the interaction site previously reported for the monomer (15). The molecular docking of the β-lactoglobulin dimer with lactose and succinic anhydride was performed to determine the probable binding site of the β-lactoglobulin dimer with *K. lactis*β-galactosidase.

Methods and Materials

Three-Dimensional Modeling

A three-dimensional (3D) bovine β-lactoglobulin dimer model was built with PyMOL software (DeLanoScientific, 2009) using the X-ray diffraction coordinates of a bovine β-lactoglobulin triclinic

crystal grown at pH 6.5 and at 1.8 Å resolution in the Protein Data Bank (PDB) database (PDB ID: 1BEB) (7).

Molecular Docking

Blind docking was performed to estimate the binding energies between all of the β-lg dimer amino acid residues and lactose or succinic anhydride using AutoDock 3.05 software (The Scripps Research Institute, 2006). The runs were submitted at 25ºC and clustered at a range of RMSD of 5 Å. The 3D models for lactose and succinic anhydride were constructed at the Dundee PRODRG2 Server site (http://davapc1.bioch.dundee.ac.uk/programs/prodrg/)

Results and Discussion

A 3D model of the β-lg dimer was built to study the exposure and the possible steric hindrance of the different lysines in the molecule during interactions with lactose, succinic anhydride and/or another protein (Figure 1). Potential β-lg binding sites for small hydrophobic molecules have been studied and have been shown to be located in highly hydrophobic regions of the monomer (17-20). The binding site may vary as the dimer is formed, but it is always located in the same region of the protein, as shown in Figure 1,whereas the binding site of small charged molecules appears to be nonspecific (7,21). Lysine ε-amino groups are some of the most exposed and reactive residues in β-lg, with Lys^{47} and Lys^{138} being the most exposed (22). It has also been reported that the lactolation of β-lg occurs through lysine, specifically Lys^{47} (23,24).

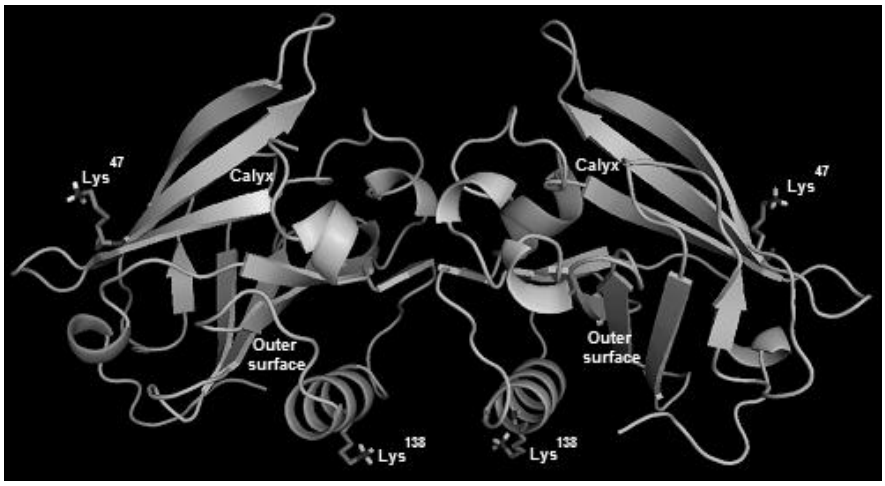

Figure 1.The dimer of bovine β-lactoglobulin showing potential hydrophobic binding sites (calyx and outer surface) and reactive lysines.

Although the function of β-lg has not been well established, its probable function as a transporter protein has led to many studies focused on determining the binding sites of β-lg for different ligands. Most ligand-protein interaction studies have been performed under conditions involving the dimeric state of β-lg; however, the techniques for determining the ligand-binding sites require the digestion of β-lg and, therefore, the loss of the dimer. Because the interactions in many studies occur under conditions in which the dimer is formed and because of the difficulty in determining binding sites without hydrolyzing the protein, the blind docking of the β-lg dimer with lactose and succinic anhydride was performed to compare the probable binding site of the dimer with those reported for the monomer and hydrolysates.

The docking results for lactose and the β-lg dimer (Figure 2) showed that Lys^{138} and Lys^{141} were the amino acids with the most favorable interaction energy at pH 7.0 (FDE=-3.30 kcal mol^{-1}) and thus the most probable residues to interact with lactose. Because a high variation in binding sites has been found, it was recently proposed that ligand exchange among different sites of β-lg might occur in solution and that

Lys^{138} and Lys^{141} are involved in such phenomena (18). Our results agree with this finding, but it is remarkable that the molecular docking showed that among all of the amino acids involved in the interactions, Lys^{138} and Lys^{141} had the same docking energy, but they corresponded to different monomers (Figure 2). This result suggests that the dimeric state of β-lg is important for the interaction because both residues are required. This result may also explain the variation in binding sites found by other authors when hydrolysates are used because upon hydrolysis, the ligand may remain in either monomer.

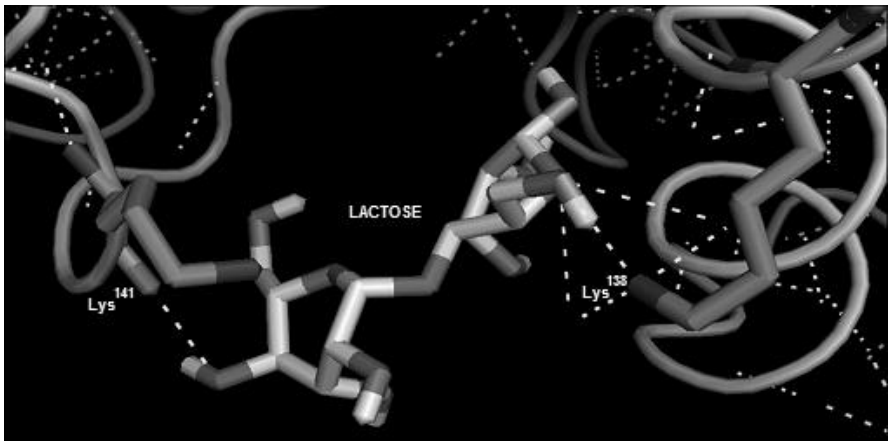

Figure 2.Molecular docking of lactose and the dimer of β-lactoglobulin showing the interaction of lactose with Lys^{141} of one β-lactoglobulin monomer (left) and Lys^{138} of the other β-lactoglobulin monomer (right).

Léonil et al. (23) reported for the first time a binding site for the lactolation of β-lg. Binding occurring only through lysines suggests the importance of β-lgε-amino groups in reactions in which a nucleophilic attack must occur between an amino group and an electrophile, such as lactose. As shown in Figure 2, in addition to binding to Lys^{138} and Lys^{141}, lactose forms hydrogen bonds with other nearby exposed amino acids of both monomers. This binding may strengthen the interaction by approaching and stabilizing lactose in a claw-like structure so that nucleophilic attack can occur. The stabilization of lactose can explain experimental evidence of the strong and stable interactions found between β-lg and larger proteins, such as KLβ-gal, in which β-lgε-amino groups of exposed regions of the protein are involved (14,15).

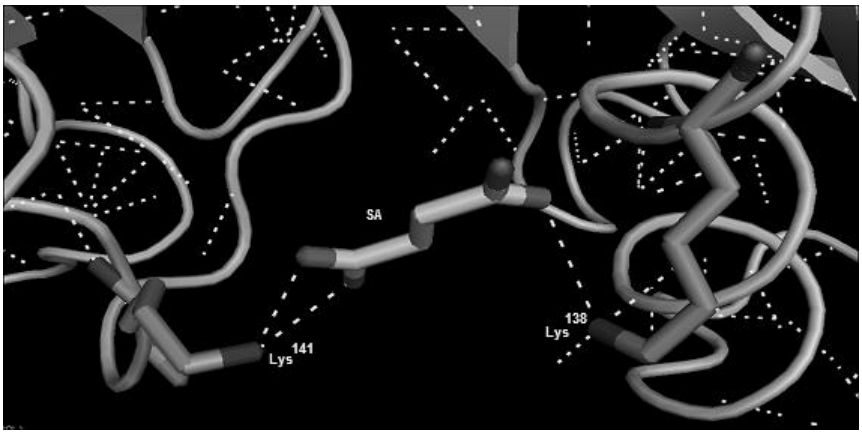

Figure 3. Molecular docking of succinic anhydride (SA) and the dimer of β-lactoglobulin showing the interaction of lactose with Lys^{141} of one β-lactoglobulin monomer (left) and Lys^{138} of the other β-lactoglobulin monomer (right).

To determine the region in which nucleophilic attack would occur in the β-lg dimer, succinic anhydride (SA) was used as a stronger electrophile for docking studies. Similar to lactose, Lys^{138} and Lys^{141} were the

amino acids with the most favorable interaction energy at pH 7.0 (FDE=-3.11 kcal mol^{-1}) and thus the most probable residues to interact with SA (Figure 3) via the same mechanism proposed for lactose, in which the ligand is stabilized by a claw-like structure forming hydrogen bonds with nearby amino acids. Our results suggest that in addition to being a highly electrophilic region, Lys^{138} of one β-lg monomer and Lys^{141} of the other monomer may be specific binding sites for electrophilic molecules.

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Effect Of Ph On The Interaction Of β-Lactoglobulin With *Kluyveromyceslactis*β-Galactosidase And Its Effect On Enzymatic Activity

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Introduction

Kluyveromyceslactis β-galactosidase(KLβ-gal) is by far the most important commercial lactase used in the dairy industry. Several reports have established that the presence of some proteins in the reaction medium may affect the activity of β-gal (1, 2). Jiménez-Guzmán et al. (3) studied the effect of whey proteins in β-gal activity and demonstrated that the activity of *Kluyveromyceslactis* β-gal increased when it was measured in the presence of either β-lactoglobulin (β-lg) or bovine serum albumin; this finding is particularly interesting since these proteins are available in milk and whey, which are the natural reaction media for this enzyme in dairy processing.

Recent studies have demonstrated that β-lactoglobulin (β-lg), the major whey protein in cow milk, enhaces lactase activity up to 230% by binding to KLβ-gal through lysine ε-amino groups probably involving $Lysine^{138}$ of β-lg(3, 4). It is well known that β-lg monomers can associate to form different oligomeric structures and then dissociate into the monomers as a function of pH by the phenomenon known as Trandford Transitions (5). It has been found by different experimental techniques that at room temperature and pH values below 4.0 and above 5.2 the protein consists predominantly of monomers and dimers. Larger oligomeric structures as octamers are formed at around pH 4.7 (6). At pH 6.8, which is cow's milk pH, β-lg exists as a dimer (5). It has been recently reported that β-lg dimmer can interact with electrophilic molecules such as lactose and succinic anhydride and that the dimer formation of β-lactoglobulin is necessary to strengthen these interactions due to the participation of $Lysine^{138}$ of one of β-lg monomers and Lys^{141} of the other. Since β-lg interactions with KLβ-gal occur through ε-amino groups, it is very likely that the region of β-lg dimmer involved in the interaction with electrophilic molecules may be the same involved in the interaction with KLβ-gal.

Although there is few information on KLβ-gal structure, in 2005 Tello-Solis et al. (7) found by circular dichroism that KLβ-gal is mainly a β-type protein consisting of 22% turns, 14% parallel β-sheet, 25% antiparallel β-sheet, 34% unordered structure and only 5% α-helix, they also showed that there is a structure-activity relationship as a function of pH.

This work aimed to study the effect of pH on the interaction of β-lactoglobulin with *Kluyveromyceslactis*β-galactosidase and its effect on enzymatic activity since both, structure-activity relationship of KLβ-gal and dimer formation of β-lg are pH dependent.

Methods and Materials

Enzyme Activity Measurement

The source of KLβ-gal was a commercial enzyme preparation, Maxilact LX-5000, (Gist Brocades, Delft, The Netherlands). All reactions were carried out at 37°C by dilution of 1:400 of Maxilact LX-5000 into 0.05 M phosphate buffer at pH values of 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5. A solution of 0.034 M *ortho-nitro-phenyl-β-D-galactoside* (ONPG) (Sigma Chemical Co. St. Louis MO, USA) was used as substrate, and

enzyme activity was measured spectrophotometrically at 410 nm on the basis of *ortho-nitro-phenol* (ONP) release after mixing 0.2 mL of ONPG solution with 0.1 mL of enzyme solution in 2.7 mL of phosphate buffer at each pH value. The hydrolysis rate (v_o) for each pH value was calculated from the linear portion of data of ONP production versus time. One enzyme unit (U) was defined as the amount of enzyme that hydrolyses 1 μmol of substrate (ONPG) in 1 min at 37°C and the corresponding pH (6.0, 6.5, 7.0, 7.5, 8.0 or 8.5). Specific activity was calculated dividing the enzyme units by the protein concentration of the sample determined according to Lowry, Rosegrough, Farr, & Randall (8).
KLβ-Gal activity of Maxilact LX-5000 was also measured in the presence of 0.3 mg/mL of native β-lg, at different pH (6.0, 6.5, 7.0, 7.5, 8.0 and 8.5).

Protein-Enzyme Interaction
Affinity chromatography was used to determine β-lg interaction with KLβ-gal using aEupergit (Röhm GmbH & Co. Darmstadt, Germany) support with immobilized β-lg at pH 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5. Immobilization was performed following the instructions of the producer. Controls without ligand were prepared at each pH by blocking the active oxyrane groups of the support with glycine. After immobilization, the amount of immobilized protein was calculated through the difference between the protein in solution before and after interacting with the support the efficiency of immobilization was 0.640, 0.634, 0.687, 0.712 0.699 and 0.705 $\mu mol/g_{support}$ at pH 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5, respectively.
Maxilact LX-5000was used as the KLβ-gal sample. Samples were prepared to have a concentration according to a molar ratio of 0.05 $mol_{\beta\text{-gal}}/mol_{immobilized\beta\text{-lg}}$ for each pH. Immobilized ligands and the controls were allowed to interact with β-gal by directly adding 0.1 g of support with immobilized ligands to 3 mL of *KL*β-gal sample and shaking for 1 h, then the supernatant was separated from the support by centrifugation, and its specific activity was measured using ONPG as a substrate.

Statistical Analyses.
Each experiment was performed three times. Data were analyzed by means of a variance test (ANOVA); in some cases, a Tukey test was performed. All statistical analyses were carried out using the statistical analysis software Statistica 5.0 (Stat Soft, Tulsa OK, USA), with $p < 0.05$ used as a threshold of statistical significance.

Results and Discussion

Figure 1 shows the variation of ONPG hydrolysis rate (v_o) by KLβ-gal as a function of pH. It was observed that lactase activity is low at around pH 6.0 and 8.5 consistent with that reported by Tello et al. (7) and Kim, Lim, & Kim (9). The maximum v_o was found at pH 7.5, and activity variation found at different pH values from 6.0 to 8.5 is consistent with that reported by Tello et al. (7) who found an important increase in lactase activity when pH changed from 6.5 to 7.0 and suggested that pH changes cause small modifications in KLβ-gal secondary structure and that the increase in enzyme activity is mostly caused by small local changes in structure such as the charges in some residues. Furthermore, when pH increases from 7.0 to 7.5 (Figure 1) hydrolysis rate reaches its maximum, suggesting that this change in pH may cause an important modification on KLβ-gal secondary structure and thus a considerable increase in lactase activity.

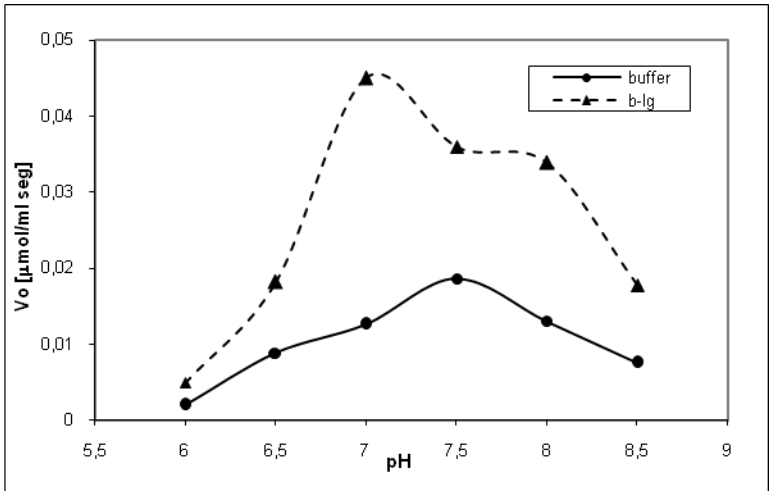

Figure 1. Effect of pH on ONPG hydrolysis rate (v_o) by KLβ-gal and on the effect of β-lg on KLβ-gal activity

When lactase activity was measured in the presence of 0.3 mg/mL β-lg at different pH values (Figure 1) an increase in v_o was observed at pH between 6.5 – 8.0 ($\alpha < 0.0002$), confirming that the presence of β-lg enhances lactase activity as reported by Jiménez-Guzmán et al. (3) and Del Moral et al. (4). At pH values of 6.0 and 8.5 β-lg did not activate KLβ-gal ($\alpha > 0.05$) showing that the activation effect is dependent on pH. Optimum pH, at which the maximum activity was reached, shifted from 7.5 to 7.0 in the presence of β-lg.

Some reports establish the activating effect of some whey proteins such as β-lg or bovine serum albumin on KLβ-gal activity (3, 10). Jiménez-Guzman et al (3) suggested that this effect could be related to the ability of the protein to bind to the enzyme. It is very likely that as pH changes, charges in the residues of β-lg are modified, changing the protein's ability to bind to the enzyme. In order to study the effect of pH on the interaction of β-lg with KLβ-gal, affinity chromatography was performed at different pH values immobilizing β-lg on Eupergit, and allowing the immobilized protein to interact with a solution of Maxilact LX5000 at different pH values. A control for each pH was used in which no protein was immobilized, but the reactive groups of the resin were blocked with glycine.

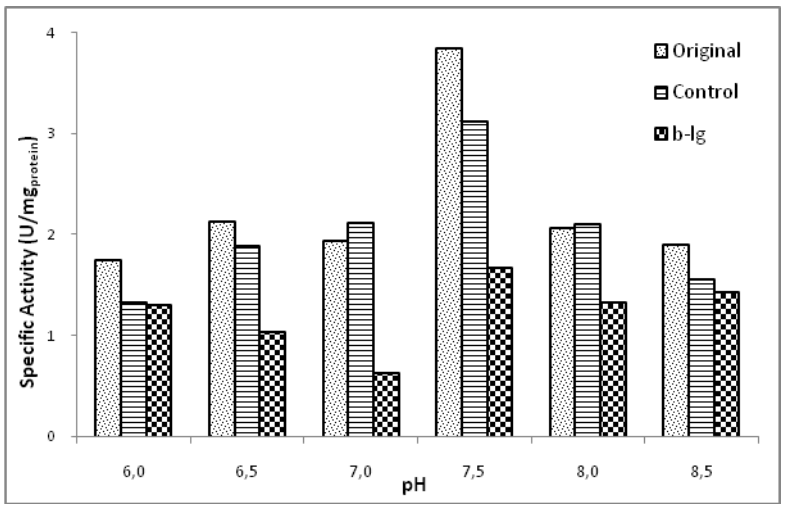

Figure 2.Effect of pH on the interaction between β-lg and KLβ-gal determined by affinity chromatography.

The specific activity of the control was not significantly different from that of the initial lactase solution at any of the pH values tested ($\alpha > 0.05$ for all cases) meaning that lactase was not specifically bound to the support without protein (Figure 2). When the same solution of lactase (Maxilact LX5000), containing eight proteins visible by electrophoresis, was mixed with the support containing β-lg as ligand, it was observed that at pH 6.0 and 8.5 the specific activity of the supernatant remained the same as that of the control (α= 0.9794 and 0.5430 for pH 6.0 and 8.5 respectively) demonstrating that at these pH values, there is no specific interaction between β-lg and KLβ-gal. At pH between 6.5 and 8.0 the specific activity of the enzyme solution that interacted with immobilized β-lg changed significantly with respect to the control (support without ligand, $\alpha<0.00033$ in all cases), reaching a maximum diminution of 70% at pH 7.0 showing a specific interaction between β-lg and KLβ-gal, which decreased as pH increased further more. This suggests that KLβ-gal bound specifically to β-lg and that the interaction was favored at pH 7.0. At pHs below or above 7.0, the interaction gradually decreased until it was not observable at pH 6.0 or 8.5. When comparing the changes on the activation caused by pH with the changes on the interactions between β-lg and KLβ-gal (Figure 3), a direct correlation was observed: as the interaction between β-lg and KLβ-gal is stronger the activation increases.

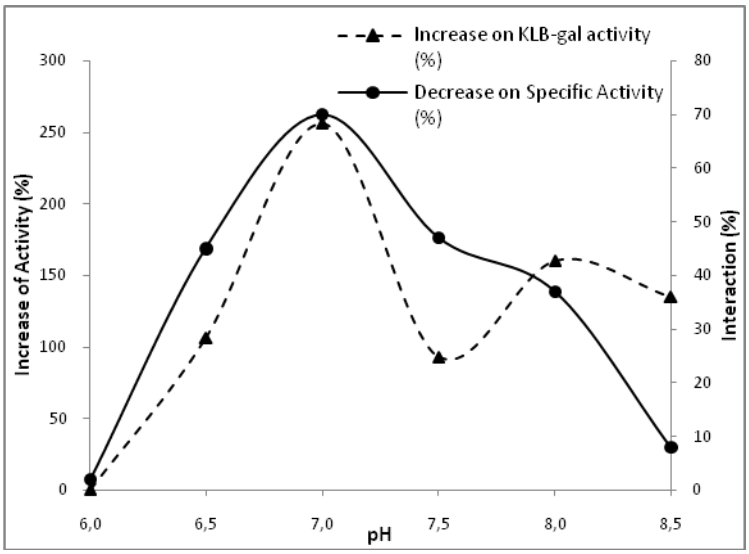

Figure 3.Relationship between the activating effect of β-lg and its interaction with KLβ-gal.

Tello et al (7) established that small changes in KLβ-gal structure may cause significant changes in enzyme's activity. Mbuyi-Kalala, Schnek, &Léonis (11) reported that β-galactosidase activity is influenced by some ionizable groups, prototropic groups and by conformational changes. Jurado, Camacho, &Luzón (12) reported that the effect of pH on lactase activity is usually described by a kinetic model which involves the dissociation of one or two protons in β-gal. Although KLβ-gal has its most stable conformation at pH 7.0 (7) this is not the conformational state at which it reaches the highest activity which is at pH 7.5 (Figure 1) and in which conformational changes caused by pH variation may leave the active site more accessible to the substrate, increasing enzyme activity. The interaction between KLβ-gal and β-lg could cause a structural change on the enzyme leading to the exposure of the active site and thus a more active conformation.

Many studies have attempted to establish the ligand-binding site of β-lg (13) and despite of remaining uncertain, two potential binding sites have been postulated for hydrophobic and small molecules: one inside the calyx and the other at the outer surface of the protein between the α-helix and the β-barrel (14, 15). Jiménez-Guzmán et al., (3) and Del Moral-Ramírez, et al. (4)showed that besides binding small, polar and non-polar ligands, β-lactoglobulin can also bind to *Kluyveromyceslactis*β-galactosidase increasing its

activity and that the interaction between these proteins is very likely to occur through lysine ε-amino groups of β-lg, more specifically Lys^{138}.
Sawyer, Kontopidis, & Wu (16) reported that lysine, tyrosine and tryptophan residues show a variable reactivity that is dependent on the media, and that $Cysteine^{121}$ (Cys^{121}) has a free thiol group with a reactivity that is dependent on pH and structural changes that take place at pH values from 6 to 8.

It has also been reported that the quaternary structure of β-lg undergoes structural changes when pH is modified (6). At pH 6.0 and 8.5 at which no activation or interaction with KLβ-gal was observed, β-lg exists as a monomer, while between 6.5 and 8.0 where both interaction and activation were observed, it exists as a dimer, showing that the dimeric state of β-lg is essential for both the interaction and the activation of the enzyme.

In buffer solution, as pH approximates 7.5 the most active conformation of KLβ-gal is reached (Figure 1); however, in the presence of the β-lg dimer the interaction between both proteins could help to reach an even more active conformation of KLβ-gal, shifting the optimum pH from 7.5 in the absence of β-lg to 7.0 in the presence of the protein and achieving a rate of hydrolysis significantly higher ($\alpha = 0.00018$) than the one obtained in the optimum pH in the absence of β-lg (142% activation with respect to optimum pH in buffer solution), suggesting that at this pH β-lg promotes the KLβ-gal conformational state in which the active site is more accessible to the substrate.

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Determination Of The Role Of Carboxyl Groups Of *Kluyveromyceslactis*β-Galactosidase In The Interaction With β-Lactoglobulin And Its Activating Effect

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Introduction

Among several microbial lactases, *Kluyveromyceslactis*β-galactosidase (KLβ-gal) is by far the most important commercial lactase used in the dairy industry (1-3). KLβ-gal is mainly a β-type protein consisting of 22% turns, 14% parallel β-sheet, 25% antiparallel β-sheet, 34% unordered structure and only 5% α-helix (4). Enzymatic hydrolysis of lactose glycosidic bond by β-galactosidase takes place via general acid catalysis that requires two critical residues: a nucleophile and a proton donor, which in most β-galactosidases is the same amino acid residue, glutamic acid (5). *Kluyveromyceslactis*β-galactosidase has two glutamic acids (Glu^{482} and Glu^{551}) as the proton donor and the nucleophile at the same time in the enzymatic reaction (5).

Several reports have established that the presence of some proteins in the reaction medium may affect the activity of KLβ-gal. Jiménez-Guzmán et al. (6) studied the effect of whey proteins in KLβ-gal activity and demonstrated that the activity of this lactase increased when it was measured in the presence of either β-lactoglobulin (β-lg) or bovine serum albumin; this finding is particularly interesting since these proteins are available in milk and whey, which are the natural reaction media for this enzyme in dairy processing. Affinity chromatography assays demonstrated that KLβ-gal bound specifically to β-lg, resulting in enzyme activation (6). Several reports have established that the most reactive groups of β-lg are lysine ε-amino groups (6-8) and that blocking these groups with either lactose (lactolation) or succinic anhydride results in the loss of the binding capacity of the protein to the enzyme and therefore its activating effect (6-8).Since the interactions of β-lg with KLβ-gal occur through ε-amino groups, it is very likely that the region of β-lg involved in the interaction with electrophilic molecules may be the same involved in the interaction with KLβ-gal via electrophilic groups such as the side-carboxyl groups of the enzyme.

The aim of this work was to determine the role of the side-carboxyl groups of *Kluyveromyceslactis*β-galactosidase on both the interaction with β-lg and the activating effect.

Methods and Materials

Kluyveromyceslactisβ-galactosidase three-dimensional modeling

*Kluyveromyceslactis*β-galactosidase three-dimensional (3D) model was built by homology modeling with Modeller v9.2 software (9-12). The template corresponded to X-ray diffraction coordinates of *E.coli*β-galactosidase A chains (PDB ID: 1JZ7 and 1JZ8) (13) at 1.5 Å ($E=9x10^{-125}$ and $E=3x10^{-124}$ respectively) with a 48% of homology. The best model had a DOPE of -103795.781 kJ/mol.

Molecular docking

Blind docking was performed to estimate the binding energies between all KLβ-gal amino acid residues and lactose or lysine using AutoDock 3.05 software (The Scripps Research Institute, 2006). The runs were submitted at 25ºC and were clustered at a range of RMSD of 2 Å. The 3D models for lactose and lysine were drawn at The Dundee PRODRG2 Server site (http://davapc1.bioch.dundee.ac.uk/programs/prodrg/).

Kluyveromyceslactisβ-galactosidase esterification

Methyl alcohol blocks gradually and specifically the side-chain carboxyl groups of KLβ-gal. Maxilact LX 5000 containing 461.92 $mg_{protein}$/mL was used as source of KLβ-gal. An alcoholic solution of methyl alcohol (Sigma Chemical Co., St. Louis, MO, USA) containing 1% protein and 0.05 N hydrochloric acid (Sigma Chemical Co., St. Louis, MO, USA) was prepared. The mixture was left under vigorous agitation for two hours at room temperature according to Fraenkel-Conrat&Olcott(14). Samples of KLβ-gal ester derivatives were taken at the beginning of reaction (E0), after 1 hour (E1) and two hours (E2) of reaction; they were centrifuged for 5 minutes and diluted with deionized, distilled water and cold dialized against HCl for 4 hours. In order to determine the degree of esterification, a general method to quantify carboxyl groups of proteins employing the formation of a colored hydroxamate-ferric ion complex was used according to Halpin& Richardson (15). Absorbance of the hydroxamate-ferricioncomplex was measured spectrophotometically at 475 nm within 30 min. An equimolar concentration of KLβ-gal served as blank.
L-Glutamic acid γ-monoethyl ester (Sigma Chemical Co., St. Louis, MO, USA) was used to prepare a standard curve for molar absorption of the ferric ion-hydroxamate complex, using the foregoing procedure. The blank contained an equimolar amount of L-glutamic acid (Sigma Chemical Co., St. Louis, MO, USA) treated in the same manner as the corresponding ester.

Enzyme activity measurement

Maxilact LX-5000 was used as source of KLβ-gal (Gist Brocades, Delft, The Netherlands). All reactions were carried out at 37°C by dilution of 1:10000 of Maxilact LX-5000 or ester derivatives (E0, E1 and E2) into 0.05 M phosphate buffer at pH 7.0. A solution of 0.034 M *ortho-nitro-phenyl-β-D-galactoside* (ONPG) (Sigma Chemical Co. St. Louis MO, USA) was used as substrate, and enzyme activity was measured spectrophotometrically at 410 nm on the basis of *ortho-nitro-phenol* (ONP) release. The hydrolysis rate (v_o) was calculated from the linear portion of data of ONP production versus time. One enzyme unit (U) was defined as the amount of enzyme that hydrolyses 1 μmol of substrate (ONPG) in 1 min at 37°C and pH 7.0. Specific activity was calculated dividing the enzyme units by the protein concentration of the sample determined according to Bradford (16).
In order to evaluate the activating effect of β–lg, activity was determined in the presence of 0.3 mg/mL β–lg.

Protein-enzyme interaction

Affinity chromatography was used to determine β-lg interaction with KLβ-gal using anEupergit (Röhm GmbH & Co. Darmstadt, Germany) support with immobilized β-lg at pH 7.0. Immobilization was performed following the instructions of the producer. Controls for each derivative without ligand were prepared by blocking the active oxyrane groups of the support with glycine. After immobilization, the amount of immobilized protein was calculated through the difference between the protein in solution before and after interacting with the support, the efficiency of immobilization was 1.6568 $\mu mol_{\beta\text{-}lg}/g_{support}$ at pH 7.0.

Derivatives E0 and E1 were used as the KLβ-gal samples, prepared to have a concentration according to a molar ratio of 0.05 $mol_{\beta\text{-}gal}/mol_{immobilized\beta\text{-}lg}$. Immobilized ligand and the controls were allowed to interact with β-gal by directly adding 0.1 g of support with immobilized ligands to 1.5 mL of *KL*β-gal samples and shaking for 30 min, then the supernatant was separated from the support by centrifugation and its activity was measured using ONPG as substrate.

Statistical Analyses.

Each experiment was performed three times. Data were analyzed by means of a variance test (ANOVA); in some cases, a Tukey test was performed. All statistical analyses were carried out using the statistical analysis software Statistica 5.0 (Stat Soft, Tulsa OK, USA), with $p < 0.05$ used as a threshold of statistical significance.

Results and Discussion

Recent studies have demonstrated that β-lactoglobulin (β-lg), the major whey protein in cow milk, enhances lactase activity up to 230% by binding to KLβ-gal through lysine ε-amino groups, probably

involving Lysine^{138} of β-lg(6-8) and suggesting that the interaction mechanism might be via the nucleophilic attack of Lys^{138} of β-lg to an electrophile group in KLβ-gal.

In order to study how KLβ-gal would interact with nucleophiles such as lactose, lysine and/or other protein, a 3D model of KLβ-gal was built using the X-ray diffraction coordinates of *E.coli*β-galactosidase A chains (PDB ID: 1JZ7 and 1JZ8) as template (13).

Blind docking of KLβ-gal with lactose showed that the amino acids with the most favorable interaction energy at pH 7.0 were Glu^{482} and Glu^{551} (FDE= -4.82 kcal/mol) which are consistent with the catalytic sites reported for KLβ-gal (5).

Since Lys^{138} of β-lg has been suggested to be the amino acid most likely responsible for the interaction between KLβ-gal and β-lg, blind docking of KLβ-gal with lysine was performed, and 250 possible binding sites were displayed. It has been reported that at pH 7.0, which is the pH at which our studies were performed, KLβ-gal exists as a dimmer (4), because of this, a 3D model of the KLβ-gal dimer was built in order to select the possible amino acids interacting with lysine (Figure 1). The selection criteria for the possible interaction site of KLβ-gal with lysine were based on the most favorable binding energy as well as on the exclusion of those amino acids located at the dimer interface and those of the catalytic site, which due to steric hindrance, or the loss of enzyme activity would clearly not be available to interact with another protein. With these selecting criteria, Glu^{592} was found to be the most probable interaction site with lysine (FDE= -4.55 kcal/mol).

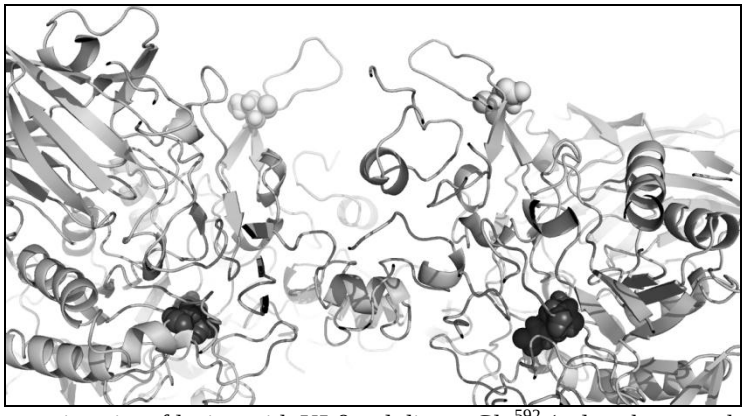

Figure 1.Probable interaction site of lysine with KLβ-gal dimer. Glu^{592} (pale spheres at the top) is far from the catalytic site (dark spheres at the bottom) and is highly exposed.

It is well known that carboxyl groups of proteins can be chemically modified through gradual esterification with alcohols of low molecular weight by adding low concentrations of hydrogen ions (0.02 to 0.1 M) to catalyze the reaction in mild conditions (15, 17). *Kluyveromyceslactis*β-galactosidase has 157 esterificable groups per monomer (156 combined glutamic acid and aspartic acid residues and 1 carboxyl end group). In order to determine the role of side-carboxyl groups of KLβ-gal in the interaction with β-lg, KLβ-gal carboxyl groups were blocked by gradual esterification for 1 (E1) or 2 (E2) hours. A sample was taken immediately after mixing all reagents (E0) in order to discard the effect of the reagents in lactase activity.

Esterification was followed according to Halpin& Richardson (15) to determine the degree of side-carboxyl groups blocked in the different esterified derivatives. After 1 and 2 hours of reaction, 3.9% and 16.5% of carboxyl groups of KLβ-gal were esterified respectively. It is very likely that after 1 hour, the first side-chain carboxyl groups to be esterified were those most reactive and exposed, thus the most probable to interact with β-lg. According to the results of the blind docking, such exposed and thus reactive side-carboxyl group in KLβ-gal, could be Glu^{592} (Figure 1).

Activity of KLβ-gal ester derivatives obtained at 1 and 2 hours of esterification (E1 and E2) was measured to ensure that the enzyme was still active after the esterification process. It was observed that activity

significantly decreased after 1 hour of reaction (α=0.007) and completely disappeared after 2 hours; because of this, E2 was excluded of all further experiments. As reported by (5), *Kluyveromyceslactis*β-galactosidase has Glu^{482} and Glu^{551} at its catalytic site, it is very likely that after 2 hours of esterification these residues were esterified and thus activity was lost.

Jiménez-Guzmán et al. (6) demonstrated that the activating effect of β-lg could be due to the interaction of β-lg with KLβ-gal. In order to study the effect of KLβ-gal side-carboxyl groups in the interaction with β-lg, the capacity of KLβ-gal ester derivative (E1) to bind to immobilized β-lg was compared with that of unesterified KLβ-gal (E0) through affinity chromatography.

Interaction tests were performed immobilizing β-lg on Eupergit, and allowing the esterified derivatives (E0 and E1) to interact with the immobilized ligand. In order to discard unspecific interactions, a control for each derivative (CE0 or CE1) was used in which no β-lg was immobilized, but the reactive oxyran groups of the resin were blocked with glycine.

Specific activity relates the activity ratio to the amount of protein present in a given sample, and therefore helps to establish the proportion of enzyme among other contaminating proteins; when the interaction with the ligand is unspecific any of the proteins may be bound to the support, but their proportions in the eluting mixture would not change; on the other hand, if there were a specific interaction between one protein (in this case KLβ-gal) and the ligand, its proportion in the eluting mixture would decrease. Results showed that the specific activity of the control was not significantly different from that of the initial KLβ-gal solution (α = 0.1483 and 0.2841 for CE0 and CE1 respectively) meaning that KLβ-gal was not specifically bound to the support without protein (Figure 2). When E0 was allowed to interact with the immobilized β-lg, lactase activity of the supernatant significantly decreased (α = 0.03) with respect to the control, suggesting that while the carboxyl groups of KLβ-gal are not esterified, there is still a specific interaction between KLβ-gal and β-lg. On the other hand, when E1 (containing the esterified KLβ-gal) was allowed to interact with immobilized β-lg specific activity of the supernatant remained the same as in the control (Figure 2, α = 0.061), suggesting that when the carboxyl groups of KLβ-gal were blocked by the esterification, its ability to bind to β-lg was lost.

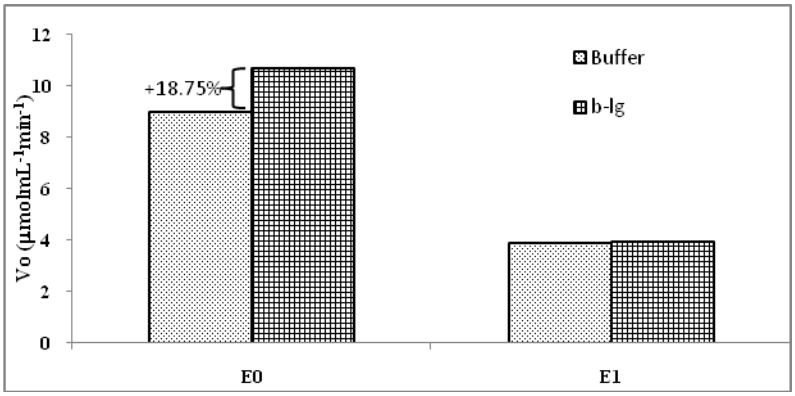

Figure 2. Specific activity of KLβ-gal in esterified derivatives (E0 and E1) after affinity chromatography with immobilized β-lg. Initial refers to the specific activity in the solution before affinity chromatography; control refers to the specific activity obtained when no ligand was immobilized in the support; b-lg refers to the specific activity obtained after affinity chromatography with immobilized β-lg.

In order to evaluate the effect of KLβ-gal esterification in its activation by β-lg, lactase activity of the esterified derivatives (E0 and E1) was determined in the presence of 0.3 mg/mL β-lg (Figure 3). Results showed that when KLβ-gal is not esterified (E0), the presence of β-lg in the reaction media induces a significant activation of the enzyme (18.75%, α=0.0254) which is in accordance with Jiménez-Guzmán et al (6) and Del Moral-Ramírez et al (8) who had reported an important activation of this enzyme by β-lg. On the other hand, when KLβ-gal was esterified (E1), the presence of β-lg did not induce any significant

activation of the enzyme (α=0.4808), suggesting that the availability of carboxyl groups of KLβ-gal is essential for the activation.

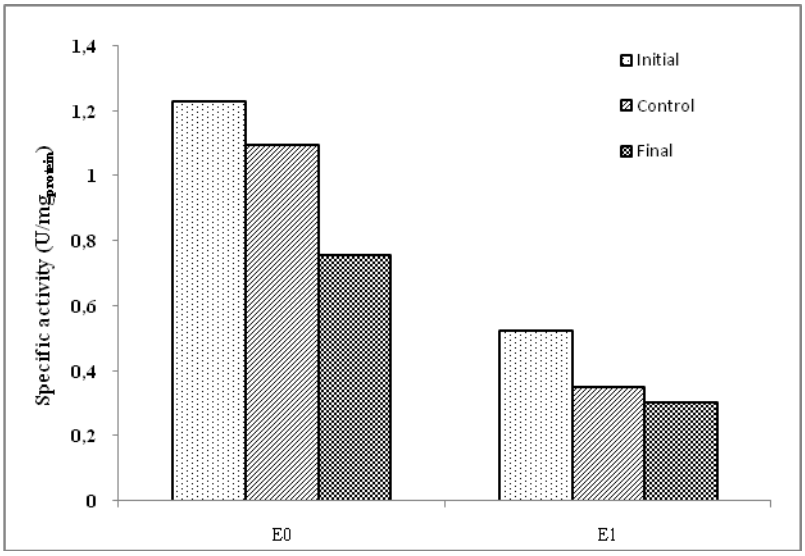

Figure 3.Effect of β-lg on the activity of esterified (E1) and non-esterified (E0) derivatives of KLβ-gal.

When comparing the changes on the activation caused by esterification of KLβ-gal with the changes on the interactions between β-lg and KLβ-gal a direct correlation was observed: as long as β-lg may interact with KLβ-gal its activating effect is observable.Tello-Solis et al. (4) established that small changes in KLβ-gal structure may cause significant changes in enzyme's activity; these changes may be induced by different factors, such as pH or an interaction with another protein. Our results suggest that the interaction with β-lg promotes a conformational change of KLβ-gal leading to a state in which the active site is more accessible to the substrate and thus, activity is enhanced.

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Amylase Inhibitors From Mexican Medicinal Plants To Diabetes Treatment

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Introduction

Diabetes mellitus, or simply diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. The cause of diabetes continues to be unknown, although both genetic and environmental reasons, such as obesity and deficiency of exercise,appear to be considered[1]. It has now become an epidemic with a worldwide incidence of 5% in the general population. The number of adults with diabetes in the world will rise from 135 million in 1995 to 300 million in the year 2025[2]. The greater part of the increase is likely to occur in developing countries[1], where there are many patients in the community with undiagnosed diabetes. Diabetes is being projected as main killer in the next 25 years[2].

One therapeutic approach for treating diabetes is to decrease postprandial hyperglycemia. This can be achieved by delaying the absorption of glucose through the inhibition of carbohydrate hydrolyzing enzymes (α – amylase and α – glucosidase) in the digestive tract[3]. The α-glucosidase inhibitors can retard the liberation of glucose from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial plasma glucose levels and suppress postprandial hyperglycemia[4]. The α-amylase and glucosidase inhibitors are drug-design targets in development of compounds for the treatment of diabetes, obesity and hyperlipaemia[5]. These inhibitors decrease the action of α-amylase leading to a reduction of starch hydrolysis that shows beneficial effects on glycemic index[3].

Plants have always been an important source of drugs and many of the currently available drugs have been derived directly or indirectly from them. Ethno-botanical reports indicate about 1200 plants in the world with anti-diabetic potential, of which more than three hundred have been reported in the literature[1].

The purpose of this study is to evaluate the presence of amylase inhibitors in Mexican medicinal plant extracts to possible elaboration of dietary supplement as alternative treatments for diabetes.Plants selection was performed after analysis of data that reported the presence of amylase inhibitors in the plants of other countries, as well as empirical data that reported traditional use plants to control of diabetes in Mexico.

Materials& Methods

Chemicals

The α – amylase (EC 3.2.1.1) was purchased from MP biomedical, dinitrosalicylic acid from Sigma – Aldrich, other reagent were analytical grade from Baker Co..

Plant material and extract preparation

The *Bidensodorata, Equisetum laevigatum, Cnidoscoluschayamansa, Guazumaulmifolia, Odontotrichumsinauntum, Opuntia, Celbaacuminata, Hintonialatiflora, Agave lechuguilla, Tecomastans, Eupatorium aschemborniaum* plants were bought in naturist store (Saltillo, Coahuila, Mex.). Previous to extraction the biological material was dried at 37° C for one week to constant weight, once dried they are crushed (particle size 1 cm) by dynamic maceration. The extraction was carried out at room temperature (25°C approximately) for one period of 5 husing a stirring speed of 400 rpm. The ethanol (70%) was applied to biological material at a ratio 10:1 (v/w). Finally the solvent was evaporated until the samples were completely dried and could be sprayed again[6].

α-amylaseInhibition Assay

An enzyme solution was prepared with 39.56 U (1.72mg) per test in a volume of 20 ml of cold deionized water. The reaction mixture contained: 0.5 ml of starch (1%), previously heated to boiling in 20 mM PBS, pH 6.9 , for 15 minutes; 0.5 ml of extract prepared at 2000 µg/ ml (o water in the case of control 1 and 2). The hydrolysis was initiated with 0.5 ml enzyme solution (o water in blank assay o control 2) addition. The mixture was incubated at 20 ° C for 3 min. The reaction was by addition of 0.5 ml of DNS-reagentat 96 mM(3,5-dinitrosalicylic acid and potassium sodium tartrate in 2 MNaOH). The mixture was boiled in a water bath for 15 min and then cooled in an ice bath for 5 min. Finally it was diluted with 4 ml of deionized water and absorbance was read at 540 nm[6]. The percentage of inhibition of α-amylase was calculated from absorbance data according the equation: % Activity = (B-D) / (A-C) * 100

Wherein:

B = absorbance in the presence of the sample

D = absorbance in the blank assay (without enzyme)

A = Absorbance in control 1assay (without inhibitor)

C = absorbance in control 2 assay (without inhibitor and enzyme)

The method for evaluation of enzymatic activity inhibition was standardized using acarbose as synthetic drug considered as control.

Results and Discussion

The acarbose - synthetic drug, applied to standardize the analytical technique, was characterized by IC50 (concentration applied to decrease α-amylase activity at 50%) nearly to 1000 ppm. The concentration twice higher than detected for acarbose (purified pharmacological preparation) was selected to carry out the assays with plant extracts.

Figure 1 presents the results of α-amylase inhibition by studied ethanolicextracts. Extracts from *Equisetum laevigatum, Guazumaulmifolia, Celbaacuminata, Odontotrichumsinauntum, Agave lechuguilla* inhibited the enzyme at 5-10%. It may be caused by low concentration of inhibitors in obtained complex mixture. With extracts from *Bidensodorata,Tecomastans,Opuntia*the inhibition was at 13-21%, while with extracts from *Eupatorium aschemborniaum*and *Cnidoscoluschayamansa* the inhibition was nearly to 30-35%. The higher inhibitory effect was observed in the case of extract from*Hintonialatiflora*: in the presence of 2000 ppm the α-amylase activity was decreased at 67%.

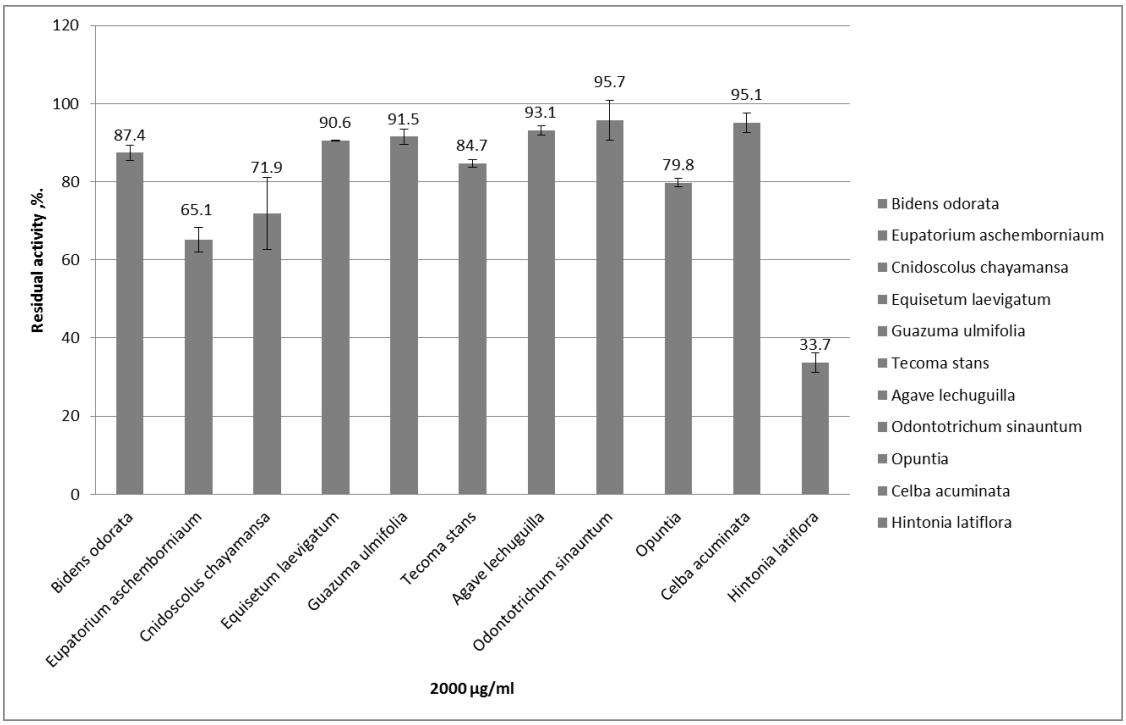

Figure 1. The inhibitory activity of extracts from *Bidensodorata, Equisetum laevigatum, Cnidoscoluschayamansa, Guazumaulmifolia, Odontotrichumsinauntum, Opuntia, Celbaacuminata, Hintonialatiflora, Agave lechuguilla, Tecomastans, Eupatorium aschemborniaum*detected on α-amylase.

The inhibitory effect of *Hintonialatiflora*(known in Mexico as quinaamarilla) was higher than detected in the presence of 1000 ppm of acarbose – synthetic drug. To establish better characterization of plants extracts further analysis is needed.

In Type 2 diabetes, hyperglycemia is a condition characterized by abnormal postprandial increase of blood glucose level[3]. Many natural resources have been investigated for the suppression of glucose production from carbohydrates in the glucose absorption from the intestine to try reducing postprandial hyperglycemia. The inhibition of carbohydrate absorption with plant extracts based on α – amylase inhibitor offers a prospective therapeutic approach for the management of type diabetes mellitus 2. Previously Jayasari showed the antidiabetic activity of *C. pictus*: four different extracts were studied to find possible mechanisms of its antidiabetic action[3]. In the present study it was demonstrated that Mexican plants have a great potential for further research, especially with *Hintonialatiflora*, *Eupatorium aschemborniau, Cnidoscoluschayamans, Opuntia,* and *Tecomastans*. More studies are required to characterize the mode of action of this plant extracts on α – amylase enzyme.

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The Enzimatic Activity Of Peroxidase And Catalase From *Coriandrum sativum* And *Petroselinum crispum* Fresh

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Introduction

Coriander (*Coriandrum sativum*) is one cultivated species that joins the group of medicinal herbs, aromatic and seasoning of higher consumption, is also known for being bug spray at field-level and storage [(6)]. Parsley (*Petroselinum sativum*) is a herbaceous plant that is used as a medicinal herb and is distributed worldwide and is grown for use as a condiment, contains flavonoids, compounds that provide antioxidant and diuretic properties [(3)]. Both plants are basic condiments in Mexican cuisine, used in different dishes which gives flavor to enrich its color, aroma and flavor, along with the therapeutic qualities that meet to bring benefits to the human body [(5)]. In these plants, during storage, handling or processing may cause physical damage to the tissue, causing aerobic darkening of the food this is a enzymatic browning reaction caused by polyphenoloxidase or peroxidase enzyme [(1)].

In the loss of fruit quality plays an important role the balance between reactive oxygen species (ROS) and the antioxidant system, within the ROS superoxide radicals are located (O_2-.), singlet oxygen (O_2), hydroxyl radicals (.OH) and hydrogen peroxide (H_2O_2). Although production of ROS is a normal process, this can be increased not only during ripening and senescence of the tissues, but also for stress-causing agents such as heavy metals, radiation, alteration of the atmosphere, extremes temperature and the attack by microorganisms [(2)]. With respect to peroxidase catalyzes the oxidation of phenols typical of the cells to quinones, which are highly reactive with proteins, nucleic acids, flavonoids and other quinones; in addition these reactions generate brown colors, reduce the sensory properties of texture, color and flavor, decreasing the nutritional quality of the food [(4).]

Catalase (CAT) is an enzyme that catalyzes the decomposition of hydrogen peroxide (H_20_2) into oxygen and water. Hydrogen peroxide is a waste product of cellular metabolism of many living organisms, but given its toxicity should quickly become less dangerous compounds. The peroxidase (PER) is an enzyme that catalyzes the oxidation of a wide range of organic and inorganic substrates [(9)].

In this paper we evaluate, crude plant extracts of coriander and parsley, to evaluate the role of CAT and PER.

Objectives

Determine the activity of catalase and peroxidase in herbs such as coriander (*Coriandrum sativum*) and Parsley (*Petroselium sativum*), divided into leaf, stem and combination of both.

Materials and methods

Raw material. The material was obtained from a local market in the city of Mexico City, Mexico. Fresh product was used for coriander (*Coriandrum sativum*) and Parsley (*Petroselinum sativum*).

Sample preparation. Coriander and parsley were washed with water, then took off the dark and yellow leaves, and dark or dry stems. Then disinfected with a microbicide containing bio-distilled water, animal gelatin and ionized silver at 0.35%. Using 8 drops in one liter of water for a period of 10 minutes.

Consecutively leaves and stem are separated, weighing them. 50 g of each sample are ground, and is added a solution of 5% NaCl, allowed to stand 48 hours, then filtered with satin fabric and stored in amber vial at 4 ° C.

Determination of peroxidase activity (Mahely y Chance, 1954 The determination of peroxidase was performed in duplicate for each sample. The substrate was prepared by diluting 1 ml of hydrogen peroxide (H_2O_2) at 30% in 100 ml of water. To use 1 mL of the above solution to make 100 ml with 0.01 M phosphate buffer, pH 6.0, the dye was 1% o-dianisina in methyl alcohol. For process 0.05 ml of dye solution were added to 6.0 ml of substrate. 2.9 ml were transferred to the cell and a control is performed. At zero time was added 0.1 ml of diluted enzyme. Subsequently the enzyme was introduced to the cell with the pipette tip under the surface. It was stirred and measured the absorbance of oxidized o-dianisina varying amounts of peroxide in the presence of the enzyme. Under these conditions the molar absorbance of the period was equal to the factor of 11.3 at 460 nm.

$$Unidades\ mg = \frac{\frac{\Delta A_{420\ nm}}{minuto}}{11.3\ mM\ mg\ enzima\ \frac{\square}{ml} de\ la\ mezcla\ de\ la\ reacciòn}$$

Determination of catalase (Mahely y Chance, 1954). The catalytic activity of catalase is independent of pH between the limits 3 to 9. This is because the enzyme is constituted by subunits and rapidly loses activity at 35° C. This spectrophotometric method is used where the decomposition of H_2O_2 is measured at 240 nm. The determination of catalase is done in duplicate for each sample. Hydrogen peroxide was used as substrate in 0.059 M phosphate buffer pH 7.5, 0.05M. Using 1 ml of this solution with 1.8 ml of phosphate buffer pH 7.5, 0.05 M and 0.2 ml of extract. The reaction temperature was 25 ° C and prepared for setting a blank at zero absorbance at 240 nm. Subsequently the enzyme solution is added, homogenized and stirred. Reading is taken every minute (5 min). The unit of catalase is the rate of decomposition of 1µmol of hydrogen peroxide of between 10-70 seconds at 25 ° C.

$$\frac{Unidades}{mg} = \frac{\frac{((A_{240\ nm})}{minuto}\ x\ 1000}{43.6\ mg\ catalasa \frac{\square}{\square} mlmezcla\ de\ reaccion}$$

43.6 = molar absorption coefficient for H_2O_2 at 240 nm in a cell with 1 cm light path.

Results and discussion

Determination of peroxidase. The peroxidase is an enzyme not very easy to inactivate, since it presents greater stability in comparison with other oxidative enzymes. The peroxidase activity is given in units per ml of enzyme extract for five minutes. In Figure 1 are shown the results obtained for the sample of parsley, which shows that the stem is 41% lower with respect to the leaves.

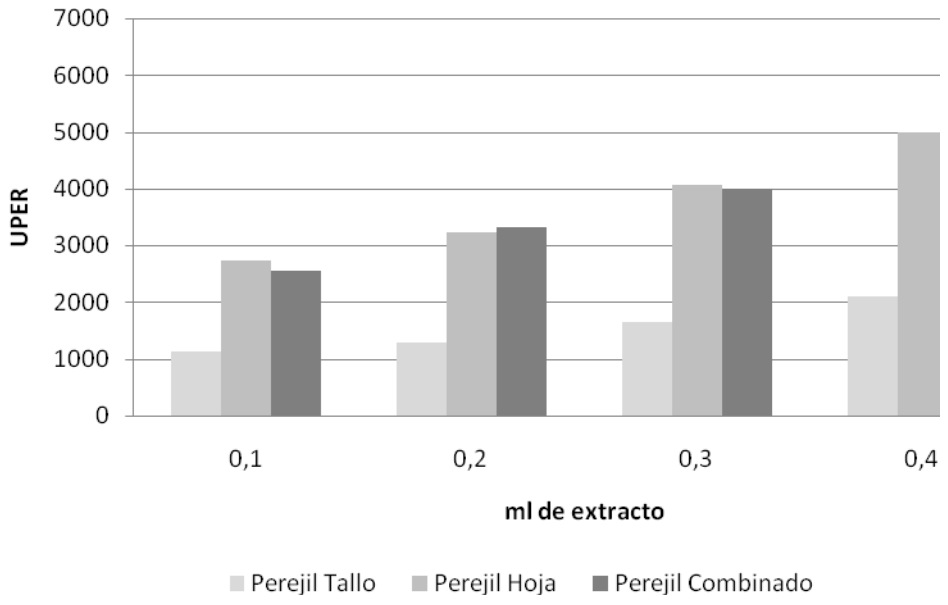

Figure 1. Peroxidase activity in parsley.
Conditions: hydrogen peroxide at 30% in water. Reaction temperature at 25 ° C.
NaCl extract at 5%.

Figure 2 shows the enzymatic activity of peroxidase in coriander samples, showing that there is a great difference between the leaf and the stem, which is 5 times greater the leaf than the stem. There was an increase from the enzyme extract.

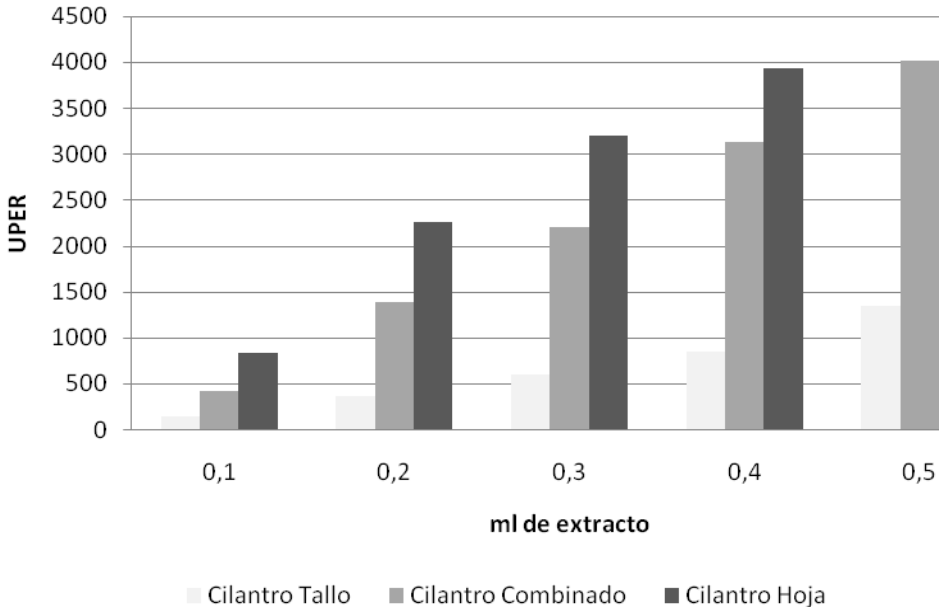

Figure 2. Coriander peroxidase activity
Conditions: hydrogen peroxide at 30% in water. Reaction temperature at 25 ° C.
UPER Units of peroxidase in ml of extract

Peroxidases are widely distributed among higher plants, part of its importance is that they catalyze the oxidation of several electron donors phenols in the presence of hydrogen peroxide, generating free radicals which react together and produce dimers. Some of the physiological functions in the plants of peroxidases are participating in the biosynthesis of ethylene, defense against infection, in wound healing and in the cell wall lignification [(7)].

Determination of Catalase. For the determination of catalase, with respect to the sample of parsley, no catalase activity occurred. However activity was obtained in coriander. In Figure 3, results are shown of this plant, which shows that the activity was constant in stem and leaf shows an increase relative to the amount of extract.

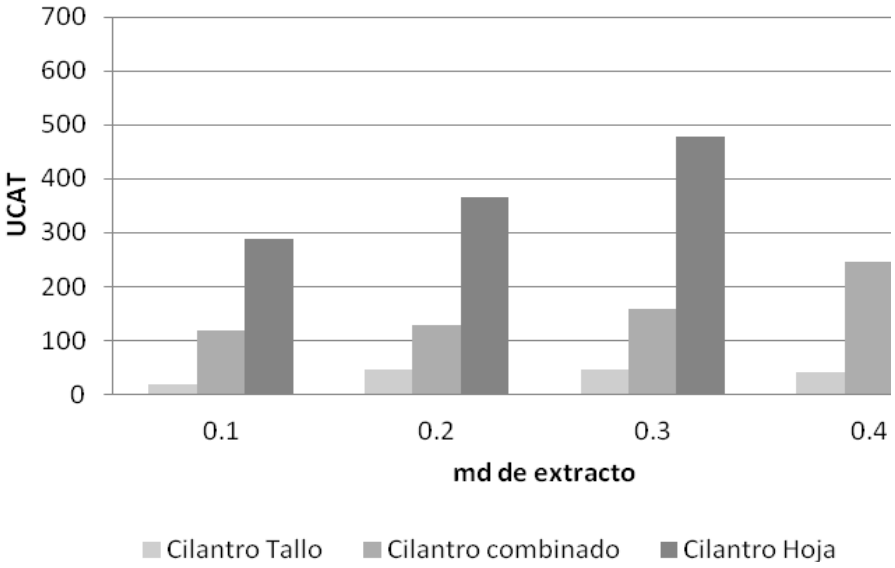

Figure 3. Coriander peroxidase activity
Conditions: Hydrogen Peroxide at 30% in pH 7.0 phosphate buffer, 0.05 M.
Reaction temperature 25 ° C UCAT, units of catalase in ml of extract.

Conclusions

The PER are distributed in the leaves of the plants, that is why they gave greater activity and catalyzing the oxidation of various electron donors phenols in the presence of hydrogen peroxide. The functions which have the PER in plants is its participation in ethylene biosynthesis, defense against infections, wound healing [(8)].

It can be observed a deterioration of the plants when cut, it may be that CAT is more effective in degrading the H_2O_2 than the PER and this acts on the degradation of peroxides that generate enzymatic browning. CAT generally has low affinity for the H_2O_2 when comparing with PER.

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Evaluation Of Enzymes Of The Starch Biosynthesis During The Development Of Bread Wheat *(Triticum aestivum*) Seeded With And Without Physiological Disorder Yellow Berry

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Introduction

Globally it is more consumed wheat that any other grains and it is used to prepare bread, tortillas, crackers, pastries and pasta. (5).

A physiological disorder known commonly as yellow berry exists in the wheat. (12) were one of the first researchers to define "yellow berry" as the appearance of wheat grains in a light yellow, opaque, soft and starchy. The yellow berry could be covering the whole grain or a small fraction. Other researcher, (16) established that it is an important physiological disorder in wheat, which causes the production of grains without color. The problem of yellow berry in wheat has been subject of investigations in some agronomic aspect for many years. Researchers have sought to identify environmental factors and planting of wheat that affect this physiological disorder. Roberts and Freeman (12), discussed that the yellow berry is due to inheritable and climatic conditions (mainly rain), this increase the presence of this physiological disorder. In subsequent studies (13, 6, 14), showed that soil conditions and in principle of nitrogen availability, determine the presence of yellow berry in the wheat grain. (1), found that this physiological disorder is not hereditary, but is due to climatic factors such as humidity, air and sun on the grain when the it is still in the straw. But also It found that the yellow berry in wheat is not hereditary. This author observed that the yellow berry not only is lack of nitrogen but also for an excessive application of potassium. For many years it has been observed that the starchy in wheat is associated with a shortage of nitrates in the soil (4). The content of vitreous grains in hard and crystalline wheats is an important factor, of quality as cooking pasta, grinding and baking. The decrease of the protein content of grain for yellow berry, affect the quality of baking, pasta and milling of baking and durum wheat. (4). Great efforts have been made in recent decades in understanding the biosynthesis of starch cereals that have presented a clearer image of the enzymes that are directly related to the synthesis of these complex molecules. There are 2 classes of enzymes called SSs synthases in plants: starch synthase essential for the synthesis of amylose bound starch synthases the granule, and starch synthases responsible for the synthesis of amylopectin, requirements debranching activity on starch synthesis is very clear, the mechanism. by which the debranching enzymes involved in starch synthesis is not yet fully defined. (11). The objective of this research was to evaluate the enzymes: debranching, synthase of starch 11A and 11B, besides synthase of starch 111, during the development of wheat seeded with and without yellow berry in bread wheat (tarachi cultivar)

Materials and Method

One sample was obtained from commercial bread wheat (tarachi cultivar) with 60% of yellow berry.

This wheat sample manually separated for two samples: wheat with yellow berry and wheat without yellow berry. These samples were seeded with 50, 150 and 250 kg / ha of N, in four blocks and for triplicate, in the school field of agriculture of the University of Sonora. Km 16.5, road to Kino Bay.

Sampling grains

Sampling began after 8, 14. 21. 28 and 35 days after anthesis. In each sampling 10 spikes were cut at least of each of the blocks, for a total of 24 samples.

Oligonucleotide Synthesis

The enzymes tested were: Debranching enzyme (DE), starch synthase III (SSIII). branching enzymes (BE) IIA and IIB, besides 18S III as control, genes whose mRNA were obtained from the NCBI Sequence Viewer v2.0. (www.ncbi.nlm.nih.gob/blast). (Table 1). Of these genes were synthesized oligonucleotides which were used for the Primer 3 program (http://frodo.wi.mit.edu/cgi-bin/primer 3).

Obtaining RNA

The spikes were taken frozen at least 3 per treatment were extracted by one of a deep freezer, placed in a small cooler with ice to which they are detached wheat grains formation (approximately 30), were withdrawn from wheat husks with sterile tweezers to leave the endosperm tubes were placed in sterile, DNAse and RNAse free from which RNA was extracted using the following technique: 50 mg of sample was brought to a column of Lyser pearls Roche Magna equipment were added 200 ul of tissue lysis buffer Was added 40 ul of proteinase K, the samples are placed in the equipment Lyser Magna stirred at 6000 rpm for 5 min, incubated for 2:30 hrs at 55 ° C. Then was added 200 ul of binding buffer, vortex mixing in 10 seconds and incubated 10 min at 70 ° C, then 100 ul of isopropanol added, mixed by vortexing for 10 sec, the supernatant was placed in a column extraction, centrifuged at 8000g x 1 min. The column was removed and placed in another fresh tube precipitate was discarded and the liquid is again added 500 ul of wash buffer, centrifuged at 8000g for 1 min, the column was removed, the liquid was discarded centrifuged at 13.000 g for 1 min and placed in a tube column 1.5 mL sterile, free of DNase and RNase, was added 60 ul of elution buffer, preheated in the center of the column. RNA was quantified at 260nm.

Preparation of cDNA

From the mRNA obtained from the endosperm of wheat samples, cDNA was obtained. With the reagents and the quantities in Table 2.

Real time RT-PCR

Reaction was performed RT-PCR to measure quantitative gene expression of enzymes, the equipment used was a Light Cycler ®, Brand Model 480 System from Roche., Which used the control gene and oligonucleotide and cDNA their preparation described in previous paragraph. The RT-PCR assays were performed at 55 ° C. To carry out the retotranscription for 30 min, then at 95 ° C to activate the polymerase for 5 min followed by 45 cycles that started at 90 ° C for denaturation for 30 sec, 65 ° C for 30 sec and alignment after at 72 ° C for 10 min extension.

Experiment Design and Statistical Analysis

For the purpose of obtain cp real time PCR was used software that is included in ligth cycler equipment. A completely randomized experimental design was utilized with three factors (Nitrogen, development time and type of wheat) to data generated from this research was performed an analysis of variance (ANOVA) with a significance level of 0.05%. To see differences between specific means was used Tukey test. For statistical analysis, we used the SAS 9.0 statistical software (15).

Table 1. Enzyme genes

Enzyme	Genes
SS llA	Sense 5'-TGGTTTCGATGGCACTGATA-3' Antisense 5'-GGGCATTCCACTGACATCTT-3'
SS llB	Sense 5'-CTTAGACTCGGACGCTGGAC-3' Antisense 5'-GTATTTGGGGGAGGTTTCGT-3'
DE	Sense 5'-AGTTACAGAGCGTTCAGA-3' Antisense 5'-AAGAAGCGATGACCAGTT-3'
SS111	Sense 5'-GGGAAGTGGATGCAAGTGAT-3' Antisense 5'-ACAAGTCGCAGCTCAGGATT-3'
18S*	Sense 5' -TTCATATCACGTGCTGCATGG-3' Antisense 5'AGACGACTTCGGTGAGACG-3'

* Used as a control gene, synthesized by Hurkman, William J. et al., 2003

Table 2. Component, quantity and final concentration of reaction superscript III, for real time PCR..

	Quantity (µL)	Final concentration
The reaction mixture 2X	12.5	1X
Template of RNA	5	
Primer Sense (10uM)	0.5	0.2 uM
Primer Antisense(10uM)	0.5	0.2 uM
RT-PlatinoTaq mixture	0.5	
FHR[a] mixture	5	
H_2O PCR Grade	1	
Final concentration	25	

[a] Fusion high resolution

Results and Discussion

In figure 1, is the expression levels of the DE. It is observed that begins to increase its level of expression from 14 days of development after anthesis (AA), showing maximum values at 21 days AA. Expression was higher in samples of wheat seeded with yellow berry (WSYB) with 3 levels of nitrogen and some authors (2,3). DE mention that catalyzes the hydrolysis of α 1-6 glycosidic bonds which are important for maintaining the crystallinity of the starch granule. On the days of high level expression of the DE in a study by (2) in hexaploid wheat where the highest expression were also obtained at 21 days, starting at 15 days increased development AA.

In figure 2, found the values of enzyme SS 111, where can see that the maximum values occur at 21 days AA, increasing its level at 14 days, of samples of wheat seeded without yellow berry (WSWYB) with 150 and 250N and to 28 and 35 still are expressed in WSYB sample 50N. One of the models proposed for the operation of this enzyme by (7) that the SAIII can interview to produce longer chains that extend between clusters and may involve the introduction of the ramifications before they can proceed extension.

Figures 3 and 4 are graphically the results of the BE IIA and IIB, in the first days of sampling is very little expression could detect the higher amounts are between 14 and 21 days AA, but the enzyme BE 11A, at 35 days continues to show levels of expression. Higher values are given in the WSWYB with different levels of nitrogen at 14 days shows a higher expression level in the WSWYB 50N, at 21 days were more treatments showed a good level of expression. These enzymes play an important role in the structure of amylopectin, due to catalyze the hydrolysis of one link α 1-4 - and forms a bond glucan α 1-6 glycosidic on carbon 6 of a fraction of a glucan glycosidic α 1-4, to form a branch. These results may be due to the amount of enzyme that is expressed in WSYB is sufficient to give the branches necessary for this type of wheat starch compared with WSWYB, which also transfers the BE IIA longer chains than IIB during extension. (9). 10 and 17 found that the lack of this enzyme affects the fine structure of amylopectin. In **figure 5**, observed the agarose gels, showing the presence of the enzymes studied, corresponding to sampling 3.

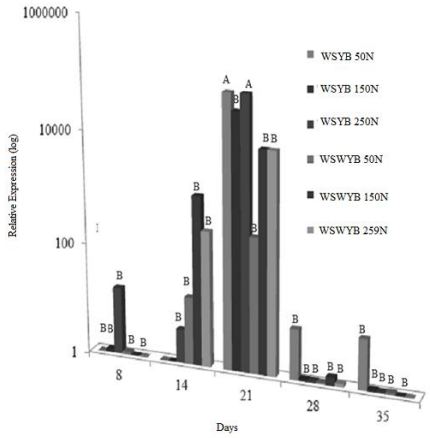

Figure 1. Relative expression of debranching enzyme of wheat seeded with and without yellow berry at different days after anthesis

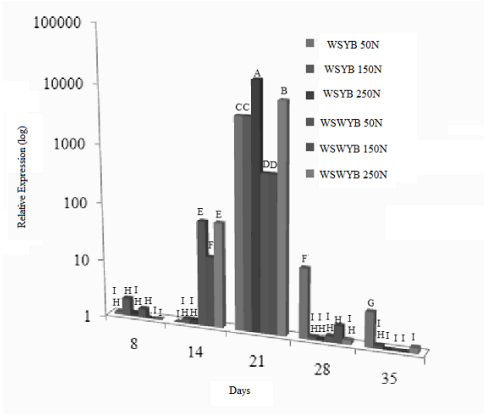

Figure 2.Relative expression of synthase of starch 111 of wheat seeded with and without yellow berry at different days after anthesis

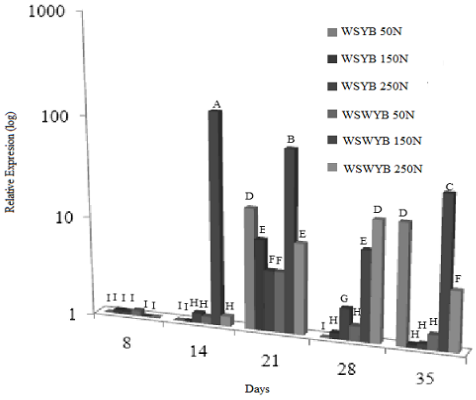

Figure 3.Relative expression of synthase of starch IIA enzyme of wheat seeded with and without yellow berry at different days after anthesis

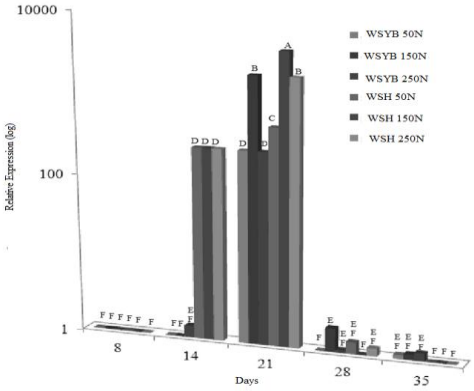

Figure 4 Relative expression of synthase of starch IIB enzyme of wheat seeded with and without yellow berry at different days after anthesis

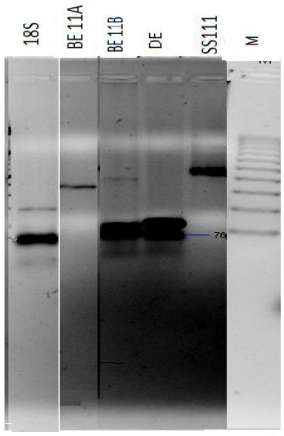

Figure 5. Agarose gel electrophoresis of the enzymes studied corresponding to three sampling

Conclusions

According to the results obtained in the DE, SS III, and BE IIA and IIB, starch wheat with yellow berry sample presents a higher crystallinity, amylopectin is the component which would be most affected and more specifically the fine structure of amylopectin, respectively. Results agree with those presented in the characterization of starch in article (8). The crystallinity is higher in starch with yellow berry.

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Assessment Of Antioxidant Activity Of Hydrolysates As Obtained In Amaranth Stubble

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Summary

Amaranth (*Amaranthus hypochondriacus* L.) is one of the oldest crops in Mexico, and is mainly consumed as a cereal grain as well as a vegetable. However, once the grain is harvested, the plant residue, known as stubble, becomes a waste product, which is usually an ecological issue. It may partially be used as a fuel, substrate for preparing compost or used as animal forage. Thus, this research focused on studying the optimization of acid hydrolysis experimental conditions as obtained in amaranth stubble (AS), its chemical composition and its antioxidant activity. Proximal chemical analysis, total dietary fiber, cellulose, hemicelluloses and lignin as obtained in AS were performed according to A.O.A.C. and TAPPI methods, respectively. Insoluble fiber was isolated and subjected to a chemical hydrolysis using 2.5, 5 and 7.5% (p/v) H_2SO_4 at 121°C during 30, 60, 90, 120 and 150 min. The hydrolyzates were neutralized, then underwent an extraction with ethyl acetate and, finally, two phases were obtained: an aqueous one (PA) and an organic one (PO). Both of them were assessed to get the total polyphenol concentration with the Folin-Ciocalteu method. Eventually, the optimal hydrolyzates were analyzed for antioxidant activity, expressed as IC_{50} DPPH inhibition (DPPH•). AS dietary fiber was made up of 30±0.5% cellulose, 18.4±0.35% hemicelluloses and 9.5±0.5% lignin. Statistical analysis showed that the different hydrolysis conditions influenced significantly on the reducing sugars content, which was released from acid cleavage of hemicelluloses (xilooligosaccharides) and cellulose (glucose), respectively. The highest antioxidants concentration was obtained at 5% H_2SO_4 during 90 min at 121°C. Statistical analysis showed that the different content of the PA was 44.165±8.38%; and PO was 15.49±0.65 mg GAeq./g of total polyphenols. IC_{50} of the PA was 0.97±1.5 mg eq AG/g ; and PO was 0.039 mg eq AG/g. Amaranth stubble might be used as a source of xilooligosaccharides, which might in turn be utilized as prebiotic or as carbon source into a further fermentation to obtain xilitol. Additionally, lignin might also be a source of antioxidant compounds to obtain nutraceuticals.

Introduction

The amaranth plant is classified as a pseudo-cereal given that it yields cereal-like grains. It was one of the main pre-Hispanic crops. Its presence in Mexico dates to 4000 BC which makes it one of the oldest Mesoamerican crops. Throughout history amaranth leaves have been consumed as vegetables and its seeds as cereal (7). At an industrial level enriched cereals, flour, concentrates, starches, oils and colorants are all obtained from amaranth derivatives. These serve as base products for other food and beverage industries to produce amaranth products as well as raw materials for industrial sectors (chemical, cosmetic, pharmaceutical etc). Amaranth is one of the richest sources of protein, minerals and natural vitamins (A,B,C,B1,B2,B3) in the vegetable kingdom. It also contains folic acid, niacin, calcium, iron and phosphorus as well as being one of the foods with a very high presence of amino acids such as lysine. A study completed in 1975 by the National Academy of Sciences in the United States to identify under-exploited crops with great potential, indicated that amaranth is one of 36 crops with the greatest potential in the world. The aforesaid academy described it as 'the best food of vegetable origin for human consumption.'

It belongs to the Dicotyledons class, Caryophyllales order and Amaranthaceae family (2), comprises more than 60 genera and approximately 800 species of herbaceous annual and perennial plants. There are three

Amaranthus genes that produce large inflorescence, A. *hypochondriacus* and A. *cruentus* which are principally cultivated in Mexico and Guatemala respectively and A. *caudatus* which is cultivated mainly in Peru (7).

In Mexico, yields of 1.1 to 6 tons of grain per hectare and 3.8 to 4.1 tons of leaves per hectare have been reported equating to 271,100 tons of agro-industrial residues each harvest cycle of the amaranth grain. The cultivation period of amaranth is from 150 to 180 days allowing 2 cultivation periods per year which results in the annual production of approximately 542,200 tons of residues called 'amaranth stubble'. The current use of amaranth stubble is very limited; growers leave it in the field as a vegetable fertilizer and in some cases it is used as animal fodder (8). However, in the majority of cases it is burnt causing significant contamination. Amaranth stubble is considered a lignocelulosic material. It consists mainly of cellulose, hemicelluose and lignin meaning that it could be a source of dietary fiber, antioxidant compounds and xilooligosaccharides.

Fiber has been studied for centuries and through the years the health benefits of a diet high in fiber have become known. Antioxidants are used as conservatives of various food products to prevent their deterioration. In fact, food requires phenolic components which generally have elevated antioxidant activity resulting in positive effects on the preservation of food quality and human health. For these reasons they are generally recommended to be included in one's diet (1). Today, the use of natural antioxidants is common not only for their reductive properties but also, being natural they are appreciated by the consumer's organism (6). The effects of the xilooligosaccharides on health are mainly related to their action on the gastro-intestinal flora acting as a source of carbon for the bifido-bacterias, promoting their growth. These properties allow the oligosaccharides to satisfy the requirements for the prebiotic components of foods and to be used as active ingredients of biofunctional foods (5, 9).

Methods and Materials

The stubble was obtained from growers of *A. hypochondriacus* L. amaranth in the Tulyehualco zone of Mexico City in November 2010. The enzymes used were: α-amilasa (EC 3.2.1.1), Type XII-A of Bacillus licheniformis, Pepsin A; (EC 3.4.23.1) 1:10000 extracted from the mucous membrane of the stomach , pancreatin extracted from a porcine pancreas, amyloglucosidase of Aspergillus niger solution (Sigma®). Folin-Ciocalteau reagent, prepared with sodium molybdate was used, sodium tungstate, concentrated chloridic acid, phosphoric acid (85%) and distilled water, sodium sulphate (150g). Radical 2,2-difenol-1-picrilhidrazil (DPPH) of Sigma-Aldrich was also used. All the reagents were of analytic grade.

Obtention of insoluble dietary fiber (IDF).

1 gram of fat-free flour was mixed with 25ml of sodium phosphate buffer 0.1M, pH 6. 100 µL of Termamyl was added and then incubated for 15 minutes in a wáter bath. 200ml of distilled water was added and the pH was adjusted to 1.5 with HCl 4M. 100mg of Pepsin was added and then incubated at 40 ºC for 1 hour. The pH was adjusted to 6.8 with NaOH 4M. 100mg of pancreatin was added then incubated at 40ºC with constant agitation for 1 hour. The pH was adjusted to 4.5 HCL 4M. 35 µL of amyloglucosidase was added and incubated at 60 ºC for 30 minutes. The mixture was centrifuged at 5000rpm for 15 minutes at 4ºC. The supernatant is discarded and the resisdues are washed with 10ml of distilled water (twice), 20ml of ethanol (twice), and 10ml of acetone. It is dried on a stove at 105ºC. The resultant IDF underwent proximal chemical analysis in accordance with the official methodologies of the A.O.A.C. (2000). The chemical analysis was carried out in accordance with the official methodologies of TAPPI (Technical Association of the Pulp and Paper Industry).

Hidrolysis acid

A sample of amaranth IDF was placed in test tubes and mixed with sulphuric acid 2.5, 5 and 7.5 % (v/v) in a relation 1:16 (p/v). It was taken to isothermic conditions of 112ºC with pressure of 15lb/pg for 30,60,90,120 and 150 minutes to obtain phenolic compounds. The resultant amaranth hydrolysates were neutralized with NaOH 4M. It was centrifuged at 5000 rpm for 10 minutes. The residues were discarded and the supernatant kept at 4ºC. The extraction was carried out in accordance with the description by Conde 2009. The solvent is recuperated with a rotary evaporator Yamato RE200. Polar (PA) and non-polar phases (PO) are obtained with both phases maintained at 4ºC until their use.

Quantification of total polyphenols and evaluation of antioxidant activity

Quantification of total polyphenols was determined by Spectrophotometry based on the method of Folin-Ciocalteu. For the evaluation of antioxidant activity the radical inhibitor method 2,2-difenol-1-picrilhidrazil (DPPH) was used in accordance with the description by Huang *et al*, 2005 (4), using a standard curve of galic acid.

Results and Discussion

In Table 1 the proximal chemical analysis of amaranth IDF is shown. The results are expressed in humid base (HB) and dry base (DB). A factor of 6.25 was used for the conversion of total nitrogen in raw protein.

Table 1. Proximal Chemical Análisis of amaranth IDF

Análisis	Stubble	
	HB	DB
Humidity	4.1±0.12	-
Proteín	13.5±0.25	14.1±0.26
Ethereal Extract	4.3±0.18	4.4±0.2
Fiber	8.4±0.59	8.5±0.59
Ash	9,7±0.18	10.2±0.19
NFE	60.0	62.8

*HB= Humid Base, DB= Dry Base, * NFE= Nitrogen free extract, calculated by difference of 100*

In Table 2 the chemical analysis of amaranth IDF is shown. It was determined in the Department of Cellulose and Paper "Ing. Karl Augustín Grellmann", of the engineering division of the 'Centro Universitario de Ciencias Exactas e Ingenierías (CUCEI) of the University of Guadalajara. The analysis is expressed in dry base. The α-cellulose was determined from holocellulose.

Table 2. Chemical Análisis of amaranth IDF

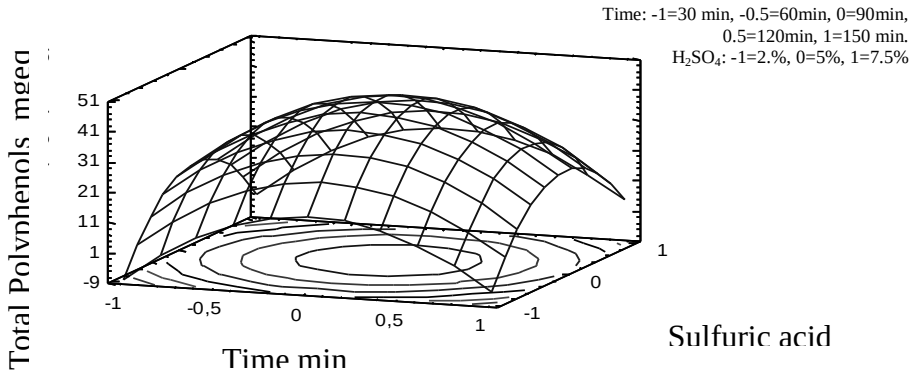
Analysis		Amaranth IDF (g/100g)	Corn Cob* (g/100g)
Ash		1.95±0.08	1.5
EXTRACTABL	*Benzene ethanol*	7.6±0.3	ND
	Ethanol	4.5±0.09	2
	Water	11.1±0.48	2.6
	Material free of extracts		
Lignin		21.2±0.5	16.3
Holocellulose		85±0.54	73
α-Celullose		64.4	64.8
Pentosans		21.1±0.42	28.7

**Cordoba et al.,2010*
***α-Celulosa was determined from celluolose*

In the results obtained we can observe that amaranth IDF has a significantly higher content of extractables, lignins and holocellulose than corn cobs and similar contents of ash and cellulose. For their high content of cellulose corn cobs are used in the energy industry as a raw material for the obtention of biocombustibles. Therefore, amaranth IDF could be of study interest for this sector. On the other hand a significant quantity of pentosans were found in the IDF indicating that xilo- oligosaccharides included in the holocellulose could be obtained and used as prebiotics.

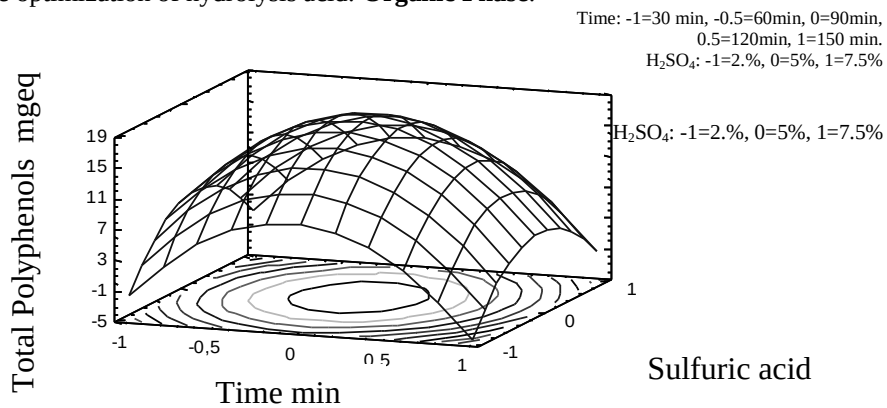
Figure 1 shows the response area of the aqueous phase and figure 2 shows the organic phase of the hydrolysates of amaranth IDF. In general the theoretical and experimental results show an optimal hydrolysis acid at 90 minutes using 5% of H_2SO_4, obtaining a maximum of 15.49±0.65 mgEAG/g for the hydrolysates of the organic stage and 44.16±8.38 mg EAG/g for those of the aqueous phase of quantification of total polyphenols.

Figure 1. Graph of the optimization of hydrolysis acid . **Aqueous Phase**.



*Total polyphenols = 44,165 + 2,142*time + 4,509*sulfuric acid - 22,7952*time^2 + 0,547*time*sulfuric acid - 23,3837* sulfuric acid^2*

Figure 2. Graph of the optimization of hydrolysis acid. **Organic Phase**.



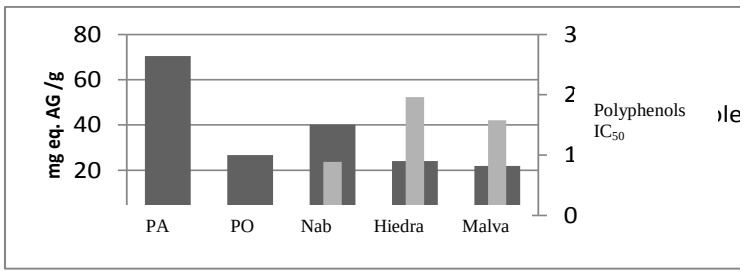
*Total Polyphenols = 18,2928 - 1,29729*time + 1,5103*sulfuric acid - 12,0629*time^2 + 0,0737333*time*sulfuric acid - 7,75637*sulfuric acid^2*

Upon comparison of the polyphenols present in fruits such as mango and pineapple, which are sources of natural antioxidants (2.66 y 1.74 mg EAG/g respectively, 10*)*, it is seen that those found in amaranth IDF are above these levels. The phenolic compounds obtained were liberated from the lignin matrix which indicates that amaranth stubble represents an important source of natural antioxidants presenting an obtention alternative of these compounds for the food industry.

The PA and PO of amaranth IDF hydrolysates do not present a proportional relation in the content of polyphenols and antioxidant activity as shown in Figure 3. This could be attributed to the great variety of

compounds present in each of the vegetative parts. On the other hand, comparing the PO of the organic stage with the extracts of Hiedra it is seen that there is a similar content of polyphenols (3). However, their IC_{50} are completely different. Having a lesser IC_{50} in PO, clearly seen in the graph, does not necessarily correlate to a low level of antioxidant activity. The phenolic compounds liberated from the lignocellulosic matrix by means of hydrolysis acid are capable of reducing the radical DPPH to low concentrations in comparison with molasses used in animal fodder. Consequently it can be presumed that they have a potent antioxidant activity.

Figure 3. Value of IC_{50} of the radical DPPH for different extracts.



Conclusions

Owing to the discoveries in the present work we can infer that *Amaranthus hypochondriacus* L. stubble represents a source of oligosaccharide that could have prebiotic activity and antioxidant compounds applicable to the food industry. The work also presents a simple and practical method for the extraction of these. For these very reasons it is proposed to take advantage of the agricultural residues generated by the harvest of amaranth and to promote the cultivation of amaranth to allow for more options in its commercial use.

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Nutraceutic Potential Of The *Agave atrovirens* Aqueous Extract

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Introduction

Mexico is a country with an important herbal tradition;unfortunately most of the plants used for this purpose are poorly studied and undervalued as a potential source of new drugs.Compoundslike fructanes and saponins have been reported in different Agave genus, fructanes have shown capacity of lowering the blood levels of glucose and cholesterol in mice [1], biological activity of different plant saponins have been reported as molluscicidal [2, 3], hypoglycemic[4], antimicrobial [5], cytotoxic [6-14].On the other hand, phenolic compounds have been extensively reported as natural antioxidants present in a great variety of plants and more recently they have been studied in Agave species in order to evaluate their antioxidant capacity [15, 16].In this work we evaluate the antioxidant activity as well as the phenolic compounds, reducing sugars and saponin content of an aqueous extract obtained from the leaves of *Agave atrovirens* in order to suggest it as a possible nutraceutic extract.

Methods and Materials

***Agave atrovirens*extrac.**The aqueous extract from *Agave atrovirens* leaves was kindly provided by the company Nutracéuticos de Agave S. P.R de R.L as a clear light brown liquid with 2 º Bx, posteriorlywas concentrated using a single effect evaporator until it has 20 ºBx, and evaluated for the content of saponins, phenolic compounds, reducing sugars and antioxidant activity using the ABTS and DPPH methods.

Physicochemical assays. Total phenols content was estimated using the Folin-Ciocalteu colorimetric method[17] and results were expressed as mg of gallic acid equivalent per mL of extract. Saponins were analyzed according to Hernández S.R et al[18] and expressed as mg of Quillaja saponin equivalents per mL of extract. Reducing sugars were evaluated with the Dinitrosalicylic acid method [19] and expressed as mg of Glucose equivalents per mL of extract. The antioxidant activity was evaluated trough the free radical DPPH scavenging capacityaccording to Fukumoto and Mazza[20] and the Radical Cation $ABTS^{\cdot+}$Scavenging Activityaccording to Re et al[21] and expressed as percentage of DPPH·discoloration[22] and Trolox-equivalent antioxidant capacity (TEAC) respectively. Finally, a partial identification of sugars was done by thin layer chromatography (TLC) and HPLC comparing the sample against the appropriate standards.

Results and discussion

After concentration until 20ºBx, a dark brown liquid was obtained; the results of the physicochemical determinations and antioxidant evaluation are shown in table 1 and 2 respectively. According with these resultsreducing monosaccharides are present in a considerable amount in the extract and this could be a consequence of the hydrolysis of polymeric carbohydrate chains due to the thermal process. Fructose seems to be the principal sugar present in the extract (see Figure 1), this is logical because fructanes are the most abundant water soluble carbohydrate in Agave species [23] and as we suppose before, fructose may be produced by the hydrolysis of fructanes type compounds. The other compounds evaluated are present in less quantities specially phenolic compounds but according to Ben Hammissa et al; [15] exist a significant correlation between phenolic compounds obtained from *Agave Americana* leaves and antioxidant activity, and as we can see in table 2, the *Agave atrovirens* extract has a bigger activity than the synthetic antioxidants used as controls in both assays.

On the other hand, there are no similar reports in Agave species for comparing the saponin content obtained in this work. Saponins amounts varying from 0.17 to 16.926 mg/g of *Agave lechuguilla* were

obtained by Hernández S.R et al; [18] evaluating different conditions of extraction, unfortunately a direct comparison is not possible with the results obtained in this work because our results are not expressed in dry basis.

Table 1. Physicochemical determinations in the Agave atrovirens extract

Assay	Results
Appearance	Dark Brown liquid
Brix grades	20 ºBx
pH	6.03 units
Phenols	0.423 ± 0.006 mg of Gallic acid equivalents/mL
Reducing sugars	339.27 ± 2.23 mg of Glucose equivalents/mL
Saponins	59.82 ± 7.02 mg of Quillaja Saponin equivalents/mL

Table 2. Antioxidant activity of *Agave atrovirens* extract

Sample	ARA[b]	TEAC[c]
BHT (500 µM)	55.83 ± 3.85	ND
Gallic acid (500 µM)	98.46 ± 0.54	ND
E1 (15.63 µM)[a]	ND	139.83 ± 12.14
E2 (31.25 µM)[a]	ND	257.40 ± 9.15
E3 (62.50 µM)[a]	49.42 ± 1.91	373.94 ± 20.22
E4 (125.0 µM)[a]	66.45 ± 3.78	ND
E5 (250.0 µM)[a]	75.13 ±3.86	ND

[a]Agave extract concentration expressed as µM equivalent of Gallic acid
[b] ARA = Antiradical activity expressed as % of DPPH* decoloration
[c] TEAC= Trolox Equivalent Antioxidant Capacity expressed as µM Trolox equivalents
ND= Not determined

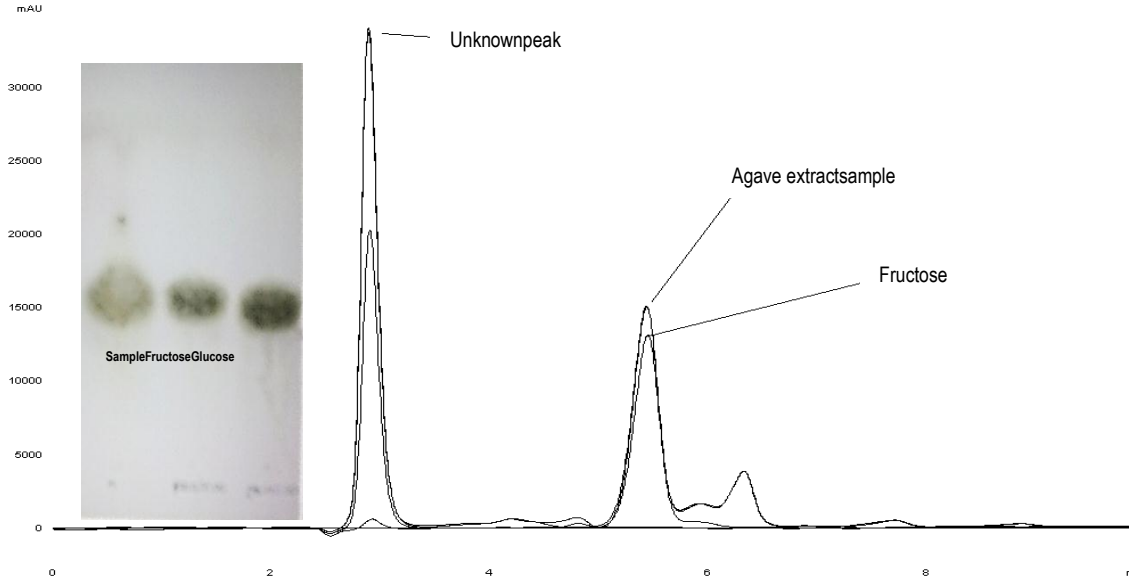

Figure 1. TLC and HPLC Chromatograms of the Agave *atrovirens* extract. (HPLC conditions: Movil phase: ACN:H_2O (75:25), Flux: 0.8 mL/min, Injection volume: 5 µL, Column temperature: 35°C, Run time: 10 min.TLC

conditions: Silica 60, plates Movil phase: Butanol:Aceticacid:Water [2:1:1], Revealing agent 2% H_2SO_4 v/v in Ethanol.)

Conclusion

The antioxidant activity observed in this study and the reported biological properties like antimutagenic, anti-inflamatory, antimicrobial, and hypocholesterolemic activities for phenolic compounds and saponins may contribute to increase the evidence for the beneficial effects of Agave extract and after a purification process could support its use as an antioxidant in different industries.

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Preparation Of A Low-Cholesterol Mayonnaise Type Through The Replacement Of Egg Yolk By Chitosan

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Introduction

The mayonnaise is considered a product for all social strata of different countries and is one of the most popular sauces. It is an oil in water emulsion, which contains as basic ingredients vegetable oil (70-80%), egg yolk, vinegar, lemon juice, and additives such as salt, spices, stabilizers, thickeners, etc.. (1,2,3). However, its high content of cholesterol is one of the egg yolk's major problem. According to recent studies high cholesterol has been linked as one of the main causes of death from cardiovascular disease (4). It is for this reason that consumers are interested in low cholesterol foods (5). The use of chitosan as an alternative emulsifier in the preparation of mayonnaise has the advantage of decreasing the cholesterol content. In previous studies, chitosan has shown good properties as emulsifier (6,7,8). Chitosan is derived from the deacetylation of chitin, a natural polysaccharide and is also the second most abundant polymer after cellulose. Chitin is the major component in crustacean's shells such as shrimp and crab (9) and for this reason, crustacean shells, which are waste products from the food industry, are commercially employed for the production of chitosan with different degrees of deacetylation and molecular weight (9,10). Considering these aspects, the development of a low cholesterol mayonnaise with chitosan as an emulsifying agent, proves to be a good alternative.

Materials and methods

Chitin and chitosan were obtained from shrimp shells following the protocol of our laboratory (11). The molecular weight of chitosan was determined by intrinsic viscosity and the degree of deacetylation was obtained by potentiometric titration (12).

Subsequently a traditional mayonnaise was developed and served as a control, soybean oil was used as well as egg yolk, vinegar, water, salt, sugar and lemon drops (13) using a blender Braun MR 430 CA model. To prepare the low in cholesterol mayonnaise the chitosan was incorporated into the formulation of mayonnaise previously disolved in vinegar at a 2.5% w / w concentration and was replaced by 30% of egg yolk.

Once the mayonnaises were prepared they were stored in sterile glass containers for further analysis, which consisted in the determination of physical, chemical, microbiological and stability parameters. These tests were performed in triplicate obtaining an average, standard deviation and coefficient of variation.

The emulsion stability was determined by droplet size of the mayonnaise prepared recently and after accelerated aging for 2 days at 40 ° C (14), using a ZEIZZ Axioskop 40 microscope fitted with a Evolution VE cooled Media Cybernetics camera and an image analyzer Qcapture Pro 6.0 The 40X objective was used.

Creaming stability was also determined by measuring the cremated volume at the top (VC) and its relation to the total sample volume (VT) after accelerated aging conditions (50 ° C for 48 h) and centrifugation at 1100 g for 3.5 h. Additionally, by visual observation for 30 days, it was determined whether there was phase separation in the mayonnaise.

Percentage of fat was evaluated by the Gerber method, the moisture by a digital thermobalance OHAUS MB45, the protein percentage was determinated using the Sorensen Walker Method and the peroxide index (IP) according to the AOAC method 41.1.16 (15), accelerating the oxidation of the sample to 40 ° C for 7 days (16). The water activity (Aw) was obtained using a water activity meter Testo 650, the pH was

measured with a potentiometer HORIZON 5997-20 ECOLOGY CO, and the acidity was considered as total acidity and acetic acid was determined by titration using the AOAC method 935.57 (15).
The viscosity of the mayonnaise was determined using a Brookfield Viscometer RVDV-II + PRO with needle S06. Because the mayonnaise has a thixotropic rheological behavior (17), a speed of 1 rpm for 5 minutes was applied to assess the decrease of viscosity with respect to time. The test was conducted at 25 ° C one day after both mayonnaises were prepared.
Microbiological determinations were performed for molds and yeasts, mesophilic aerobes, total coliforms and E. coli, using microbiological kits 3M Petrifilm Plate.

Results and discussion

Whereas the degree of deacetylation of commercial chitosan is from 70 to 90% (12) it can be said that chitosan obtained has a high degree of deacetylation, with a value of 83.88% and this is very convenient because it takes less time to neutralize the negative charges on the surface of an emulsion droplet, generating a more stable emulsion (18). The molecular weight of chitosan was 90,297 kDa and it is considered low (19). This result is beneficial in this research, since a chitosan with low molecular weight exhibits a better emulsifying activity (20).
Figure 1 shows micrographs of the droplets of mayonnaises, both once prepared and after aging. The droplet size of the mayonnaises was found to be highly dispersed, thus, there was carried out 10 measurements of droplet diameter of the mayonnaises and then an average was calculated, the results are shown in Table 1. The freshly prepared mayonnaise type got a smaller diameter than the control mayonnaise with significant difference ($p < 0.0001$), and this effect was attributed to the increased surface activity of the continuous phase caused by the addition of chitosan (21). There was an increase in the average droplet diameter of aged mayonnaise relative to that of freshly prepared mayonnaise, this increase is because, mayonnaise is a highly concentrated emulsion of oil, and for this reason, tends to have coalescence of oil droplets, which occurs after the droplets have contact for a long period of time (13).

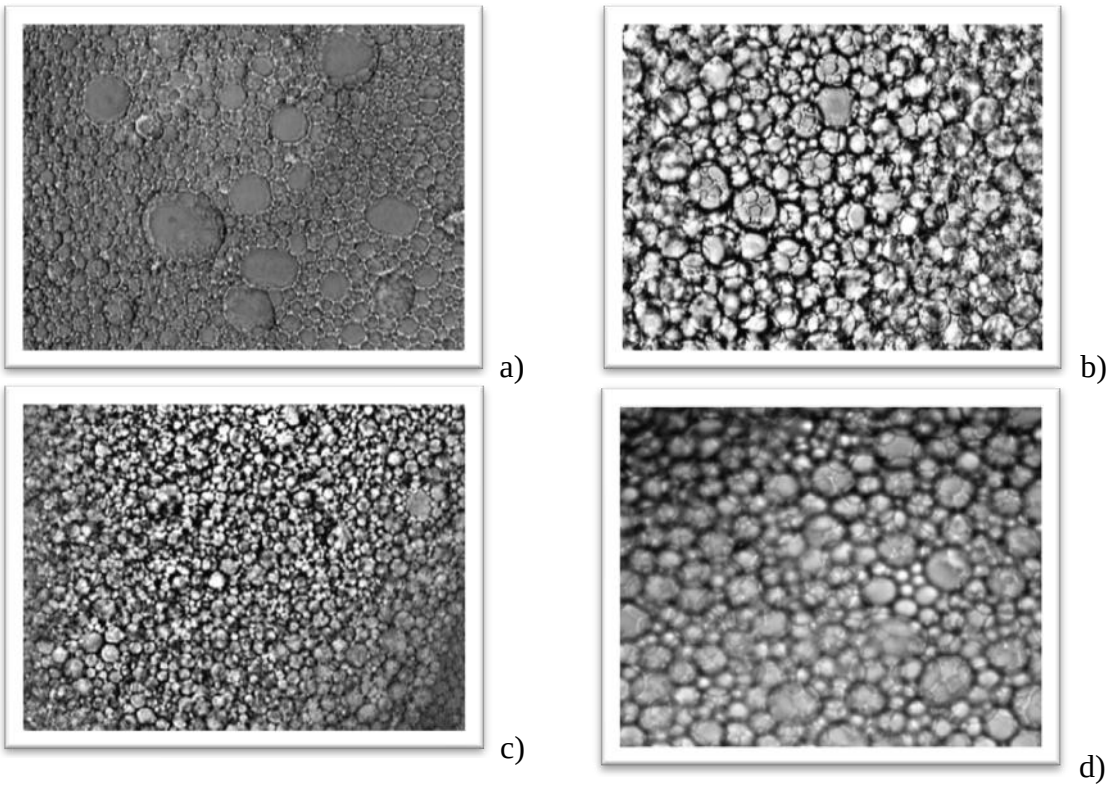

Figure 1 Micrographs (40X), a) Freshly made mayonnaise control , b) Aged mayonnaise control, c) Freshly made mayonnaise type, d) Aging mayonnaise type.

Table 1 Average diameter drop of mayonnaise-type control and freshly preparedand aged

Mayonnaise	Droplet diameter (μm)
Control	**5.0477**
Aged control	**13.5987**
Type	**4.4610**
Aged Type	**8.6430**

Creaming degree of control mayonnaise was 13.59% and that of the mayonnaise type has a lower value (11.25%) with a coefficient of variation of 1.60% and 2.58%, respectively. These results indicate that there is less coalescence of oil droplets in the mayonnaise type, which is consistent with the results of particle size, resulting from the addition of chitosan and the relatively thick and electrically charged interfacial bilayer produced by this biopolymer, since such interfacial film around the oil droplets regulates interaction between the droplets, preventing the oil drops could coalece (22). There was no phase separation in any of the mayonnaises after 30 days.

Table 2 presents the results of fat, moisture, protein, IP, Aw, pH and acidity of mayonnaise. The percentage of fat in the mayonnaise type is only slightly less than the percentage of fat in the mayonnaise control; this occurs because, in the formulation of the mayonnaise type, a lower percentage of egg yolk is added. Mayonnaise control presented a lower percentage of moisture that the mayonnaise type. The percentage of protein was lower in the mayonnaise type than that of mayonnaise control, due the first one possess minor amount of egg yolk. The I.P. of the mayonnaise type was higher than that of mayonnaise control because oxidation takes place on the surface of the drops, therefore a smaller drop, as in the mayonnaise type, provides a high rate of oxidation because surface area that is exposed to the aqueous phase is bigger (16). Aw of the mayonnaise type was lower than that of mayonnaise control, and both values were higher that the value of Aw that require microorganisms which may develop in the mayonnaise, which are molds and yeasts, with Aw values of 0.80 and 0.88, respectively (23). The pH of the mayonnaise type was lower than that of mayonnaise control. This lower value of pH in the mayonnaise type is beneficial, since in this condition, most of the bacteria do not develop because they grow at pH close to neutrality (23). The mayonnaise type presented a higher value of percentage of acidity (0.5%). This higher percentage of acidity may be due to the addition of chitosan, being cationic when dissolved in the aqueous acid solution, the concentration of active ions H+ is higher, thus increasing the acidity of mayonnaise (24). These results are consistent with the results of pH, since having a higher percentage of total acidity the pH is lower, which is also the result of the addition of chitosan.

Table 2 Chemical and physical properties of the control mayonnaise and low-cholesterol mayonnaise type

Mayonnaise	Fat (%)	Moisture(%)	Protein (%)	IP($meq_{peroxide}$ /kg_{sample})	Aw	pH	Acidity (%)
Control	81	12.06	1.71	1.19	0.9206	4.1	0.41
Type	78	14.52	1.13	2	0.9196	3.93	0.50

Figure 2 shows the decrease of the viscosity of both mayonnaises with respect to time describing a thixotropic behavior. The mayonnaise type showed higher viscosity values than mayonnaise control during the test time. The higher apparent viscosity of the mayonnaise type is due to the addition of chitosan in the formulation, because the addition of chitosan increases the viscosity of the continuous phase and therefore the stability of the emulsion (21).

Microbiological determinations showed no presence of molds and yeasts, or mesophilic aerobes or total coliforms and *E. coli* was negative in both mayonnaises. These results show that the sanitary quality of

raw materials employed in developing the mayonnaises was good, as there was adequate and hygienic processing handling and packaging of mayonnaise.

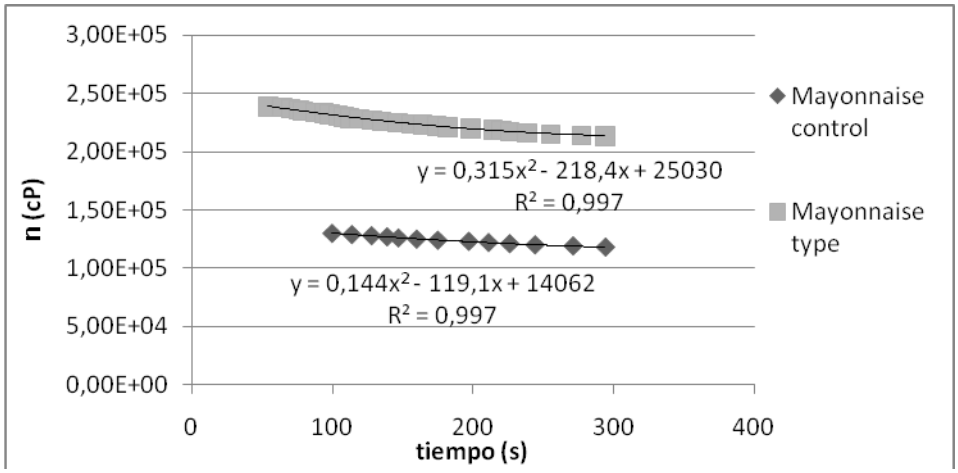

Figure 2 Apparent viscosity versus time at 1 rpm of control mayonnaise and mayonnaise type.

Conclusions

The low molecular weight and high degree of deacetylation of chitosan worked well for the development of a more stable emulsion. The droplet size obtained in the mayonnaise type is smaller and less likely to coalesce. The mayonnaise type has less fat and protein and because the antimicrobial properties of chitosan, the product is maintained in a sanitary good condition.

Acknowledgment. To the Academic program PAPIME PE203211 for the facilities provided to carry out this work and financial support.

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Effect Of Drying Temperature On The Pigmentsand Antioxidant Activity In Squash Flowers (*CucurbitaPepo*)

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Introduction

Squash flowers (SF) are highly appreciated as a traditional food ingredient in Mexican cuisine. SF color ranges are from dark yellow to light orange, andhas been associated with the presence of natural antioxidants such as carotenoid compounds[1,2,3].Epidemiological studies have also shown a strong and consistent protective effect of vegetable consumption against the risk of several age-related diseases this effect is attributed to the antioxidant vitamins and other phytochemicals [4,5].

SF have a limited shelf life due to their high moisture content, causing important postharvest losses attributed to chemical and microbial deterioration. Therefore, a feasible way of increasing SF shelf life is by developing reduced moisture products. However, even though air drying is the most widely used technique for fruit and vegetable dehydration, it may have detrimental effects on both nutritional and sensory quality of heat-sensitive components, including antioxidants. The aim of the study was to assess the effect of drying temperature on pigments and antioxidant activity of SF.

Methods and materials

Sampling and flowers characteristics

In order to choose the squash flowers (*Cucurbitapepo*) for this study, flowers var.GreyZuchinni with significantly specific characteristics, that is, flesh color (yellows and oranges), along with size (8-10 cm) and weight (3-5 g) were selected. 100 male flowers were cultivated and harvested in a farm in San Andres Cholula, Puebla, Mexico. All flowers were to the laboratory the same day right after the picking. All flowers were delivered to the laboratory on the same dayright after picking.

Dryning process

Samples were subjectedto convectivedrying at temperatures of 70, 80 and 90°C and resulting drying curves were mathematically described with Page's model in order to estimate the required drying time 420, 330 and 240 min, respectively, to achieve a final free moisture fraction of 0.05.

Analytical Assays

Samplesoffresh (F) and dried (D) SF were crushed in a coffee grinder for 2 min, to avoid heating of the flowers. Powdered plant samples were extracted by acetone to analyzetheir pigments (total carotenoids, chlorophyll *a* and chlorophyll*b*).

Determination of Pigments

The concentration of chlorophyll a, chlorophyll b and total carotenoids was determined quantitatively by its absorbance at 662, 645 and 470 nm, respectively.Each component (mg/L) was calculated as described by Lichtenthaler and Wellburn (1985)[6].They were calculated as follows (1):

Chlorophyll a $11.75Abs_{660}$-$2.35Abs_{645}$

Chlorophyll b $18.61Abs_{645}$- $_{3.96}$ Abs_{662}

Total carotenoids 1/227 1000 Abs_{470}- 2.27 Cl*a*-81.4Cl*b*

Determinations of Total Antioxidant Activity

The antioxidant activity of themethalonic extracts, on the basis of the scavenging activity of the 2,2-Di(4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) free radical, was determined by the method described by

Mongkolsilp*et al.*, 2004 [7]. An aliquot of 500µl of each extract solution was mixed with 2ml of 0.1mM methanolic DPPH radical to give final concentrations of 2-9 mg extract/ml DPPH. By using Jenway spectrophotometer at 515 nm, the reduction of DPPH radical was measured with continuous monitoring of the decrease of absorption every minute up to 30 min.
Similarlythe ABTS (2,2'azinobis- 3-ethylbenzothiazoline-6-sulfonic acid diammonium salt) radical was generated with manganese dioxide.Then, 1.8 ml of the $ABTS^+$ solution was added to 150µl of sample (dilutedas required) and vortexed for 10 s. The decolourisationcaused by reduction of the cation by antioxidants fromthe sample was measured at 734 nm at exactly 6 min after initialmixing.
BHT (butylatedhydroxytoluene, mg/ml) and 2.5mM Trolox(6-hydroxy-2,5,7,8tetramethychroman-2-carboxylic acid) were used as standard antioxidants.
All determinations were carried out in triplicate at each separate concentration of the standard and samples. The antioxidant potential results are expressed as IC_{50} values, which were calculated from the linear regression of the percent inhibition versus extracts concentrations.

Statistical analysis
The effect of air-drying temperature on pigments contents and IC50 of antioxidant activitywas estimated using Statistix 8.1. The results were analyzed by an analysis of variance (ANOVA), with a significancelevel of $\alpha = 0.05$ and a confidence interval of 95% ($p < 0.05$). In addition, the multiple range test (MRT) included in the statisticalprogram was used to demonstrate the existence of homogeneous groups within each of the parameters.

Results and Discussion

Variations of the moisture content with the drying time *t* for varying values of the drying parameters (mainly, the temperature *T* and the relative humidity Ψ) were studied. Figure 1 shows a typical characteristic dying curve (moisture content compared with time) of flowers samples during drying operation at different temperatures (70, 80 and 90°C)to achieve a final free moisture fraction of 0.05.

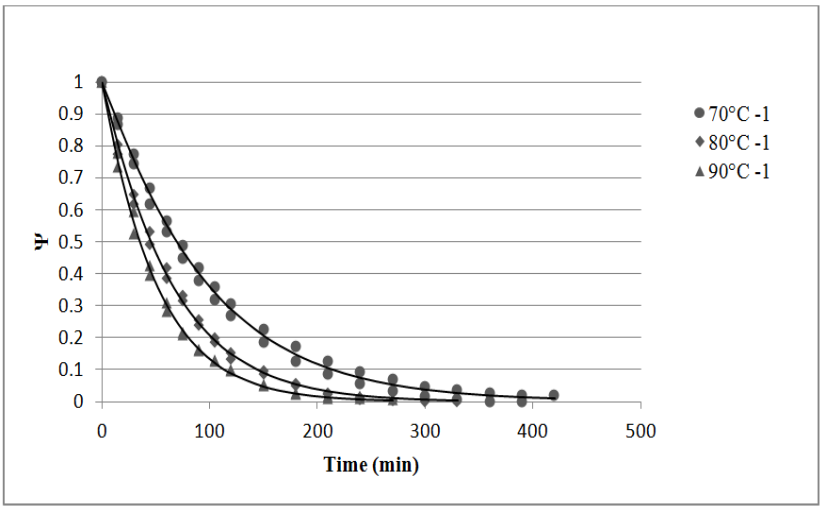

Figure 1 Drying curves in squash flowers at 3 different temperatures described with Page's model in order to estimate the required drying time.

The coloring genus *Curcubita* flowers are mainly due the high content of carotenoid compounds, particularly of β-carotene[8].While the chlorophyll contentsare found in low

concentrations,resulting inthe yellow colorationcharacteristicofthistypeofflowers. In this study carotenoid, chlorophyll *a* and chlorophyll*b*contents in dried products were in the ranges of 0.91-1.70 mg/100 g, 0.0019-0.004 mg/100g and 0.001-0.0032 mg/100 g dry product, respectively. A higher retention of these compounds was obtained with drying at 70°C ($p < 0.05$) (Fig 2A).Carotenoidcompoundsare relatively stableduringdrying processes, however fortemperatures of 80°Corhighertend to degrade[9]. This effect ofdrying temperatureonthetotal carotenoidcontentoftheflowerswas observedin the treatments of 80and90 °C (Fig 2B).

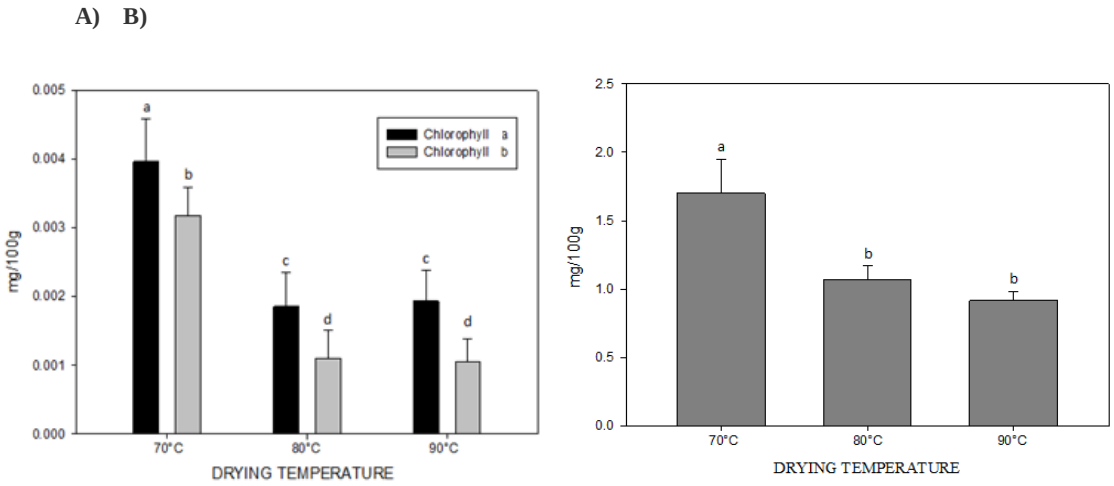

Figure 2 Mean values of pigment contents in squash flowers during dying processing at 3 different temperatures. A) Chlorophyll concentration. B) Total carotenoids concentration.

Antioxidant activity (expressed as the amount ofsample neededto reduce by50% the free radical scavenging capacity) ranged from 4-4.7 mg dry product for both ABTS and DPPHmethods, observing the highest residual antioxidant activity at 90°C ($p < 0.05$).
DPPH and ABTSscavenging activities of squash flowers may be explained by the presence of flavonoid andphenylpropanoid glycosides-compounds known as potential hydrogen donors and radical scavengers[10].

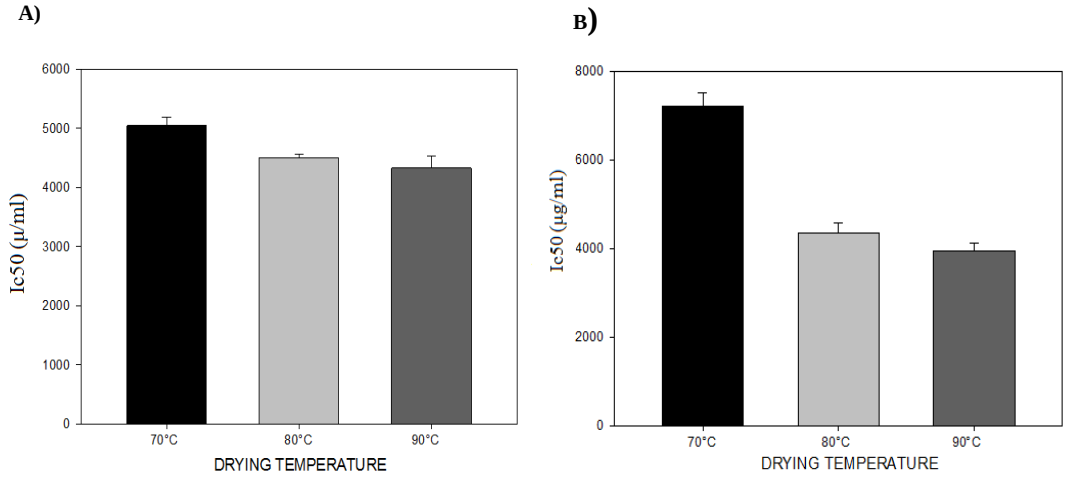

Figure 3 IC $_{50}$values of squash flowers extracts. A) DPPH radical method and B) ABTS radical method.

These results may be related to those reported by Jacobetal. (2010) [11] who mentioned that the thermal processes can affect the antioxidant activity of vegetable products depending on the time duration instead of temperature of the drying process (Fig 3A and 3B).

This study has confirmed that the drying process may be used to conserved squash flowers with increased shelf-life and wider marketability, increasing their consumer appeal by stressing the potential health benefits associated with antioxidant-rich foods.

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Determination Of Antioxidant Activity And Phenolic Compounds Of *Hamelia Patens*

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Introduction

Currently there are reports that demonstrate the use of medicinal plants in the treatment and prevention of various diseases (1). It is also known that our ancestors used herbs in order to alleviate ailments and cure diseases, to strengthen the body's defenses, to control the physiology and emotions of consumers (2). These facts have been arranged that, today, plants have become the basis of research. This is due, in large part, to the presence of bioactive compounds in the stem and the aerial parts of plants.The most ubiquitous in the plantae kingdom and numerous bioactive compounds are polyphenols, same to be essential to their physiology, contributed in the morphology, growth, and reproduction of plants (3). Polyphenols are compounds responsible for providing the antioxidant activity (4) and thereby, are involved in the prevention of diseases such as cancer and cardiovascular diseases (5).

Plants are a potential source from antioxidants natural, however, the antioxidants of aqueous extracts of plants have not been studied extensively (6), is for this reason that, in the present investigation, it was evaluated the antioxidant capacity and phenolic content of aqueous extracts of the plant known as ripe banana (*Hamelia patens*).It is believed that *Hamelia patens* is native to United States of America (7). However, it has had a good develop in large part of the Mexican Republic (8). Ripe plantain is also known as mazamora (7), coralillo, or herb santa, cimarron, and belongs to the Rubiaceae genus. It is a shrub to 3-6 m tall, it has leaves opposite on the stem which are 5-15 cm long (9). Their flowers are dark yellow, orange or red, while the fruit of *H. patens* is red, turning to black in maturity (7).*H. patens* have antihemorrhagic and healing qualities. In addition, anti-inflammatory and analgesic properties are attributed to him (10), also has qualities anti-pyretic, antimicrobial and anti-fungical. Also used against cancer, diarrhea, dysentery, jaundice, malaria, and wounds, among others (11), also it is used as antibiotic for the treatment of infectious processes, especially in rural areas, where preparations obtained from plants (12) are applied.

The family Rubiaceae is a primary source of natural, medicinal and hallucinogenic products. Studies fromquimiotaxonomiaof alkaloids, reported three structural types of alkaloids in this plant: quinolinics, isoquinolinics and indolics (13). The leaves of *H. patens* contain saponins, steroids, and tannins. The flower contains flavonoids such as apigenin and narirutin (10). Likewise, it was identified stigmasterol, ursolic acid, aricin, oxindol, rotundic acid and catechin (14), also found: ephedrine, rosmaric acid and the flavones: petunidin, malvidin, and narirutin; the alkaloids, oxindolicspalmirin and rumberin, maruquin, isomaruquin and alkaloid A, isopteropodin, pteropodin, speciofilin and alkaloids indolicstetrahidroalstonin and aricin (15).

Materials and methods

Preparation of the samples. The leaves of *H. patens* were collected in city Valles, S.L.P., were subsequently performed aqueous extracts through an infusion accumulate in proportion 1:50 w/v,

using freshly cut leaves and distilled water at a constant temperature of 95 ± 2°C, for periods of 3, 5, 8, 10, 12, 15 and 30 minutes corresponding to the times of infusion. Finally, the extracts were leaked using whatman filter paper N ° 2.

Evaluation of antioxidant activity. The antioxidant activity was evaluated with the methodology described by Brand-Williams *et al.* (1995) (16), through inhibition of the stable radical DPPH. To do so, 3 mL of the methanol solution DPPH $6.1x10^{-5}$M with 100 µL of the aqueous extract, the absorbance was measured at 5 seconds for 30 minutes, at 515 nm. The antioxidant activity was expressed in $–D.O.^{-3}$ /min/$mg_{m.f.}$ it was established from the determination of the equation proposed by Manzocco *et al.* (1998) (17).

Inhibition of free radicals (RL). The percentage inhibition of RL was determined taking the initial (DPPH) and the final absorbance (DPPH-extract complex study) respectively.

Quantification of total polyphenols. The polyphenols content was assessed in accordance with the methodology of Folin-Ciocalteau (18). To quantify the polyphenols from diluted aqueous extract in water deionized in proportion 1: 10, then mixed 1 mL of the above dilution with 5 mL of Folin-Ciocalteau reagent at 10%, and they were left to stand for 8 minutes. Then it was added 4 mL of solution of Na_2CO_3 to 7.5%, followed by homogenization, finally the solution incubate for 2 hours at 25°C. Past this time we read the absorbance of the solution to 740 nm. The results were expressed as equivalent of Gallic acid (GAE/L).

Statistical analysis. The data obtained were expressed as the mean ± standard deviation. Determinations were made in triplicate, calculations and graphs of the data were processed using the software KaleidaGraph.

Results and discussion

The formation of difenilhidracina is an important feature in the development of antioxidant activity (19), and is the result of the ability of the plant extract to discolor the DPPH radical solution from purple to yellow. A high antioxidant activity measured by kinetic through radical DPPH is due to the presence of phenolic compounds (6). Which have proved to possess antioxidant properties (20). In table 1 are observed the phenolic content and antioxidant activity the percentage inhibition of RL the aqueous extracts of *H. patens* with respect to the time of infusion.

Table 1. Content phenolic, antioxidant activity and percentage of inhibition from freeradical of extracts of *H. patens* in respect to the infusion times.

Infusion time	Polyphenols (mg GAE/L)	*Antioxidant Activity (D.O. $^{-3}$/min/$mg_{m.f.}$)*	% Radical inhibition
3	259.11 ± 2.43	8.57 ± 0.41	92.91 ± 0.11
5	304.54 ± 2.95	10.29 ± 0.11	93.60 ± 0.26
8	391.87 ± 1.93	12.48 ± 0.54	94.01 ± 0.01
10	407.33 ± 4.83	14.73 ± 0.94	94.44 ± 0.27
12	476.61 ± 1.47	19.43 ± 0.46	96.59 ± 0.19
15	427.63 ± 1.93	18.21 ± 0.14	95.45 ± 0.14
30	294.23 ± 1.93	10.96 ± 0.59	93.77 ± 0.17

GAE: Gallic acid equivalent

The polyphenols release was increased as the time infusion pass from 3 to 12 minutes long, being at this time when the higher content of phenolic compounds was obtained (476.61 mg GAE/L). Likewise, it was observed that after 12 minutes of infusion the content of polyphenols exhibited a marked decline. This behavior is attributed to antioxidant/prooxidante activity of phenolic compounds, which depends on extrinsic factors such as the temperature, behavior chelating, pH, the structure, the number and the position of the substituents (21). So in this case, assume that the time of exposure to a high temperature resulted in the oxidation of phenols and this stopped their antioxidant action.

With respect to the antioxidant activity described to aqueous extracts of *H. patens*, we showed that the highest value of D.O. was obtained at 12 minutes of infusion and was 19.43 $\pm$ 0.46 -D.O.$^{-3}$/min/mg$_{m.f.}$ The antioxidant effect found in the aqueous extracts of *H. patens* may be attributed to the presence of tannins and catechins in the leaves of this plant (10 and 14), characterized by having a high antioxidant activity (22).

In earlier studies, was evaluated the antioxidant activity of green tea and the tea, describing antioxidant activity of 5.6 and 1.9 D.O.$^{-3}$/min/mg$_{m.s.}$, respectively (17). In contrast, the antioxidant activity observed from the aqueous extracts of *H. patens* turned out to be much higher with respect to that reported for the green and black teas which you can infer that by drinking an infusion of H. patens permit better antioxidant benefits.

The linear analysis between the antioxidant activity of aqueous extracts of *H. patens*, measured in kinetic form with the radical DPPH depending on the content of polyphenols, obtained a value of r=0. 948 (Figure 1), which indicates that there was a significant relationship between both parameters, i.e., the total phenols content presented an important relationship with the capacity to act of antioxidant agents released at different times. This behavior matches with the reported for aqueous extracts of plants (22). On the other hand, from the phenolic compounds present in plants, flavonoids are the majority antioxidant component (4), and because of this, there is a positive correlation between phenolic content and antioxidant activity.

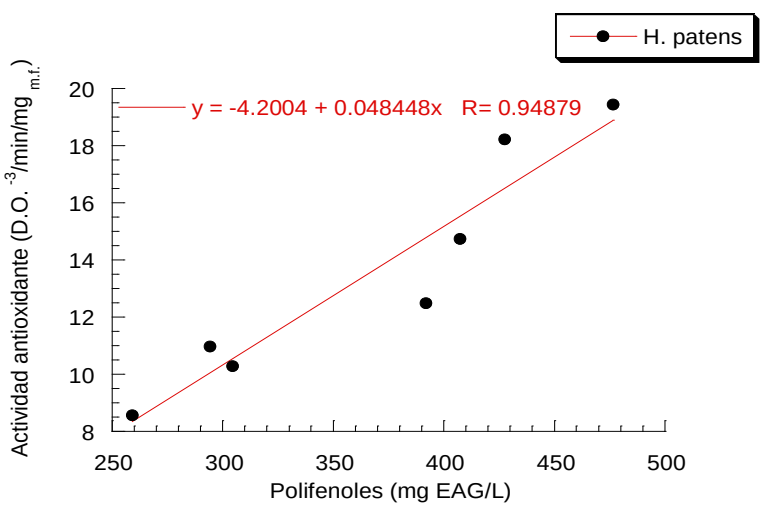

Figure 1. Correlation between antioxidant activity phenolic content of extracts of *H. patens*.

In terms of the percentage inhibition of radical free, the observed range was 92.91 to 96.59%, indicating that there was stability during the analysis period. The capacity of inhibition of radical free, he was accomplished by donating an electron from the antioxidant molecule to free radical molecule,

allowing the stabilization of the radical, with the consequent conversion of the antioxidant in a non-toxic molecule.

Recent studies indicate that tea and infusions made from plantsis the second most consumed beverage in the world, surpassed only by the water (23). Known as catechins, the flavonoids exert protective effects against diseases such as diabetes mellitus, cancer, heart disease, viral infections, ulcers and inflammation, in addition to its antioxidant, antimicrobial actions, and hemoprotectora (24). Therefore, it is important consume a diet rich in antioxidant compounds, which contribute to improving the quality of life of human beings, this fact has led researchers to establish a dose equivalent to 23 mg/day (25) of flavonoid intake, also ensures that the main source of flavonoids is contained in beverages such as tea and infusions. With this study we showed that H. patents is an important source of antioxidants.

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Production Of Antioxidant Compounds By *In Vitro* Cultures, A Promising Biosource Of Functional Food Additives

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Introduction

Many plants are appreciated for human beings as they show beneficial effects on health, such as *Buddleja cordata* Humb. Bonpl. & Kunth (Buddlejaceae), *Ibervillea sonorae* Greene (Cucurbitaceae) and *Prosopis laevigata* Humb. & Bonpl. Ex Willd M. C. Johnst (Fabaceae). Those species are commonly used by Mexican folk to counteract diseases that affect significant amounts of people, so *B. cordata* is used as remedy against stomach and skin infections, dysentery, affections related to antiinflamatory, antitumoral, antiseptic and diuretic activities (Rodríguez-Zaragoza y col., 1999); while the *I. sonorae* uses comprise skin diseases, stomach ulcers and diabetes mellitus (Hernández-Galicia y col., 2007); and *P. laevigata* is applied to treat dysentery, eye diseases, and to act as purgative, emetic and antibacterial agent (Almanza y Mova, 1986). From those plants, mainly from leaves, diverse ethnopharmacological and phytochemical studies have revealed the presence of bioactive compounds, and in some cases the specific biological activity have been elucidated, being that compounds identified as phenolic or phenylpropanoid compounds. The phenolic compounds are secondary metabolites produced by plants in response to stress conditions such as attack from pathogens, herbivores or insects, wounding, UV light, exposition to heavy metals, among others. The ability of phenols to counteract several stress conditions is related to its great reactivity, thus a phenolic compound can show a wide range of biological activities, of which can highlight the antioxidant, cytotoxic, antimicrobial, antiviral, antitumoral, antiinflamatory, analgesic, pesticide, among others. Certainly, the antioxidant activity is distinctive for health since several metabolic cellular processes produce free radicals that cause oxidative stress which if are not scavenged by own antioxidant machinery, serious damages to biomolecules can be triggered (Vermerris y Nicholson, 2006). Then, the exogenous acquisition of phenols possessing an antioxidant capability can significantly contribute to reduce oxidative cellular stress, and it is hope that their incorporation to foods will contribute substantially to develop them as functional. Moreover, to accomplish it, the development of sustainable technologies for providing a continuous and controllable biosource of phenols must be carried out in order to not undermine the natural resources, such as *in vitro* cultures. *In vitro* or tissue culture comprises several techniques based on the totipotent capability of the vegetal cells, making possible develop cultures of cells, tissue, organs or complete plants growing under environmental and nutritional conditions controlled. So, through *in vitro* cultures is possible to exert a relative control under secondary metabolite production (Georgiev y col., 2009). The aim of this work was to determine the total phenol content (TPC) and the antioxidant activity of *in vitro* cultures from *Buddleja cordata*, *Ibervillea sonorae* and *Prosopis laevigata*.

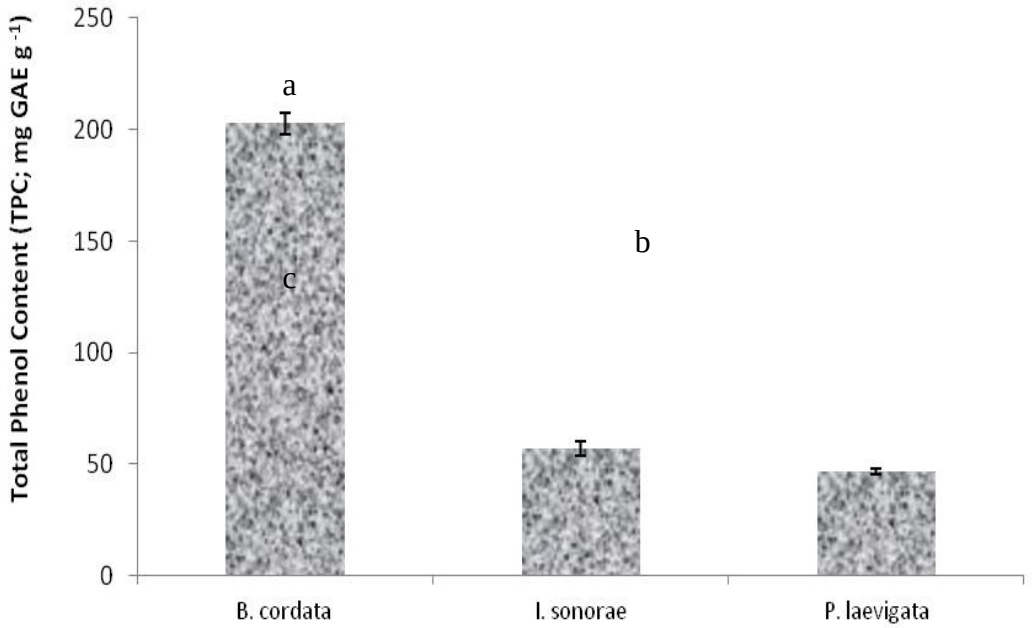
Methods and materials

Establishment of cellular lines: Callus cultures of *B. cordata*, *I. sonorae* and *P. laevigata* were provided by The Universidad Autonoma Metropolitana, Campus Iztapalapa (UAM-I), being every cellular line propagated according to previous reports (Estrada-Zúñiga y col. 2009, 2012; Buendía-González y col. 2010) through 8 subculture cycles, being the two latter used to determine the growth index (GI), total phenol content (TPC) and antioxidant activity.

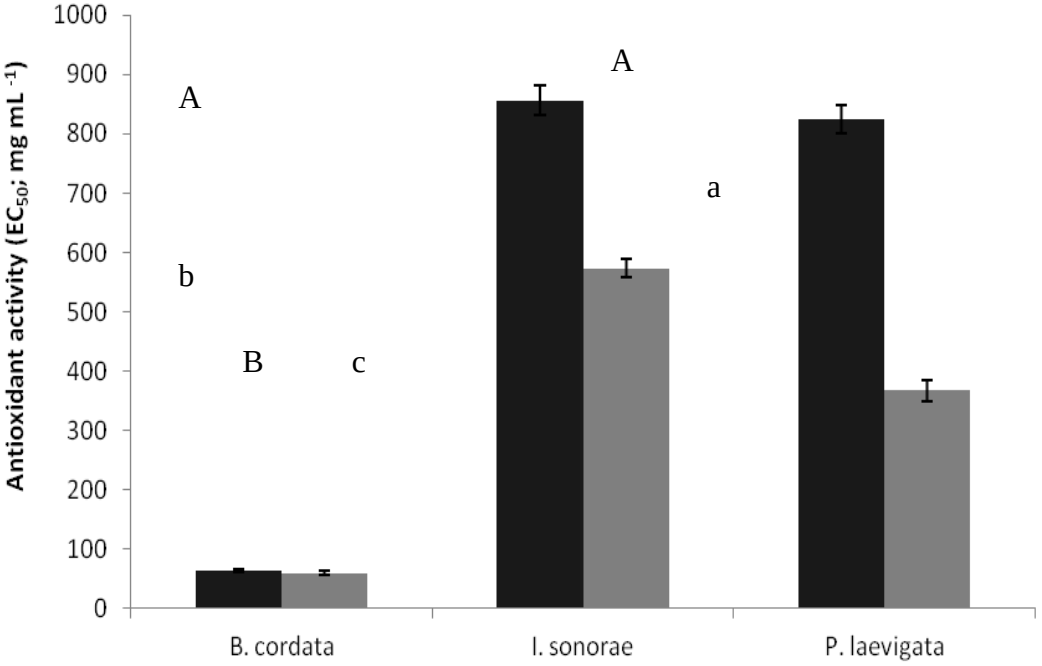
Extraction and quantification of phenolic compounds: Biomass from callus of *B. cordata*, *I. sonorae* and *P. laevigata* was lyophilized and weighted, followed of extraction with MeOH (50 mL per 100 mg of biomass) boiling for 1 h, filtration and concentration (10 mL). After, TPC was assessed by Folín-Ciocalteau method (Aslan y col., 2007) whose results were expresses as equivalent mg of galic acid per gram of extract (mg GAE g^{-1}).
Determination of the antioxidant activity: Concentrated extracts were evaporated to deliver complete dryness then several concentrations were prepared in MeOH (0-3 mg mL^{-1}) to determine the amount of extract needed to inhibit the initial concentration of free radical (EC_{50}); ABTS and DPPH assays were carried out to evaluate the antioxidant activity (Re y col., 1999; Sánchez-Moreno y col. 1998). All the results were done by triplicate and statistically analyzed with NCSS software at 5 % of significance level.

Results and discussion

The TPC and antioxidant activity were significantly different between species, and also TPC was associated to antioxidant activity. The highest TPC (202.7 mg EAG g^{-1}) was in *B. cordata*, followed of *I. sonorae* and *P. laevigata* (57.1 and 46.8 mg EAG g^{-1}, respectively) (**Fig 1a**), as well as EC_{50} corresponding to 62.5 and 59.5 µg mL^{-1} for *B. cordata*, 855.7 and 573.3 µg mL^{-1} for *I. sonorae*, and 824.3 and 366.7 µg mL^{-1} for *P. laevigata*, DPPH and ABTS assay, respectively (**Fig 1b**). The TPC values determined for callus from *B. cordata* and *I. sonorae* were similar to those reported for leaves of close species belonging to Buddlejaceae (28.2-236.7 mg GAE g^{-1}) (Sawadogo y col., 2006) and Cucurbitaceae (49.7-157.6 mg GAE g^{-1}) (Local Food-Nutraceuticals Consortium, 2005) families, respectively, whereas that TPC value for *P. laevigata* callus was lower to that reported in leaves of Fabaceae species (221.9 mg GAE g^{-1}) (Local Food-Nutraceuticals Consortium, 2005). *Tecoma stans* (Bignoniaceae) is close to Buddlejaceae species, and as well as *B. cordata*, *T. stans* is commonly used in folk medicine Mexican whose curative property is also related to phenolic compounds. However, *T. stans* phytochemical constitution in fields is highly varied, so *in vitro* cultures has been developed (López-Laredo y col., 2009). Whilst, extracts of cell cultures of *Lavandula vera* and *Rosa damascene*, other species used extensively in folk medicine, have been tested as sources of phenolic antioxidants for use in foods (Kovatcheva-Apostolova y col., 2008). Certainly, the highest TPC determined for *B. cordata* callus (202.7 mg EAG g^{-1}) was significantly superior to those reported for callus of *T. stans* (45.40-97.03 mg GAE g^{-1}) (López-Laredo y col., 2009), *L. vera* (85.6 mg GAE g^{-1}) and *R. damascena* (7.9 mg GAE g^{-1}) (Kovatcheva-Apostolova y col., 2008). On the other hand, lower amounts of extract (µg mL^{-1}) capable to inhibit at 50 % the initial concentration of free radical, means that extracts possess a high antioxidant activity. The EC_{50} results observed from all species are similar to those values reported from leaves of the corresponding close species (16.33-785.67 µg mL^{-1}) (Sawadogo y col., 2006; Chew y col., 2011). Moreover, several works comprising a variety of phenol producer species such as *Blepharis linearifolia*, *Dicliptera verticillata*, *Dyschoriste perrottetii*, *Hygrophila auriculata*, *Lepidagathis anobrya*, *Nelsonia anescens*, and *Justicia spicigera* report a TPC association with antioxidant activity (Sawadogo y col., 2006), relation that also is observed in the present work. Phenolic compounds possess a great reactivity, so they can exert a variety of beneficial effects on human health, among the antioxidant activity is highlighted. Phenolics may prevent oxidative stress occurring under a disease state and through their antioxidant activity they exert protective effect by selectively inhibiting or stimulating key protein in the cell signaling cascades, which in turn can mark the point of no return in the cell death process (Soobrattee y col., 2005). So, *B. cordata* cellular line cultures can represent a potential biosource to produce phenols which if are added to foods may exert benefits to health. More research advances over phenol production increase in these *in vitro* systems must be developed, and also those produced phenols must be proved as food additives.



a)



b)

Fig 1. Total Phenol Content (**a**) and antioxidant activity (**b**) from callus extracts of *B. cordata*, *I. sonorae* and *P. laevigata*. Means (± SD) followed by the same letter are non-signicantly different at the 5% level. ▩ TPC, EC50 regarding ■ABTS and ▩ DPPH assay.

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Chemical Compositionand Dietary Fibre Content Of Mango And By-products (*Mangiferaindica,* CVsAtaulfo And Tommy Atkins)

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Introduction

In recent years, worldwide consumption and production of tropical exotic fruits has increased, because of their attractive sensory properties their nutritional quality. *Mangiferaindica* is one of the most important tropical fruits with a global production exceeding 27 million Tonnes per year (1). Mexico ranks fourth in world production and second in exports of this fruit. *Ataulfo, Tommy Atkins* and *Kent* mango varieties are the most widely consumed (2). The Mexican variety *Ataulfo* has proven to be a success in international markets because it has unique sensory features: firm consistency, plump and sweet drupe, low acidity and intense aroma (3).Fruit changes during ripening can induce changes in the chemical composition that can be nutritionally beneficial. Exotic fruits exhibit an important content of DF (4), juice from mango is the main industrial product and the industrial by-products represent 35 to 60% of the total fruit weight (5).Furthermore, *Ataulfo* and *Tommy Atkins* varieties have highly fibrous pomace generated as a by-product of juice preparation and to the best of our knowledge, has not been characterized and could be potentially incorporated into food products as inexpensive, non-caloric bulking agents.
The aim of this work was to evaluate changes in chemical composition and DF content in two mango cultivars (Mexican *Ataulfo*and *Tommy Atkins*) at two stages of ripening and their by-products obtained during their processing.

Materials and Methods

2.1. Sample preparation

Mango fruits from two varieties (*Ataulfo*and *Tommy Atkins*) were purchased from “Cinco de Mayo” orchard near Tepic, Nayarit, Mexico. A proportion of the fruits used in this study were in mature-green stage (MGS) and another portion was used when the concentration of soluble solids was 16 °Brix, which is considered consumption stage (CS). Mango was peeledand dried at 65 °C. The dried pieces were homogenized (IKA® Works Inc, Model M20-S3, Wilmington, NC) and passed through a 0.05 mm mesh, then stored in plastic containers at -20°C for further analyses. The samples obtained henceforth are referred to as *Ataulfo*-MGS and *Tommy*-MGS and *Ataulfo*-CS and *Tommy*-CS.

Preparationof by-products

The by-products (fiber and peels) were purchased from *Ataulfo*and *Tommy Atkins* fruitsfromMexifrutas S.A. de C.V. mango processingplant, during the manufacturing of juice concentrate. Samples were freeze-dried, milled (IKA® 80 Works Inc, Model M20-S3, Wilmington, NC) and passed through a 0.05 mm mesh,then were stored in plastic containers at -20°C for further analyses. The samples obtainedhenceforth are referred to as *Ataulfo*-F and *Tommy*-F (F, Fiber); *Ataulfo*-P and *Tommy*-P (P, Peel).

2.2 Proximate composition and dietary fibre.

Ash, protein and fat were analysed according to AOAC methods 923.03, 920.87 and 920.85, respectively (6).*Total soluble carbohydrates* were assessed by the Anthrone method (7).*Total starch* was determined by the method of Goñi et al.(8). Glucose was determined using the glucose oxidase/peroxidase (GOD/PAD) assay. ***Dietary fibre analysis***.Samples were analysed by the AOAC method modified by Mañas et al.(9). Samples were treated enzymatically, followed by centrifugation, instead of filtration to separate the soluble and insoluble fractions obtained after enzymatic hydrolysis. Supernatants were quantitatively collected; pellets were washed and transferred into dialysis tubes (12000–14000 MWCO, Dialysis Tubing Visking, Medicell International Ltd., London, UK). The dialysates (soluble dietary fibre, SDF) were hydrolysed with concentrated sulphuric acid. Residues obtained after the enzymatic hydrolysis (insoluble dietary fibre, IDF) were dried and hydrolysed with sulphuric acid. Non-starch polysaccharides(NSP) from SDF and IDF were determined spectrophotometrically at 520 nm by the Englyst and Cummings method (10) and glucose was used as a standard. Residues were dried and gravimetrically quantified as Klason lignin (KL).

2.3. Statistical analysis

Differences between means were analyzed by ANOVA using SAS statistical software v. 9.0. Statistically significant differences ($p<0.05$) were evaluated using the LSD multiple comparison procedure.

Results and discussion

Changes in proximate composition in two mango varieties

Proximate composition in tropical fruits like mango, changes depending of the crop origin, variety and maturity stage (11).Proximate composition of our samples is depicted in Table 1. According to the ripeness stage, the protein content was significantly different ($p\leq 0.05$) between samples. Other authors have reported protein contents of 5.42 g/100g in *Peruano* mango variety (12), without any further reference of the maturity state. Protein content was higher in the consumption stage than on the mature-green stage. This might occur because an increase in the synthesis of cell wall enzymes that may function collectively and synergistically to impact significant texture and protein changes in fruits occurs during the ripening stage (13).

Table 1.

Changesin the proximate composition *Ataulfo* and *Tommy* varieties in their consumption stage (CS) and mature-green-stage (MGS) of mango pulps (g/100g dry base, except moisture)[a]

Component	*Ataulfo*-MGS	*Ataulfo*-CS	*Tommy*-MGS	*Tommy*-CS
Moisture	80.17±2.10c	84.86±1.3b	86.40±1.50a	85.40±0.60bc
Protein[b]	5.33±0.02b	6.40±0.04a	4.04±0.05d	5.55±0.03c
Ash	2.09±0.13b	2.57 ±0.20a	1.56±0.19c	1.48±0.05c
Fat	1.14±0.10a	1.02±0.08ab	0.92±0.48b	1.02±0.00ab
SC[c]	36.76±6.60c	48.47±4.03ab	48.85±2.60a	48.85±2.60a
Total starch	16.24±0.72a	Nd	8.53±0.37b	Nd

[a]Values are given as mean ($n \geq 3$). The values with different letters are significantly different ($p\leq 0.05$) by LSD test. [c]SC; Soluble carbohydrates. [d]Nd; not determined.

Ash content in both varieties and ripeness stage had a significant effect ($p\leq 0.05$) between samples. The main minerals identified in mango are calcium, potassium, magnesium, phosphorus and nitrogen.In general ash is incremented with the ripeness stage (14). However, the behaviour in *Ataulfo* variety in regard to ash content was contrasting and it will be considered for further research on physiology studies for this variety. The studied varieties did not show a significant ($p\geq 0.05$) difference in lipid content.

However the variations could occur during maturing when there is formation of volatile compounds from lipids (14)such as terpenes, esters and alcohols (11). Soluble carbohydrates increased in *Ataulfo*-CS, which can occur when starch in green-mature-stages is hydrolysed to simple sugars during ripening. Additionally, both pH values and enzyme activity increase as a result of ripening which is a consequence of polysaccharides hydrolysis, thereby increasing the soluble sugars content (15).

Proximate composition of mango by-products

Usually, mango fruit in consumption stage is employed to prepare juice concentrate. Fibre and fruit peel are recovered as by-products and may have a different chemical composition even if they originate from the same variety. Some nutrients may vary in unripe as compared to ripe fruit, while others remain unchanged. The proximate composition in *Ataulfo* and *Tommy Atkins* by-products (fibre and peel) are shown in Table 2. Protein content in *Ataulfo*-F and *Tommy*-F were similar than that of *Ataulfo*-CS and *Tommy*-CS (Table 1), although these were higher than in *Ataulfo*-P and *Tommy*-P. Theprotein in peel of different mango varieties has been studied, and similar values to those reported herein have been found as those by Ajila et al.(16)(3.6 g/100g, dry base)and Hassan et al.(17)(4.6 g/100g, dry base).

Table 2.
Proximate composition in *Ataulfo*and *Tommy Atkins* mango varieties by-product (g /100g dry base)

Component	*Ataulfo*-F	*Tommy*-F	*Ataulfo*-P	*Tommy*-P
Protein	5.10±0.7a	4.98±0.07a	3.27±0.05c	4.30±0.45b
Ash	2.23±0.04c	1.87±0.09d	3.18±0.11b	3.90±0.05a
Fat	0.57±0.01d	1.70±0.19c	2.08±0.20ab	2.35±0.13a
SC[b]	72.17±1.15a	65.39±1.26b	56.52±1.44c	56.65±1.64c
Total starch	8.23±0.74b	6.38±0.73c	10.69±0.29a	7.76±0.27b

Values are given as mean (n≥ 3). The values with different letters are significantly different ($p \leq 0.05$) by LSD test. [b]SC=Soluble carbohydrates. F=Fibre, P=Peel.

Ash content in *Ataulfo*-F and *Tommy*-F were similar than their corresponding pulps in the maturity stage. Peels in two reported varieties have shown higher mineral and fat content. Similar values in mango peel were reported elsewhere (17). Fat content obtained in our by-products was lower than *Citrus sinensis*peel (22.2 g/100g, dry base) (18).Soluble sugars were higher in our by-products, which could be attributed to that industrial process employ fruits that are in their optimal ripening stage.Total starch (TS) content in fibre and peel of *Ataulfo* was higher than in its counterpart of *Tommy*. In general, TS content in the by-products was low and it is remain from the starch present in the pulp that is not hydrolysed to simple sugars during ripening. The higher TS content in peel may be attribute to starch granules which are encapsulated in the cell wall and released during peel grinding.

Dietary fibre content

Dietary fibre (DF) values in samples are shown in Table 3. The total dietary fibre (TDF) content was greater ($p<0.05$) in *Tommy Atkins* peel than *Ataulfo*peel and the rest of studied samples. However, *Ataulfo*-F was higher ($p\leq 0.05$) than *Tommy*-F. These values were smaller than those from different varieties reported in *Mangiferaindica* L peels (5).Dietary fibre is constituted mainly by non-starch polysaccharides such as cellulose, hemicelluloses, xylanes, galacturonic acid and cell wall components in the peel are constituted by these structural carbohydrates (13).

Table 3.
Total dietaryfibre (TDF), soluble dietary fibre (SDF), insoluble dietary fibre (IDF), total sugars (TS), Klason Lignin (KL) and percentage (%) SDF/TDF(g /100g dry base)in*Ataulfo* and *Tommy Atkins* varieties[a].

Sample	TDF[b]	SDF	IDF		SDF/TDF
			TS	KL	
Ataulfo-MGS[c]	17.66±0.59e	7.59± 1.21c	7.60±0.12bc	0.10±0.01e	42.9
Tommy-MGS	10.87±1.27g	3.87±0.78f	6.49±1.38c	0.50±0.12e	35.6
Ataulfo-CS[d]	15.27±1.05f	9.19±0.41b	8.32±0.31b	0.14±0.01e	60.1
Tommy-CS	11.62±0.40g	10.24±0.38a	6.50±0.82c	0.09±0.00e	88.1
Ataulfo-F[e]	24.59±0.21c	6.22±0.05d	10.22±0.80a	7.39±1.59c	25.2
Tommy-F	22.50±1.23d	4.91±0.47ef	11.01±0.09a	6.57±0.30d	21.8
Ataulfo-P[f]	23.64±0.91b	11.15±1.28a	8.48±0.94b	8.05±1.70b	40.3
Tommy-P	38.71±4.8a	12.11±0.40a	11.43±0.41a	15.17±1.60a	31.2

[a]Values are given as means (n = 3). Values with different letters are significantly different ($p \leq 0.05$) by LSD test. [b]TDF; As the sum of SDF+IDF. [c]MGS=Mature Green-Stage. [d]CS; Consumption stage. [e]F; Fibre. [f]P; Peel.

The soluble dietary fibre (SDF) in the mango peel from studied samples. SDF in *Tommy Atkins* and *Ataulfo*atconsumption stage, was higher ($p \leq 0.05$) than MGS and the fibre by-product obtained.A higher content in SDF in CS could be explained by the different contents of structural and non-structural polysaccharides in the pulp and peels in MGS tropical fruits (13).During maturation these compounds changed due to a series of enzymatic de-esterification and de-polymerization reactions that tropical fruits carry out during ripening (15). These reactions could promote an increase in soluble pectic polysaccharides and the concomitant increase in soluble fibre.SDF content in this study was similar than that reported in *Mangiferapajang* fruit pulp (9.05 g/100g, dry basis) (19) and *Mangiferapajang*fruit peels (33.4 g/100g, dry basis) (17).IDF is constituted mainly by cellulose, hemicellulose, galacturonic acid and lignins.KL content was higher in by-products (peel and fibre) than samples in consumption stage and mature-green stage. This could be because during mango pulping the sections attached to the seed usually contain the components that comprise the KL. However, in *Mangiferapajang* fruit pulp and peel (17,19) KL values were higher than what we obtained our varieties. Ligninsare an indigestible constituent of plant foods. Nutrition experts recommend that at least one third of the daily dietary fibre intake should come from SDF (20).In this sense DF from tropical fruits has proved to be an excellent source of SDF. The samples *Ataulfo*-CS and *Tommy*-CS had a content of FS that exceeds this recommendation (Table 3). However, the proportion of SDF in *Ataulfo*-GMS and *Ataulfo*-P is elevated too, thus in most samples the SDF/TDF ratio was close to recommended levels. Only the fibre by-product was lower than the other samples, but if we compare against the SDF from cereals the values obtained in fibres were higher.

Conclusions

TDF content in studied mango by-products was higher than fruits in MGS and CS. However, peels in *Ataulfo* and *Tommy Atkins* varieties had similar values in SDF to that of fruits in CS. The ratio SDF/TDF was higher, in all studied samples, from the 1:3 ratio recommended by nutrition experts. Finally, our results emphasize the features of DF in the *Ataulfo* variety, and suggest the potential use of their by-products in foods.

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Chemical Composition And Physicochemical Properties Of Agroindustrial Wastes

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Introduction

This study was carried out in order to reduce the environmental impact generated by the waste obtained from agroindustry, giving an added value.Some of these wastes may be used as input during elaboration of different products. Most of agro-residues are rich in fiber, minerals and antioxidant compounds, whose functional properties help to prevent various chronic diseases as obesity, diabetes and hyperlipidemiaand improve human health. Functional properties of food or food-ingredient depend on their chemical composition and physicochemical properties (5, 8). These properties are generally determined *in vitro* and serve to give an idea of their behavior *in vivo*, since in this medium undergo a complex physiological environment and mechanisms that could modify them. The principal physicochemical properties that are evaluated are: pH, particle size, water-holding capacity (WHC), swelling capacity (SC), cationic exchange capacity (CEC), oil-holding capacity (OHC) and organic molecule adsorption capacity (OMAC).
Studies on the chemical composition of edible part of cladodes and fruits of *Opuntiaficus-indica* showed that these parts have high nutritional value, mainly due to their mineral, protein, dietary fiber and phytochemicals contents (2, 7, 9, 18). The wastes generated by the consumption of prickly pear are the shell and spines, which represent 45% of the fresh weight of the prickly pear (17). Moreover, the sotol pineapple has carbohydrates such as fructose and cellulose, the last being the most abundant component (4). However, in commercial fermentation process, cellulose fibers are not completely hydrolyzed, leaving large amount of fiber as residues known as bagasse. These wastes are deposit in the environment, so they represent a potential contaminant. For all these reasons, we decided to chemically and functionally characterize for the wastes of the sotol industry and prickly pear peels (red and green) of the region of Coahuila, as there is great interest by know the nutritional quality of these residues. All with the purpose of obtaining quality ingredients to be incorporated into functional foods and thereby increase the added value of these by-products.

Methods and materials

Material and Sample preparation. Two prickly pear varieties of *Opuntiaficus-indica* sp., red and green, purchase in the food supply center in the city of Saltillo, Coah.,Méx. The pricky pear samples were peeled in the normal way for human consumption. Spines, epidermis and glochids were manually removed. While residues of sotol were provided by the Sotolera Torrecillas, Durango. All samples were dry at 65 °C and ground. Then, samples were sieved and recovered the particles in range from 425 to 75 µm. The samples were stored in dark at room temperature until analysis.
Proximal Composition. Proximate analysis samples were analyzed using AOAC methods (1): fat (Method 985.15) and ash (Method 923.03). Total soluble carbohydrates was analyzing by the Antrona method (10), reductor soluble carbohydrates with DNS method (16) and total dietary fiber with a gravimetric-enzymatic method using TDF-KIT (MEGZYME, ®).
Macro and Microelements. For this analysis we used an atomic absorption spectrophotometer (VARIAN-AA240FS). We quantified the sodium, potassium, magnesium and calcium as macroelements; as well as manganese, cooper, iron and zinc as microelements.
Functional and physiological properties.

Water-holding capacity (WHC). Thirty milliliters of distilled water was added to 500 mg of dry powdered samples in a 50 mL centrifuge tube. The sample was stirred and left at room temperature for 18 h. After centrifugation at 3000g for 20 min, supernatant was discarded and residue was weight. (11). Results were expresses as g of water/ g dry sample.

Swelling capacity (SC). The dry powdered sample (500 mg) was weighed in a 10 mL measuring cylinder (0.1 mL graduations) and 10 mL distilled water was added. Measured the volume (mL) occupied by the sample. Then, it was stirred gently to eliminate trapped air bubbles and left on a level surface at room temperature overnight (24 h) to allow sample to settle (13). The volume (mL) occupied by sample was measured and SC and expressed as mL of water/ g of dry sample.

Oil-holding capacity (OHC). Ten milliliters of commercial virgin olive oil (0.5°) was added to 500 mg of dry powdered samples in a 50 mL centrifuge tube. The sample was stirred and left at room temperature for 18 h. After centrifugation at 3000 g for 20 min, supernatant was discarded and residue was weight (P_1) (11). Results were expressed as g of olive oil/ g dry sample.

*Organic molecule adsorption capacity (OMAC).*In centrifuge tubes of 50 ml were weighed 500 mg of each sample, were added 10 ml of commercial sunflower oil and shaken by hand for 10 min, then allowed to stand for 24 hours at room temperature, elapsed time sample was centrifuged at 3000 g for 10 min, supernatant was removed immediately and pellet was weighed (g) (11). Results were expressed as mL/ g dry sample.

Cation exchange capacity (CEC). In centrifuge tubes of 50 ml were placed 2 g of each sample in an excess of 2N hydrochloric acid (20 mL) for 24 h at room temperature; hydrochloric acid was removed by filtration and was added a saturated solution of chloride sodium to the sample and washed with distilled water. H^+ ions captured by the sample were determined by titration, reporting the result in milliequivalents of H^+ / g sample.

pH. One hundred mL of deionized, boiled and cold water was added to 10 g of sample. Mixed for 30 minutes and discarded the supernatant. Sample was allowed to stand for 10 min and pH was measured using a potentiometer (Thermo Orion 420) (**NMX-F-317-S-1978**).

Statistical analysis. All determinations were made in triplicate under a randomized complete block design, when needed mean comparisons were performed using the Tukey Range Test ($P < 0.05$). Differences between means at the 5 % level were considered significant.

Results and discussion

It was decided to work with a particle range from 75 to 425 microns, since particles smaller than 50 microns may cause lumps in food and particles larger than 500 microns would be difficult to chew.

Proximal Composition.The prickly pear peels presented high moisture content (around 90 %) and a low content of total dietary fiber (red: 14.03 % and green: 33.13% dry base) compared with bagasse of sotol (moisture: 9.65% and TDF: 77.88% dry base). All studied wastes had low amounts of fat (< 2.33% dry base). The high content of total dietary fiber of bagasse of sotol is due to the high content of cellulose not hydrolyzed during fermentation (Table 1). Total dietary fiber of bagasse of sotol was higher than that from passion fruit seeds (64.8 g/100 g dry base), apple pomace (51.1 g/100 g dry base) (3, 5) and similar to the grape co-products (74.5 g/ 100 g dry base) (14).

Table 1.Proximal composition of studied wastes (g/ 100 g dry sample) (Mean ± SD).

Waste	Ash	Fat	Total soluble carbohydrates	Total reductor carbohydrates	Total dietary fiber
BS	11.85 ± 0.5 **c**	1.07 ± 0.14 **b**	14.51 ± 2.1 **c**	6.00 ± 0.05 **b**	77.88 ± 2.4 **a**
GPPP	18.10 ± 0.08 **a**	1.21 ± 0.15 **b**	28.4 ± 10.2 **b**	27.74 ± 2.9 **a**	33.13 ± 1.7 **b**
RPPP	13.29 ± 0.17 **b**	2.33 ± 0.18 **a**	60.35 ± 6.8 **a**	31.78 ± 0.46 **a**	14.03 ± 1.0 **c**

* Means with the same letter are not different according to Tukey test, p = 0.05.

BS: bagasse of sotol, GPPP: green prickly pear peels, RPPP: red prickly pear peels.

However, available carbohydrates content in red and green prickly pear peels is higher than that found in sotol bagasse. These results suggest that such kind of materialscontainnutrients thatcan still beused by human body and /or beneficial bacteria.

Macro and Microelements. The waste with significant more mineral amount was has sotol bagasse (Table 2). The green and red prickly pear peels are rich in potassium and calcium and low in sodium (12), but the sodium amount was higher than that reported for the same residue (12).

Table 2. Mineral composition of studied wastes (mg/100 g dry sample) (Mean ± SD).

Waste	Na	K	Ca	Mg	Fe	Mn	Zn	Cu
BS	704.4 ± 38.5 **a**	64.9 ± 2.48 **c**	1519.1 ± 61.07 **a**	266.64 ± 25.97 **a**	7.65 ± 1.14 **a**	4.74 ± 0.0 **b**	5.01 ± 0.36 **a**	45.97 ± 2.08 **a**
GPPP	21.28 ± 3.9 **b**	3071 ± 120.9 **a**	1158.3 ± 43.21 **b**	288.72 ± 16.74 **a**	4.1 ± 0.36 **c**	23.04 ± 0.81 **a**	0.91 ± 0.1 **b**	0.48 ± 0.0 **b**
RPPP	36.04 ± 0.84 **b**	1895.4 ± 7.05 **b**	717.96 ± 9.4 **c**	134.35 ± 30.0 **b**	5.8 ± 1.43 **b**	1.63 ± 0.3 **c**	0.57 ± 0.19 **b**	0.22 ± 0.0 **b**

* Means with the same letter are not different according to Tukey test, p = 0.05.

BS: bagasse of sotol, GPPP: green prickly pear peels, RPPP: red prickly pear peels.

Functional and physiological properties

Sotol bagasse showed the highest pH value (5.95), followed by red prickly pear peels (5.26) and green prickly pear peels (4.58). Functional properties of plant fiber depend on particle size, extraction conditions, structure of plant polysaccharides and vegetal source. The highest swelling capacity was observed in green prickly pear peels (4.58 ml/ g dry sample) (Table 3), this capacity is higher than that reported for commercial fibers such as apple (3.1 ml/ g dry sample) and oat (2.4 ml / g dry sample) but lower than other dietary fiber such okara (9.44 ml/ g dry sample) (15) or wheat (7.1 ml/ g dry sample) (19). This capacity is related to production of satiety after eating. The water holding capacity for green (7.03 ml/ g dry sample) and red (7.48 ml/ g dry sample) prickly pear peels was higher than that reported for wastes like pomegranate juice bagasse (4.9 ml/ g dry sample) but lower than that reported for chia /(15.41 ml/ g dry sample) (20). Moreover, oil holding capacity observed in this study was lower than that reported for pomegranate juice bagasse (5.9 ml/ g dry sample) but highest than that reported for okara (0.27 ml/ g dry sample) (15). The highest organic molecule adsorption capacity (4.4 ml/ g dry sample) was observed with red prickly pear peels, which was higher that than mentioned for chia (1.09 ml/ g dry sample) (20), wheathusk (1.98 ml/ g dry sample) and corn husk (1.6 ml/ g dry sample) (20).This trait has been associated with decreased blood cholesterol.

Table 3.Functional properties of studied wastes (Mean ± SD).

Waste	SC (ml/g)	WHC (ml/g)	OHC (ml/g)	OMAC (ml/g)	CEC (meq H^+/g)
BS	2.1 ± 0.51 **b**	4.4 ± 0.26 **b**	3.32 ± 0.17 **a**	2.97 ± 0.33 **b**	8.3 ± 0.26 **a**
GPPP	4.58 ± 0.34 **a**	7.03 ± 0.07 **a**	1.68 ± 0.17 **c**	1.4 ± 0.02 **c**	6.74 ± 0.12 **b**
RPPP	3.99 ± 0.0 **a**	7.48 ± 0.7 **a**	2.21 ± 0.36 **b**	4.4 ± 0.7 **a**	6.66 ± 0.37 **b**

*(WHC)*Water-holding capacity, *(SC)Swelling* capacity, *(OHC)* Oil-holding capacity, *(OMAC)* Organic molecule adsorption capacity and *(CEC)* Cation exchange capacity.

The highest cation exchanging capacity was observed in sotol bagasse (8.3 meq H^+/ g dry sample) and this amount was similar to that reported for apple fiber (8.9 meq H^+/ g dry sample). This capacity may be the mechanism to explain bile acid sequestration by hydrophobic interactions and / or ionic that is involved in calcium and aluminum ions (8). The fiber containing uronic acids or phenolic acids may increase fecal excretion of bile acids which may have a hypocholesterolemic effect (8) and thereby decreasing fat absorption by the human organism.

The results of this study indicated that green and red prickly pear peels may be considered a potential ingredient in food products, increasing their content of total dietary fiber and improving their technological properties. The addition of fibers to food products is of great interest not only as a means of improving functionality of food products, but also as a means to create functional foods with health benefits.

Acknowledgment
The authors are grateful to the CONACYT for financial support for this project.

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Rheological And Sensory Properties Of Ice Cream Low In Fat And Carbohydrates Added With Different Concentrations Of Prickly Pear (Opuntia spp.)

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Introduction

Obesity is one of the main risk factorsto develop diabetes type 2, cardiovascular diseases, hypertension, dyslipidemia and certain types of cancer (1). Diabetes is the most serious metabolic disorder characterized by high levels of blood glucose. One way to treat diabetes is delaying absorption of glucose in the blood, which can be achieved by reducing food absorption of glucose into the blood, or by inhibiting the enzyme α-glycosidase (α-D-glucosidglucohydrolase) (2). The prickly pear is used as food in the Mexican diet since pre-columbin times, but also has been used to treat diseases like diabetes, gastritis and hypercholesterolemia. The composition of the prickly pear is essentially carbohydrates, with a considerable proportion of fiber of these carbohydrates (5-15%) (3). However,prickly pear mucilage containing complex carbohydrate is composed of a large capacity to absorb water. It might be considered as a potential source of hydrocolloids (4). The health benefits provided by the consumption of prickly pear, or of the fruit (pear) open new perspectives in their use as an ingredient for functional product design, they can be directed in a future for people with obesity, diabetes or hypercholesterolemia.

Materials and methods

Two types of ice creams were manufactured: ice cream added with raw prickly pear, and ice cream added with steamed prickly pear (Table 1). All the treatments were subject to compositional analysis, according to AOAC methods. The color of the ice cream samples were analyzed with a colorimeter measuring the *L*, *a*, and *b* components. The rheological characteristics of the ice creams were measured by two different tests: 1) Stresssweepsteposcillatingtodeterminethelinearregionviscoseice cream(LVR)and 2)lowtemperaturescanningphasedconstant strainamplitude, to obtain the parametersG '(storage modulusorelastic) and G" (loss modulus). Finally,the sensory evaluationof the ice creams was carried out using 60 non-trainedjudges. The flavor was measured by the panel using a non-structured (hedonic) scale of 9 cm.

Table 1. Ingredients used in the formulation of ice creams

Ingredient	**Control Ice cream**	**Ice cream with raw prickly pear**			**Ice cream with steamed prickly pear**		
		1%	**2%**	**3%**	**1%**	**2%**	**3%**
Milk fat (%)	2	2	2	2	2	2	2
Milk solids (%)	15.910	14.910	13.910	12.910	14.910	13.910	12.910
Emulsifier (%)	0.144	0.144	0.144	0.144	0.144	0.144	0.144
Stabilizing additives (%)	0.144	0.144	0.144	0.144	0.144	0.144	0.144
Maltodextrin (%)	6	6	6	6	6	6	6
Sucrose (%)	1	1	1	1	1	1	1
Glucose (%)	3.723	3.723	3.723	3.723	3.723	3.723	3.723
Aspartame (%)	0.020	0.020	0.020	0.020	0.020	0.020	0.020
Acesulfame-K (%)	0.013	0.013	0.013	0.013	0.013	0.013	0.013
Solids from Prickly pear (%)	0	1	2	3	1	2	3
Water (%)	70.860	70.860	70.860	70.860	70.860	70.860	70.860

Results and discussion

According to the values presented in the table above, there was no significant difference between treatments with raw and steamed prickly pear.In reference to the rheological characteristics of the ice creams, it was observed that when prickly pear is added into the ice cream formula, there is an increase in the viscoelastic properties of the ice cream (figure 1). The elastic (G´) and viscous (G´´) modulus increased with the amount of prickly pear, probably by the effect of mucilage provided by the prickly pear. The prickly pear was added in two different was, as a raw vegetable and prickly pear with a blanching treatment in hot water. When the prickly pear is added in low concentration (1 and 2%) into the ice cream formula, the viscoelastic properties of the ice cream with raw or steamed prickly pear is the same. But, when the prickly pear is added in three percent, the ice cream added with steamed prickly pear had higher viscoelastic properties that the ice cream manufactured with raw prickly pear. Probably, because during the treatment of blanching the polisaccharides of mucilage are hydrolyzed.

The change in the color of the ice cream by the addition of prickly pear is shown in the Figure 2. An analysisof variancefor the parameters*L*(lightness), *a* (color space between redand green) and*b*(spaceof colorbetween blueand yellow), concluding that the addition ofsolidcactussignificantly altered thecolor of the samples, especially thebrightness;which are lessbrightwith increasingconcentrationof prickly pear. Likewise,thevalues of the parameter *a*were negativebecause thegreen colorof the samples, and the samples with the higher values of the parameter *a*were the ice cream added with raw prickly pear.

In reference to the energy contributionofeach ice cream formulated, the ice creams were formulated with low fat and sugar content. The ice cream with prickly pear had in average 117 kilo calories per 100 grams of product, whereas commercial full fat ice creams have 196 kilo calories per 100 grams of product.

Table 2. Compositional analysis of ice creams

	Ice Cream Treatments	
Components	**Raw Prickly Pear**	**Steamed Prickly Pear**
	%	**%**
Humidity	91.87 ± 0.66[a]	91.21 ± 0.106[a]
Ashes	0.87 ± 0.05[a]	0.95 ± 0.116[a]
Fat	0.10 ± 0.04[a]	0.21 ± 0.071[a]
Protein	1.21 ± 0.08[a]	0.94 ± 0.261[a]
Fiber	0.60 ± 0.02[a]	0.59 ± 0.066[a]
Carbohydrates	5.35 ± 0.71[a]	6.10 ± 0.378[a]

± Standard deviation; [a,b] Mean values in the same row with different superscripts, presents statistical difference (Tukey test)

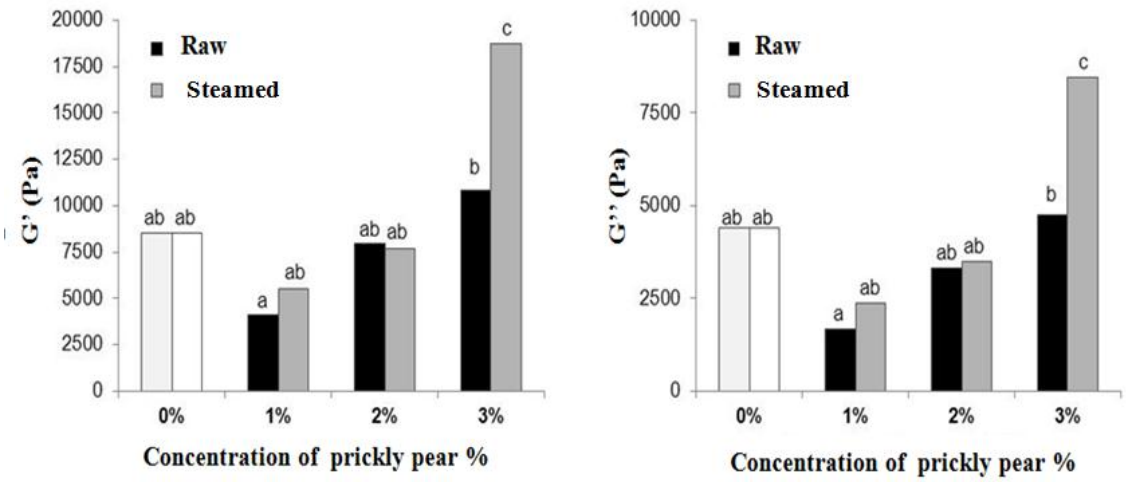

Figure 1. Changes in viscoelastic parameters of ice creams with steamed and raw prickly pear. Treatments with the same letters are not significantly different (P >0.05).

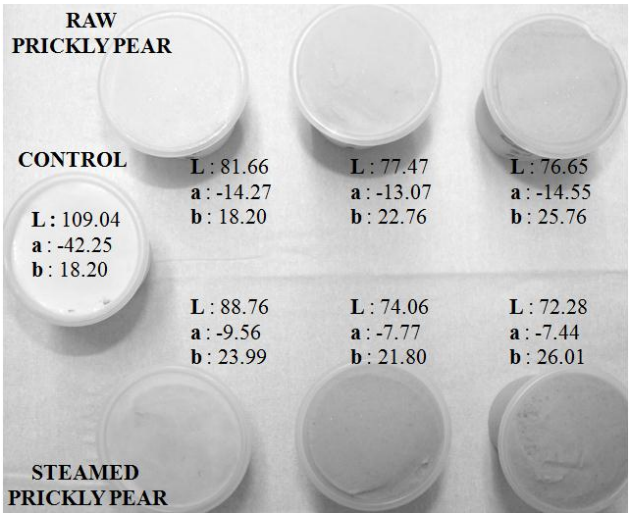

Figure 2. Results of the change in color by addition of prickly pear into the ice cream formula.

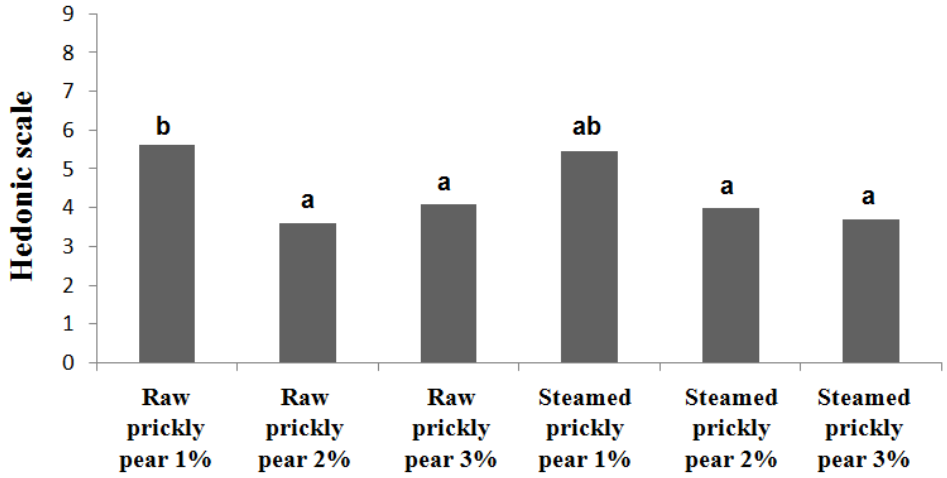

Figure 3. Results of the sensory evaluation for flavor in ice cream;[a,b]treatments with the same letter are not significantly different (P> 0.05).

In the sensory analysis were assessed the 6 different types of ice creams added with prickly pear at different concentrations. Clearly it could be noted (figure 3) that judges preferred the ice cream with lower amount of prickly pear (raw or steamed). The prickly pear produced a fresh flavor, but with a typical flavor of a green vegetable.

Conclusion

In conclusion low-fat ice cream added with solids of prickly pear could be a healthier option than regular ice-creams, but also this ingredient is an excellent option as fat replacer in low fat foods.

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Influence of Maturity Stage On Phytochemical Contents And Antioxidant Capacity Of Wild Mexican Serviceberry (*Amelanchier denticulata* (Kunth) Jones)

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Introduction

Amelanchier denticulata or "membrillo cimarrón" is also known as duraznillo, mandronillo, quince, granjenillo, manzanita and acebuche, in Spanish. In Mexican indigenous languages is called tlaxisqui, tlaxistle, tiomiscatli, tlaxioqui, clasisle, tlachistle, clalduraznо yagalán. In English is known as southern false serviceberry, big bend serviceberry, and Mexican serviceberry (1). For practical purposes of this paper, we use the name of wild Mexican serviceberry. This species grows in three main geographical areas of Mexico: Northeast Guanajuato, central and southern Querétaro and southern Hidalgo. Most of the wild Mexican serviceberry is localized in geographical semiarid areas. Production comprising 19.64%, 45.23%, 35.11% of the three areas of production cited previously, cording to data obtained from herbarium collections incorporated to REMIB of CONABIO (1). The species forms a Mexican phylogenetic resource with a wide agrifood potential. Information about their physicochemical, nutritional and nutraceutical is scarce. The fruit of this species is similar in size and shape to saskatoon berry (*Amelanchier alnifolia*) which in recent years has drawn attention for its high content of phenolic compounds and antioxidant capacity (2,3).

Evidence continues to emerge suggesting that antioxidant-rich, commonly consumed fruits are beneficial to health due to their capacity to prevent chronic diseases. The beneficial effect of fruits is attributed to antioxidant compounds such as phenolics, betalains, vitamin C, among other non-nutrients (4). However, there is a lack of information for underutilised fruits, either wild or domesticated. We have been making efforts to contribute in the knowledge of the nutritional and functional properties and the commercial potential of Mexican unexploited species (5,6). The aim of this work was to evaluate the effect of stage of wild Mexican serviceberry phenolics and antioxidant capacity (TEAC and ORAC). In addition, the characterization and quantification of phenolic acids, flavanols, and flavonols by high performance liquid chromatography-diode array detection (HPLC-DAD) were also carried out

Materials and methods

Wild serviceberry fruit was harvested in La Valenciana, Guanajuato Mexico. During the production season, any tree may bear fruits corresponding to unripe stage 1 (pink) unripe stage 2 (pink to grey-white), ripe (full grey-white) and overripe (light brown), they were harvested in July-August 2011. Fruit bulks corresponding to each maturity stage were combined to generate samples for wild quince. Immediately after, samples were freeze-dried and stored at -20 ºC until its analysis.

Total Soluble phenols (TSP):. 100 mg of dry sample was weighed, 10 mL of dilute methanol (30%) was added. The mixture was stirred for 10 minutes. Then it was centrifuged for 10 minutes and the supernatant was filtered. An aliquot of 125 µL of supernatant was placed in a test tube, was added 500 µL water and 125 µL of Folin Ciocalteu's reagent was mixed and allowed to stand in the dark for 6 minutes. Subsequently, 1.25mL of Na_2CO_3 (7%) was added and 1 mL of deionized water, it was mixed and allowed

to stand in the dark for 90 minutes, after which the readings were performed in absorbance at a wavelength of 750 nm in 6405 spectrophotometer UV / Vis, JEANWAY(7)

Condensed tannins (CT): Fruit samples (200 mg) were extracted within 1 day after grinding with 10 mL of methanol in capped, rotating test tubes for 20 min, and centrifuged. Five milliliters of reagent A (1:1, v/v, of 1% vanillin in methanol fresh daily and 8% HCl in methanol) were added to 1-mL aliquots of the sample. Five milliliters of 4% concentrated HCl in methanol were added to a second 1-mL aliquot (the blank); both were incubated for 20 min at 30 °C. After incubation the sample was spectrophotometrically measured (k = 500 nm) (Jenway 6405 UV–vis, Staffordshire, UK) and the absorbance of the blank was subtracted from the absorbance with vanillin (8). Concentration of condensed tannins was reported as mg (+) catechin equivalents/kg, fresh weight (+CE/kg, FW) after comparing with a (+) catechin standard curve.

Total anthocyanins (TA): Briefly, a fruit sample was added with 24 mL of acidified ethanol (etanol/HCl, 1N, 85:15), adjusted to pH 1.0 and agitated for 45 min. Then the sample was centrifuged (3000 rpm) and the supernatant recovered and added wotj acidified ethanol up to 50 mL. Absorbance (Jenway 6405 UV–vis, Staffordshire, UK) was recorded at 535 nm (9). TA were calculated and reported as cyanidin 3-glicoside equivalents (C3GE) as follows: $C = (A/\varepsilon)(vol/1000)\ (MW)\ (1/\text{sample weight})\ (10x10^6)$

Where
C = Total anthocyanins (mg C3GE/kg)
A = Sample absorbance
ε = Molar absortivity of the cyanidin-3-glicoside (25965 cm-1 M-1)
vol = Total volume of anthocyanin extract
MW = Molecular weight of cyaniding-3-glucoside (449 Da).

Simple phenols by HPLC: A 15 cm _ 4.6 mm i.d., 5 lm particle size Zorbax octadecylsilane (ODS-C18) reversed-phase column was utilised. Linear gradient elution was carried out by using solvent A (acetic acid/water, 2:98, v/v), and solvent B (acetic acid/acetonitrile/water, 2:30:68, v/v/v). During the analysis, the solvent gradient was programmed from 10% to 100% B in A in 30 min with a flow rate of 1.5 ml/min (10). The identification and quantification of the peaks were carried out from the retention times, the spectra derived from DAD in comparison with those from authentic standards, and by spiking with standards of the suspected compounds. Gallic, protocatechuic, 4-hydroxybenzoic, vanillic, chlorogenic, caffeic, syringic, coumaric, ferulic, benzoic and salicylic acids, as well as (+)-catechin, vanillin, epicatechin (EC), and epigallocatechin gallate (EGCg) were detected at 280 nm; on the other hand, ellagic acid, quercetin, and rutin were detected at 360 nm.

Determination of antioxidant capacities: ABTS (7 mM) radical cation (ABTS_+) solution was produced by reacting ABTS with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The ABTS_+ radical was diluted with potassium phosphate-buffered saline (PBS) to give an absorbance of about 0.700 ± 0.020 at 734 nm. For measuring antioxidant capacity 10 µL of sample was mixed with 990 µL of radical solution. Absorbance was monitored at 734 nm for 6 min. (11). Results were expressed in terms of µmol Trolox equivalent per g of sample, FW (µmol TE/g, FW). The oxygen radical absorbance capacity assay (ORAC) measures the ability of antioxidant compounds in test materials to inhibit the decline of fluorescein fluorescence that is induced by a peroxyl radical generator, AAPH (12). The final results (ORAC values) were calculated using the differences between blank and sample areas under the quenching curves of fluorescein, and were expressed as micromoles of Trolox equivalents (TE) per g of sample, fresh weight FW (lmol TE/kg, FW). All data were reported as means ± standard deviations (n = 3). Statistical analysis was performed using the JMP.5.0.1 software. Differences among means were tested for significance Tukey's test, using a level of significance of 0.05.

Results y discussion

Total soluble phenols (TSP) increased as wild Mexican serviceberry ripening (Table 1). TSP showed by overripe wild serviceberry similar to those reported by Bakowska-Barczak and Kolodziejczyk for saskatoon berry (2008) (801mgGAE/100G, FW)(13) . In contrast, condensed tannins (CT) decreased with serviceberry development. Comparing anthocyanin levels detected in saskatoon berry (251-179 mg/100g, FW) (14) with those of wild ripe and overripe fruit is not a good source of anthocyanins (2.66-5.71 mg/100g, FW). Also the TA content of wild Mexican serviceberry is lower than those reported for blueberry (*Vacciniumcorymbosum*), cranberry which has 120 and 32 mgEC3G/100 g fresh weigh, respectively. (15)
In spite of the lower content of TA and CT, ripe and overripe Mexican serviceberry is excellent TSP sources compared to fruits commonly consumed.

Table 1. Total soluble phenols, condensed tannins, and total anthocyanins of wild Mexican serviceberry in four maturity sages[a]

Maturity Stage	Total soluble phenols	Condensed tannins	Anthocyanins
1	239 ± 12.7 **d**	131 ± 1.99 **c**	4.43 ± 0.109 **b**
2	292 ± 11.1 **c**	160 ± 11.1 **a**	5.18 ± 0.374 **a**
3	445 ± 3.91**b**	149 ± 5.86 **ab**	2.66 ± 0.124 **c**
4	809 ± 15.5 **a**	137 ± 8.87 **bc**	5.71 ± 0.309 **a**

[a]Means in the same row with a common letter are not significantly different ($p < 0.05$). Total soluble phenols expressed as mg equivalents of gallic acid /100g. Condensed tannins expressed as mg equivalents of (+) Catechin/ 100g. Anthocyaninins expressed as mg equivalents of cyaniding 3- glicoside/100g

Simple phenolics detected by HPLC are shown in Table 2. As it can be seen, coumaric acid was not detected in the cultivated fruit. Benzoic, caffeic, chlorogenic, coumaric, gallic, siringic and vanillic acids were identified together with flavanols catechin, epicatechin (EC), epigallocatechin gallate (EGCg), and flavonol rutin. Of these compound caffeic, chlorogenic, coumaric siringic, and rutin were found to be the predominant ones; they comprised in edible serviceberry from 59.3% (cultivated overripe fruit) to 76.9% (wild ripe fruit) of the sum of simple phenolics. Levels of chlorogenic acid (CHA) detected in ripe serviceberry were similar to those reported in different cultivars of Saskatoon berry (16); however, some saskatoon cultivars show higher CHA levels (676.8 to 1297 mg/kg, FW) compared to those reported here. Also, the sum of flavanols catechin and EC of ripe serviceberry were two to fivefold lower compared to those reported for Saskatoon (~1100-3000 mg/kg, FW) (12). However, serviceberry showed EGCg which has not been reported for Saskatoon.

Table 2. Profile of phenolic compounds by HPLC (mg/100g FW). **Part a)**
Means in the same row with a common letter are not significantly different ($p<0.05$)

Maturity stage	Rutin	Chlorogenic acid	Gallic acid	Vanillic acid	Siringic acid	Catechin
1	31.1± 0.29[a]	34.6±2.24**a**	0.504± 0.018**b**	12.1±0.614**a**	21.7 ± 0.92 **a**	3.52 ± 0.228 **b**
2	30.4±2 **a**	29.6±1.03**ab**	0.475±0.022**b**	11.2±0.95**a**	20.8 ± 1.51 **a**	4.46 ± 0.416 **b**
3	30.7±1.6[a]	26.7±1**b**	0.418±0.023**b**	12.2±0.87**a**	18.5 ± 0.576 **a**	3.44 ± 0.269 **b**
4	31.8±2.2 **a**	40.7±2.5**a**	0.955±0.092**a**	9.49±0.84**a**	31.4 ± 1.54 **a**	6.23 ± 0.2 **a**

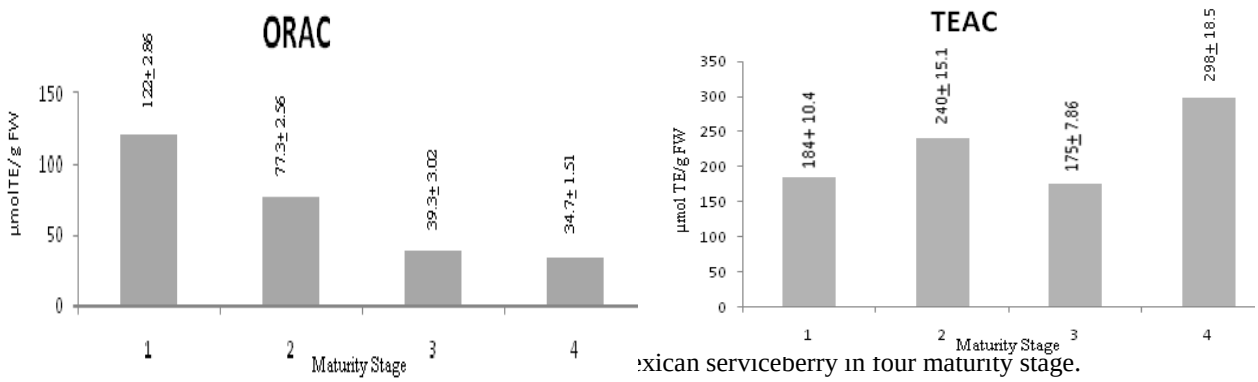
Table 2. Profile of phenolic compounds by HPLC (mg/100g FW)**. Part b)**

Maturity stage	EGCG	Epicatechin	Benzoic acid	Caffeic acid	Cumaric acid
1	3.29 ± 0.288 **a**	14.7 ± 0.774 **a**	8 ± 0.4 **a**	36 ± 1.28 **a**	53.3 ± 3**a**
2	2.73 ± 0.228 **ab**	13.3 ± 0.655 **b**	7.24 ± 0.635 **ab**	28.4 ± 1.57 **b**	19.5 ± 1.87**b**
3	1.78 ± 0.138 **b**	11 ± 0.066 **b**	5.72 ± 0.347**bc**	23 ± 1.07 **b**	16 ± 0.927 **b**
4	3.32 ± 0.29 **a**	11.6 ± 0.615 **b**	4.39 ± 0.156 **c**	----------	----------

Means in the same row with a common letter are not significantly different ($p<0.05$)

The TEAC and ORAC values of serviceberry fruit decreased as the fruit ripened; also, a higher TEAC values for wild and cultivated fruits than ORAC values were observed in all maturity stages (Figure 1). This is not all surprising, since flavanols have consistently shown lower antioxidant efficiency in terms of H-donating capacity, comparing with flavonols when evaluated in *in vitro* systems (11). These results are consistent with rutin concentration in wild samples, which represents one, to three-folds the concentration of the sum of flavanols catechin, EC, and EGCg. Also, it was observed a higher correlation between rutin and TEAC (r2 = 0.99442, p<0.0001).

When compared on a fresh weight basis, the TEAC values of *A. alnifolia* (148 µmol/g) to TEAC values reported here for edible wild serviceberry (298–175 µmol/g) (Fig. 1), it is obvious that serviceberry is by far a better source of antioxidant compounds. When compared wild serviceberry ORAC levels (122–34 µmol/g) with those reported for Saskatoon berry (124 µmol Trolox/g berry) (16), serviceberry showed similar ORAC values assuming that report of those authors is on fresh weight basis. It is pertinent to point out that unripe fruit shows the highest bioactive compounds, as well as, the highest antioxidant activities. Unfortunately, the astringency and low sweetness make the fruit, at this maturity stage, unsuitable for consumption.



xican serviceberry in four maturity stage.

Conclusions

A.denticulata is an important source of phenolic compounds (809 mgEAG/100gmf), total phenol content is very similar to A. alnifolia (801mgEAG/100gmf.
The antioxidant capacity determined by the ORAC (122 µmolET/gmf) and TEAC assay (298 µmolET/1gmf) are very similar to those of the delay saskatoon (124 µmolET/100gmf) for the former and 2 times the of default in the case of saskatoon TEAC method (148 µmolET/g FW).
The Mexican serviceberry is a good source of antioxidants and could be considered as a functional food.

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Lineal Viscoelasticity Of Gels Containing Complex Mixtures Of Biopolymers And Its Mathematical Modeling

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Some biopolymers have a wide application in food industry as additives, due to they present gelling, emulsifying and thickening properties; within the biopolymers that were used are Sodium Alginate (SA), Propylene Glycol Alginate (PGA) and Tragacanth Gum (TG). There are several studies to establish physical behavior of biopolymers, viscoelasticity is one of them, which help to determine mechanical properties of biopolymes. It presents viscous and elastic propertie, they are represented with several mathematic models. Many factors influence the selection of rheological models for the correct behavior description of biopolymers. The aim of this work was to determine gels viscoelasticity obtained with complex mixtures of SA, PGA and TG. It was used a mix design-simplex centroid, in which were obtained 10 combinations: 3 simplex, 6 binary and 1 ternary. Rheological determinations were performed in a Paar Physica Rheometer Model LS100, using paralled plates with PP 20 geometry. It was found that M1, M4 and M8 presented a behavior of macromolecular dissolution, according to rheograms of frequency scanning. M2, M3, M5, M6, M7, M9 and M10 presented a behavior of a strong gel according to frequency sweep. The gels were represented through the Burgers model equation with a Kelvin-Voigt element obtaining its dynamic parameters, emphasizing that instantaneous compliance (J_0) was used to obtain rigidity index of gels. According the rigidity index obtained from each mixture, it was determined the best mixture with $p>0.95$. The best mixture to obtain the best rigidity index values was AS 0.6061% with APG 0.3939%.

Keywords: Viscoelasticity, Biopolymers, Gels, Mixtures

Introduction

A wide application of biopolymers not only in the pharmaceutical industry, but also in the food industry like additives, since they are approve their gelling properties, emulsifiers and thickeners, among others. Among the most notable are sodium alginate (SA), propylene glycol alginate (PGA) and tragacanth gum (TG), (3).

The sodium alginate is a polysaccharide obtained from some "brown algae", among which there are *Laminaria hyperborean, Laminaria digitata, Laminaria saccharina* and *Laminaria Cloustoni*. Alginate is composed by two types of monosaccharides, guluronic acid and mannuronic acid. The algae synthesized to alginate as a biopolymer of mannuronic acid, which it is subsequently modified in to mannuronic transforming guluronic units by enzymatic epimerization (1). The final product contains zones formed by guluronic acid (G), zones formed by mannuronic acid (M), and guluronic acid and zones with alternating mannuronic acid. The relative content of each of these blocks depends the types of algae and the conditions of growth. While the block formed by acid, alternating guluronic and mannuronic acid always represented around 33.33% (1). The alginate is a derivative of propylene glycol alginate. It is obtained by esterification to propylene oxide with alginic acid partly neutralized. It has the characteristic soluble around pH 2. It is form soft gels with the addition of polyvalent cations such as calcium pH below 4. There are less sensitive to the action of the calcium than the rest of alginates and it has emulsifying power. The most common use is as stabilizing beer foam. It is important to mention that currently efficiency of biopolymers as microencapsulated agents not determined by each one, actually there are used to increase

efficiency mixtures as microencapsulated agent (1). The efficiency of the mixture depends upon the nature of the components, concentrations and proportions of each. The most important characteristic of alginate is its ability to form gels in the presence of divalent ions (calcium, magnesium) without heating. The molecular weight is related to the chain length and viscosity conditions. When the molecular weight is higher, the larger the chain length and thus higher viscosity (1).

Tragacanth gum is an exudate; it is obtained from some species as *Astragalus*. The gum is composed of a mixture of polysaccharides, one fraction is called tragacántin (arabinogalactan), it is soluble on water, which it is obtained by dissolving a hydrosol, in the same way, the second fraction is called basorin (tragacanthic acid), it is not soluble on water, its component in the gum which gives the ability to swell in aqueous medium, which represents 60-70% of the total molecule (2). It is soluble in cold water, stable to heat and low pH values (below 2). Tragacanth gum has a molecular weight around 840,000 Daltons, which it provides a high viscosity. A tragacanth property is the ability to produce high viscosity solutions at concentrations of 1% (w / v). It has highly viscous gel when in concentrations of 2-4% (w / v), (7).

To establish the physical behavior of biopolymers, we can study its viscoelasticity, which helps us to determinate the mechanical properties of biopolymers, which it has viscous and elastic properties, which it is themselves represented with various mathematical models (6).

The viscoelastic materials in small deformations, almost near-equilibrium presents a linear relationship between stress and strain, it is being in the zone known as linear viscoelasticity. While strains are increasing, this relationship let it be linear and it reaches the area known nonlinear viscoelasticity. The low strains characterized by a rheometer ensure linear viscoelastic regime, so that the same response is strongly linked with their molecular structure, which it represents a characterization. The development of the mathematical theory of linear viscoelasticity is based on the response (strain) at any time is directly proportional to its driving force (stress). Thus, if the deformation and strain rate are infinitesimal and the stress-strain relationship, time dependent, can be described by differential equations with constant coefficients, so as determined linear viscoelastic behavior. Since the rheological behavior of viscoelastic materials is difficult to visualize, mathematical models are used for representing. In such models usually represent the behavior of a Newtonian fluid and solid Hooke (6).

Objetive

The objective of this study was determined the viscoelasticity and rigidity index of the gels obtained with complex mixtures of SA, PGA and TG for subsequent mathematical modeling.

Methods and materials

For the development of work using a mix design- simplex centroid, which it was obtained 10 combinations: 3 singles, 6 binary and 1 ternary.

It was prepared gels of PGA (Cargill, Incorporated, USA), TG (Pharmacy Paris, Mexico) and SA (Cargill, Incorporated, USA) as follows: the dispersions were prepared in 100 mL of distilled water, and then depending on the components of the gel followed different ways (3) . Later the samples were taken to analyze a Rheometer model LS 100 (Paar Physica, Germany), using parallel plate geometry PP 20. The samples were placed in the rheometer, after it conducted a study of viscoelasticity, the parameters were obtained according to the Burgers equation with Kelvin-Voigt element: η, J_0, J_1 y λ (6).

$$J = f(t) = J_0 + J_1\left(1 - \exp\left(\frac{-t}{\lambda_{ret}}\right)\right) + \frac{t}{\mu_0}$$

The Rheometer determined an amplitude sweep in which it was observed the linear viscoelastic zone, then with this data is did a frequency sweep in which there are obtained the values of G 'and G", later it obtained curves of Creep and dynamic parameters obtained according to the Burgers equation with a Kelvin-Voigt element (6).

Results and discussion

Each of the mixtures that were obtained showed different linear viscoelastic zones between them. Subsequently frequency sweeps were conducted with each of these zones and its components were obtained viscous (G ') and elastic (G"). From these graphs, it obtained the behavior of the gels of mixtures. M1, M4 and M8 presented to behavior of macromolecular solutions. While M2, M3, M5, M6, M7, M9 and M10 mixtures showed strong gel behaviors; all this was based on the behavior G´and G" (6).

Also Creep curves were obtained for each of the gels, M1, M4 and M8 mixtures when left to push an strain not exhibit any recovery. While to M2, M3, M5, M6, M7, M9 and M10 mixtures when the strain failed to push to them, it was obtained almost 100% recovery. Gels were characterized by Burgers mathematical model with a Kelvin-Voigt element getting their dynamic parameters, noting that the Instantaneous Compliance (J_0) was used to obtain the index of rigidity of the gels.

Table 1. Rheological parameters according to the Burgers equation with Kelvin-Voigt element.

No Mezcla	**Torque**	**η**	**J_0**	**J_1**	**λ**
	mNm	Pas	Pa^{-1}	Pa^{-1}	S
1	0.008	$7.06X10^{1}\pm6.30X10^{-1}$	$2.40X10^{-2}\pm2.00X10^{-3}$	$2.70X10^{-1}\pm2.00X10^{-3}$	$9.83X10^{1}\pm8.80X10^{-1}$
2	0.0183	$7.62X10^{5}\pm3.74X10^{1}$	$1.00X10^{-3}\pm5.55X10^{-8}$	$6.00X10^{-4}\pm3.25X10^{-8}$	$1.83X10^{1}\pm8.90X10^{-4}$
3	0.0113	$1.57\ X10^{5}\pm1.40X10^{1}$	$1.12X10^{-2}\pm1.00X10^{-6}$	$8.047X10^{-4}\pm7.17X10^{-8}$	$3.80X10^{1}\pm 3.38X10^{-3}$
4	0.002	$5.67\ X10^{-1}\pm1.63$	$1.45X10^{1}\pm4.18$	$1.00X10^{-6}\pm2.87X10^{-6}$	$2.34X10^{-2}\pm6.76X10^{-2}$
5	0.0016	$2.77X10^{5}\pm1.40X10^{1}$	$1.34X10^{-3}\pm6.79X10^{-8}$	$1.01X10^{-3}\pm5.10X10^{-8}$	$2.10X10^{1}\pm1.06X10^{-3}$
6	0.002	$4.22\ X10^{5}\pm5.39$	$5.55X10^{-4}\pm7.09X10^{-9}$	$3.01X10^{-4}\pm3.83X10^{-9}$	$4.69X10^{1}\pm5.98X10^{-4}$
7	0.0058	$1.86X10^{5}\pm4.57X10^{1}$	$4.47X10^{-3}\pm1.09X10^{-6}$	$1.00X10^{-6}\pm2.45X10^{-10}$	$2.17X10^{2}\pm5.32X10^{-2}$
8	0.008	$1.28X10^{1}\pm6.85X10^{-1}$	1.79X10-1±9.57X10-3	$1.00X10^{-6}\pm5.30X10^{8}$	$2.34X10^{-2}\pm1.26X10^{-3}$
9	0.0079	$3.21X10^{4}\pm4.54X10^{1}$	$2.70X10^{-2}\pm3.82X10^{-5}$	$1.99X10^{-2}\pm2.80X10^{-5}$	$2.03X10^{1}\pm2.86X10^{-2}$
10	0.0016	$2.51X10^{5}\pm8.21$	$1.02X10^{-3}\pm3.30X10^{-8}$	$4.52X10^{-4}\pm1.48X10^{-8}$	$3.05X10^{1}\pm9.95X10^{-4}$

Table 1 shows the values of retardation times obtained which had a range of 0.0234 - 98.3 s. Also obtained the mixture showed higher viscosity was number 2 with $7.62X10^{5}$ Pas, while the mixture who had lower viscosity was number 8 with 12.8 Pas. The values which were obtained showed that the mixture number 4 presented a higher value of J_0 than others and it was 14.5 Pa^{-1}. In other hand the mixture 6 had a

lower value than others, which decreased by 99.9% compared to the higher value of J_0. Moreover 4, 7 and 8 mixtures showed the lowest values of J_1 with 1x10-6 Pa^{-1}, and the mixture 1 showed the highest value it is 0.270 Pa^{-1}.

Subsequently it was obtained the values rigidity were expressed as the reciprocal of J_0 obtained from each mixture, it was determined the best mixture with a confidence level of 95%, and possible antagonism between the components of the mixtures (4)

Conclusions

M1, M4 and M8 had macromolecular solution behavior, according the frequency sweep graphs; M2, M3, M5, M6, M7, M9 and M10 had a 1%, they had a strong gel behavior, based on the frequency graphs and Creep curves. All gels ware characterized by the model of the Burgers equation with Kelvin-Voigt element getting their dynamic parameters (5). The best mix for higher rigidity index values is SA 0.6061% and 0.3939% PGA.

Acknowledgements

The authors acknowledge the technical support of Miguel Marquez and Scholarship No 9980 awarded by CONACYT to ALAP.

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Expression Of The Acidic-Subunit Of Amarantin, Carrying The Antihypertensive Biopeptides VY, In Cell Suspension Cultures Of *Nicotiana tabacum* NT1

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Introduction

Hypertension is universally underdiagnosed and/or inadequately treated resulting in extensive target-organ damage and premature death. About one billion people are now suffering from hypertension and it is expected to increase 60% worldwide by 2025 (1). Thus the ability to inhibit the angiotensin converting enzyme (ACE) activity has been usually used for detection of therapeutic agents against chronic diseases such as hypertension (2). Recently, various studies on ACE-inhibitory peptides presents in natural proteins such as milk, egg, fish, soybean, spinach, and many other sources have been reported (3). The dipeptide Val-Tyr (VY) is one of the biopeptides tested *in vivo* that has hypotensive effect in humans and it easily can be absorbed into the human circulatory blood system (1).

On the other hand, amarantin, an 11S globulin, is the most predominant seed storage protein of amaranth, its amino acid composition is close to the optimum amino acid balance required in the human diet, and has remarkable heat stability and emulsifying properties (4). A His-tagged version of the amarantin acidic-subunit was expressed, purified and characterized in *E. coli*. Moreover, this acidic-subunit was further modified by inserting four Val-Tyr (VY) antihypertensive biopeptides in tandem, after that it was named bioamarantin, from which increases in the ACE-inhibitory activity of the recombinant protein expressed in *E. coli* was demonstrated (5).

Recently, the use of plant cell suspensions (PCS) has received considerable attention for the production of recombinant proteins, due to they have the benefits of systems that use animal or microbial cell cultures, and whole plants. Cell suspension of various plants species including Arabidopsis, rice, soybean, tomato and tobacco have been established (6).

The aim of the present study was to express the bioamarantin, in a tobacco NT1 cell suspension. The chimeric protein was partially purified and its *in vitro* ACE-inhibitory activity was evaluated.

Materials and methods

Nicotiana tabacum NT1 cells were gently donated by PhD J. Castillo-Reyna. The calli were maintained on NT1 solid medium containing 4.3 g l^{-1} MS salts, 30 g l^{-1} sucrose, 0.5 g l^{-1} MES, 1 mg l^{-1} thiamine HCl, 0.1 g l^{-1} myoinositol, 0.18 g l^{-1} KH_2PO_4 and 2.2 g l^{-1} 2,4-D. Cell suspension cultures were maintained in an orbital shaker (150 RPM) at 26 ^oC in the dark. Subcultures were done every 7 d by diluting 2 ml of cell suspension in 48 ml of fresh medium (7).

Cell growth kinetics were established from cell cultures by cell counting. To evaluate parameters of growth efficiency, specific growth rate (μ) and doubling time (dt) were calculated with the following formulas: $\mu = (\ln x - \ln x_0)/t$ and $dt = \ln 2/\mu$, respectively; where x_0, was the initial cell density; x, was the cell density at time t (8). Viability kinetics were determined with the number of viable cells by dye exclusion (9). Viability percentages were obtained with the following formula: Viability % = (Number of viable cells/Number of total cells) X 100.

For the transformation of NT1 cells and selection of transgenic cell lines, *Agrobacterium* strain EHA105 (containing either the vector pCAMBIA1304 (GenBank accession: AF234300), or pBioamaKDEL) inoculum were co-cultured with 4 ml of 11-days-old NT1 cell culture (containing 200 µM acetosyringone) in a Petri dish sealed with parafilm. The cells were incubated on an orbital shaker (100 RPM) at 28 ^oC, in

the dark for 72 h. Then, the cell suspension was plated on solid NT1 supplemented with 100 mg l^{-1} timentin and 15 mg l^{-1} hygromycin (NTTH medium), and incubated at 26 °C in the dark for 5 weeks (7). The hygromycin-resistant calli were transferred to suspension NTTH liquid medium to ensure the elimination of escapes, and cultures were maintained as described by (10).

For the histochemical glucuronidase assay, a small amount of cells (30 mg) were sampled from putative transformed cultures and subjected to histochemical glucuronidase (GUS) assay according to (11).

The DNA was extracted and subjected to PCR analysis to detect the presence of the *gus*A gene with a predicted fragment size of 982-bp, or the bioamarantin cDNA with a predicted fragment size of 648-bp (12). For *gus*A detection, Plasmid pCAMBIA1304 was used as positive control and DNA from *N. tabacum* NT1 Wild type (WT) callus as negative control. For bioamarantin detection, Plasmid pBioamaKDEL was used as positive control and DNA from *N. tabacum* NT1 WT callus as negative control. The amplified fragments were separated by electrophoresis on 1% (w/v) agarose gels, stained with ethidium bromide and fluorescence visualized under ultraviolet light, images were captured in a Molecular Imager ChemiDoc™ XRS+ System (Bio-Rad).

Total soluble protein (TSP) from transgenic tobacco callus was extracted as described by (13) with modifications. Recombinant bioamarantin expressed in transgenic tobacco cell lines, was immunochemically detected by Western blot analysis according to (14). The inmunodetection was done with polyclonal rabbit antibodies (0.5:30000 dilution) against bioamarantin, and as a secondary antibody anti-rabbit IgG (H+L) conjugated to horseradish peroxidase (HRP) (Invitrogen™) was added. Color development was initiated with the addition diaminobenzidine (DAB) and TBS, and the process was terminated by rinsing the blot with water.

Protein extracts of transgenic and non-transformed callus, were hydrolyzed according to (15) and the antihypertensive potential of the hydrolysates was determined *in vitro* according to (16). In order to determinate the IC_{50} of the hydrolysates, data were adjusted to a nonlinear regression model using Hill's equation using the software Graphpad Prism 5™. The IC_{50} were also calculated for proteins hydrolysates of non-transformed cells as control, data were subjected to analysis of variance and treatment means were compared using the Fisher's least significant difference test ($P \leq 0.05$) using STATGRAPHICS Plus 5.1 software.

Results

Cell counting was used as a measure of cell growth during batch culture. As shown in Fig. 1, a typical sigmoidal behavior of cell growth was observed, reaching the highest value at day 11 of culture. From the kinetic obtained, the values of specific growth rate (μ) and doubling time (dt) were calculated obtaining the following results: $\mu = 0.27\ d^{-1}$ and dt = 2.53 d. Fig. 1, also shows the viability kinetics obtained by dye exclusion; initial values of approximately 95% viability were observed on the first 3 d of culture and the viability was maintained on acceptable values close to 76% until day 11 of culture. After 12 d, a significant decrease in viability was shown. Due to this, the day 11 was selected as the ideal time to attempt the transformation experiments. The behavior observed on the growth and viability Kinects is typical for batch cell cultures, and this can be explained due to the following: according the cell number increases, toxic extracellular products are accumulated affecting cell growth and division, besides another factors such as low carbon dioxide from the bioreactor, the accumulated stress of agitation, and decreased available oxygen, which could decrease the number of viable cells.

NT1 cells co-cultured with *Agrobacterium*, harboring pCAMBIA1304 or pBioamarKDEL constructs, developed into different colonies after 4 weeks of plating on selective medium (Fig. 2a). each callus formed in each plate were considered independent transformation events and they were maintained separately on selective medium. The transformation method used in this work has been previously shown to be effective for PCS cultures and to allows a fast establishment of the transgenic cell culture in comparison with other methods (6).

For pCAMBIA1304, histological analysis detected stable GUS expression in all putative transformed calli (Fig. 2b); meanwhile, GUS staining was not detected in tissues of non-transformed calli (Fig. 2c). Some of the GUS-positive calli were subjected to PCR analysis, and the integration of the vector was confirmed by

the amplification of a 982-bp fragment (*gus*A) (Fig. 3a). For pBioamarKDEL, the identity of transformants was confirmed by the amplification of the expected fragment (648-bp) in all the clones analyzed (Fig. 3b). The accumulation of bioamarantin in calli of transformed cell lines was detected by protein gel blot analysis (Fig. 4). Nevertheless, when the immunological detection was performed, only two of the clones tested were positive for the bioamarantin (Fig. 4, lanes 4 and 5), showing that this protein was stably expressed in some of the transgenic calli. The results showed in this work, open the possibility for the further massive production of the recombinant bioamarantin by using a bioreactor based in the use of PCS.

The IC_{50} value obtained for the protein hydrolysates of transformed cells was 3.5 µg mL^{-1}, corresponding to nearly 10 times higher than the protein extracts of WT cells which IC_{50} obtained was 29.0 µg mL^{-1}, showing significant differences between them ($P \leq 0.05$). The calculated IC_{50} value (3.5 µg mL^{-1}) of transgenic tobacco cells, is far satisfactory, suggesting a higher antihypertensive potential compared with those reported in some food sources such as chickpea protein hydrolysates (3) with a IC_{50} of 190 µg mL^{-1}. Also, best values were obtained compared to the IC_{50} of 64 µg mL^{-1} obtained in hydrolysates of bioamarantin expressed in *E. coli* (5).

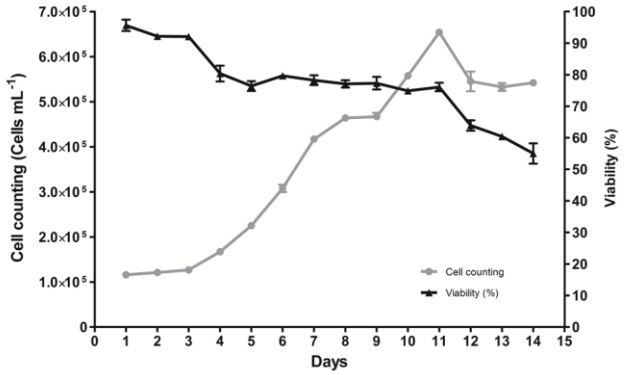

Fig. 1 Comparison of cell growth and viability kinetics. Each point is the average of three replicates. Error bars indicate standard deviation

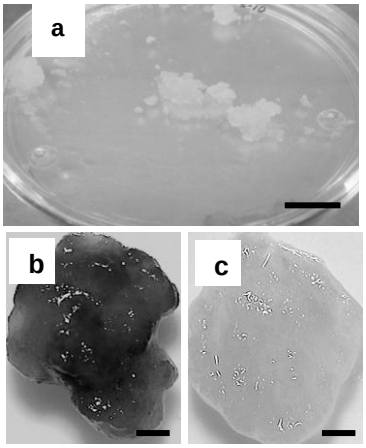

Fig. 2 Genetic transformation of tobacco NT1 cells. (a) Putative transformed calli formed from cells co-cultivated after 4 weeks of cultivation. (b) Stable *gus*A expression in transgenic callus. (c) Wild type callus as a negative control of *gus*A expression. Bars = 1 cm in a, and 0.1 cm in

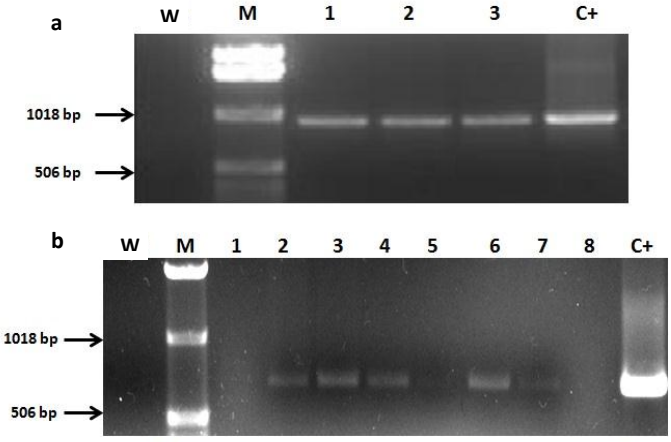

Fig. 3 DNA amplification of *gus*A gene (a), and BioamarKDEL cDNA (b), in transformed tobacco cell cultures. (a) Lanes 1 through 3, independent transformed tobacco calli. (b) Lanes 2 through 7, independent transformed tobacco cells; lanes 1 and 8: empty. WT = Wild type calli used as negative control. M = Molecular weight marker (1 kb DNA ladder). C+ = positive controls of pCAMBIA1304 (a) and pBioamarKDEL (b)

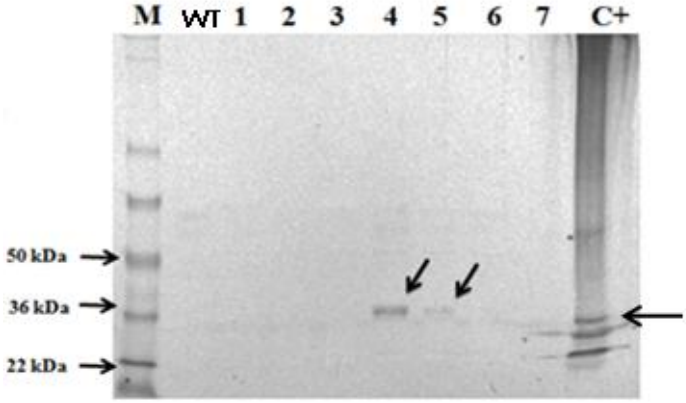

Fig. 4 Western blot analysis of total protein extract from transgenic calli. Lanes 2 through 7: protein extracts of transformed cells with pBioamarKDEL; WT: Protein extract from wild type cells used as negative control; M: Protein molecular weight marker; lane 7: empty; C+: positive control of bioamarantin expressed in *E. coli*. The arrow indicates recombinant bioamarantin. Each lane contains 20 µg of protein.

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Effect Of Protein Hydrolysates Of *Vicia Faba* On The Damage Produced By Azoxymethane In Colon

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Introduction

Colon cancer is the fifth leading cause of death in Mexico. Globally, according to the International Agency for Research on Cancer in 2008, 609,051 deaths were reported, occupying this disease the third place in deaths. Advances in early detection have been largely responsible for the reduction of mortality and morbidity in this sort of condition, but studies have shown that diet and oxidative stress are important factors in its aetiology. Numerous epidemiological studies in humans and laboratory animal experiments have directly implicated the dietary fat as a component linked to an increased risk of developing certain types of cancer, especially breast, colon and rectum, prostate and ovary(1). Also, the generation of reactive oxygen species (ROS's) and free radicals (FR) is unavoidable in aerobic metabolism, these oxidizing species cause cumulative damage to proteins, lipids and DNA, which are critical molecules to the function of the body. However, this has its own defence mechanisms to deal with the action of oxidizing species. In certain situations the antioxidant defences may be overwhelmed by excessive generation of reactive oxygen species. This imbalance between oxidant and antioxidant species is known as oxidative stress, which is associated with numerous diseases and with the normal ageing process(2). Our body is exposed to a variety of ROE's and FR that can be generated from endogenous species, related to oxygen metabolism and the various reactions of defence of our immune system, or exogenous sources, such as smoking, air pollution, UV radiation, ozone and certain medicine drugs(2). It is therefore common to relate the damage caused by the various reactive species to the pathophysiology of several diseases such as cancer and diabetes, among other pathologies(3).

Food Technology has shown that within proteins are obtainable substances capable of decreasing or counteracting the free radicals, among these substances are bioactive peptides, which are inactive within the protein, but when they are released after hydrolysis, exert beneficial effects on the body(4) which makes them an alternative to counteract the aforementioned conditions. Most research on bioactive peptides with antioxidant and anticancer activity has been conducted in animal protein, but its origin makes them expensive on the market. Good, low-cost sources of protein are the legumes. Fava bean is an important source of protein, especially for low-income sectors of the country. Its importance lies in the protein content, which is higher in comparison with other sources such as corn and beans. The protein in *Vicia faba* represents about 24-32%. The major proteins of the bean are globulins, which consist of two fractions: legumin and vicilin; these storage proteins are found in the protein bodies in a proportion of approximately 60%. Because of this reason, the peptides obtained from *Vicia faba* could serve as adjuvants to prevent the formation of free radicals generated by lipid peroxidation and to counteract diseases like cancer. This research aims to contribute to physical and technological knowledge of *Vicia faba*, besides to characterize and evaluate bioactive peptides through *in vivo* and *in vitro* assays.

Materials and methods

The protein concentrate and hydrolysates were obtained by the method of Pedroche et al. (5), and they were hydrolysed with trypsin for 0.5 h. In order to perform biological evaluation, doses of 10, 20 and 30

mg/kg were tested. For the model, male ICR mice were used weighing 25-30g, fed with normal and hyperlipidemic diets plus protein hydrolysate, intragastrically for 4 weeks; the AOM was administered intraperitoneally (10 mg/kg) in order to induce pre-carcinogenic lesions, 10 days after the treatment started, 2 times a week for 15 days, recording the weight each eight days. After treatment, the animals were sacrificed and colon was removed and washed, and opened longitudinally to be subsequently fixed with 10% formalin, keeping it for 24 h at 4 ° C and then stained with 0.2% methylene blue. Observation of aberrant crypt foci (ACF) was performed on a microscope (10X), considering the criteria mentioned by McLelland and Bird (6).

Results

Aberrant crypt foci (ACF) in mice fed with normolipidemic diet and protein hydrolysates from *Vicia faba*

As observed in Figure 1, the ACF in the normolipidemic control group are very few crypts (12 crypts). Considering this number as a baseline, since it depends on the susceptibility of the strain. By contrast, it is noted that in the AOM-treated group this number increased 7-fold compared with the control group, finding an ACF number of 70.5.

As well, in the groups treated with different doses of protein hydrolysates the number of ACF decreased significantly ($p < 0.05$) compared to AOM-treated group, from 23 to 46.

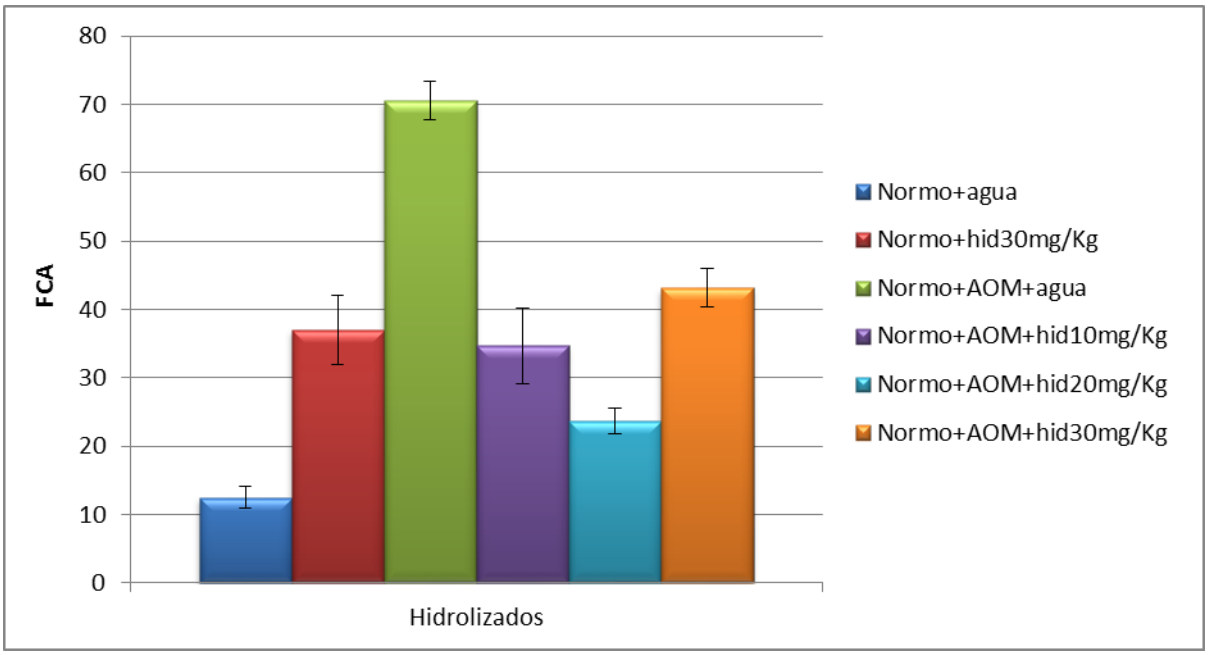

Figure 1. Aberrant crypt foci in mice fed with normolipidemic and protein hydrolysates from Vicia faba at different doses. Values are the result of averaging data from 6 mice ± SE analysed by Three way-ANOVA. Comparisons between groups were performed by Student-Newman-Keuls (SNK) test.

For hyperlipidemic mice (Figure 2), it is possible to observe that the control group of AOM had a greater number of ACF respect to the control group of normolipidemic mice.

By administering a hypercholesterolemic diet, there was an increase in the development of the ACF and this effect is further increased by administering the AOM to 77.5 crypts. As well, there was significant difference between the group treated with AOM and the group treated with 10 mg/kg dose where the

incidence of ACF is much lower, being reduced about 5 times, allowing us to consider that this dose is effective in preventing the formation of ACF in the initial stage of carcinogenesis.

On the other hand, at a dose of 20 mg/kg no significant difference respect to the group of AOM was observed; however, this dose decreased the number of foci as well as the dose of 30 mg/kg, which showed significant difference ($p < 0.05$) with respect to the group of AOM, by decreasing the number of crypts to 62.

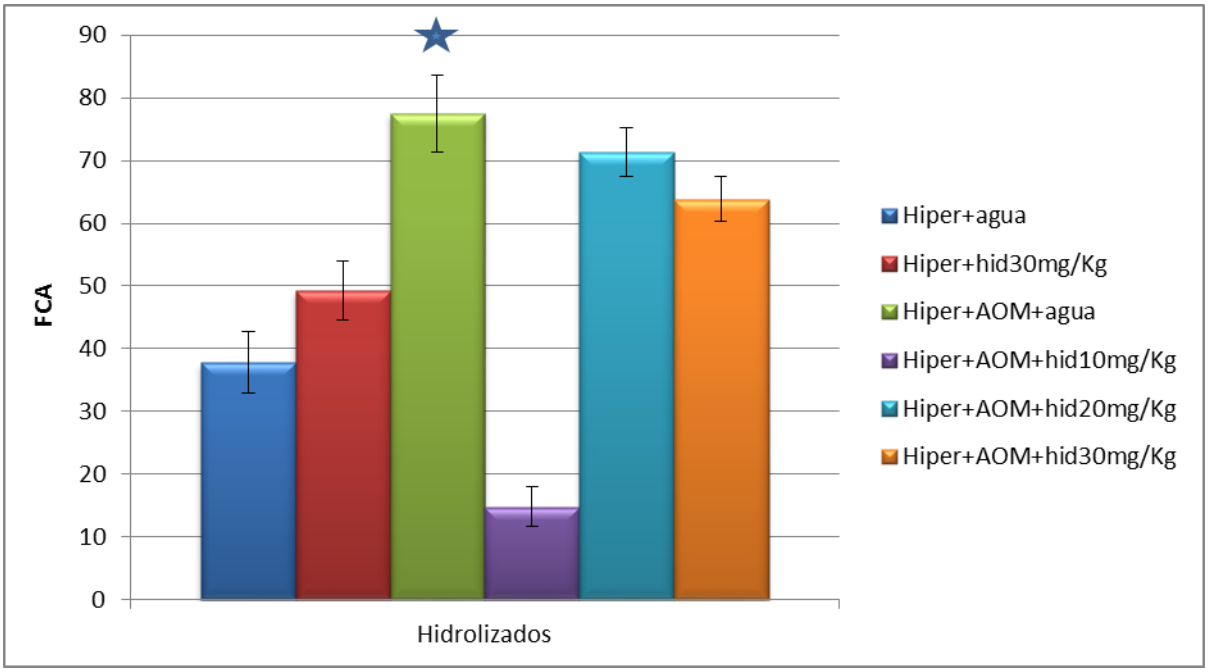

Figure 2. Aberrant crypt foci in mice fed with hyperlipidemic and protein hydrolysates from *Vicia faba* at different doses. Values are the result of averaging data from 6 mice ± SE analysed by Three way-ANOVA. Comparisons between groups were performed by Student-Newman-Keuls (SNK) test.

The AOM model for colon carcinogenesis is used to study the stages of initiation and promotion in mice. The AOM is an intermediate of the colon carcinogenic agent DHM (1,2-dimethylhydrazine) and is metabolized by cytochrome P-450. It has been shown that by administering the specific colon carcinogenic agent AOM, there is an increase in the amount and the activities of reactive nitrogen species and the activity of COX-2 in the colon mucosa(7).
The oxidation of NO to superoxide, peroxynitrite and nitrogen species such as NO_3, NO_2 and N_2O_3, leads to the formation of carcinogenic nitrogen compounds. Reactive nitrogen species and peroxynitrite are cytotoxic and can cause damage to DNA; these are generated by macrophages in inflammatory bowel disease which is a factor for developing colon cancer. Thus, studies have shown that increasing the activity of reactive nitrogen species increases colon carcinogenesis(8).

As mentioned above, the hydrolyzate at a dose of 10 mg/kg showed the greatest extent of reduction in the incidence of ACF; thereby, it can be considered to have anti-carcinogenic activity. This activity can be attributed to hydrophobic amino acids of the protein and peptides which act as antioxidants and decrease the reactive oxygen species as well as the activity of COX-2 and, therefore, the incidence of cancer.
Several studies have shown that soy protein isolate alters the overall expression of colonic genes, enhancing somatostatin, which is a known antiproliferative agent for colon cancer cells and also can inhibit tumorigenesis(9). Moreover, Hakkak (10) showed that the composition of soy proteins decreases the incidence of colon tumors induced by azoxymethane in rats, this anticancer activity may be attributed to bioactive peptides derived from soy protein, this hypothesis has been supported by models *in vivo* and

in vitro where it has been demonstrated that the hydrophobic amino acids of soy protein may have anticancer activity.

Lunasin is a chemopreventive peptide obtained from the 2s fraction of soy albumin. It contains 43 amino acids with 9 aspartic acid residues(11) . The mechanism of its anticarcinogenic activity is by serving as chemical suppressor in mouse mammary cells. In studies by Hakkak (10) in male Sprague-Dawley rats, treated with AOM at a dose of 15 mg/kg, once per week for 15 days, they found that rats fed with soy protein isolate, decreased the incidence of developing colonic tumors in relation to the control group treated with casein where the decrease was 31 to 10% in the proximal colon. Similar results were found in the distal part of the colon where the incidence of tumors was less than 31 to 10%.

The results obtained in the present work showed that protein hydrolysates from *Vicia faba* have good functional properties, showing antioxidant activity and even good prospects for the prevention of colon cancer, especially when the diet is based on foods with high contents of fat, carbohydrates and cholesterol.

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Evaluation Of Hypolipidemic Capability Of Protein Hydrolysates Obtained From *Viciafaba* In Hyperlipidemic Mouse Model

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Introduction

The main target site of oxidation processes induced by free radicals are polyunsaturated fatty acids (PUFA), so that high levels of oxidized lipids and reduced antioxidants inblood contribute to endothelial damage and dyslipidaemia. The combination of a number of cardiovascular risk factors and the association of different diseases such as diabetes mellitus, hypertension, obesity, hyperlipidaemia and atherosclerosis comprise the metabolic syndrome[1]. Dyslipidaemia is characteristic of this syndrome in which there is an increase of triglycerides (TG), decreased high density lipoprotein and increased low density lipoprotein.

Since reactive oxygen species (ROS) and free radicals (RL) are constantly and unavoidablyproduced during metabolic processes, the cell has developed a complex defence system to limit exposure to these agents and transform them to less toxic products through antioxidants. The cell protection against oxygen-derived free radicals comprises capturing aggressive intermediates,prevention of their formation, inhibition of their spread, and the repair of damages. Furthermore, antioxidants play an important role in the prevention of many diseases like cardiovascular problems, tumours, chronic degenerative diseases and even the aging process[2].

In recent years, research on functional food components has become one of the most explored. The functional components, such as phenolic compounds, flavonoids and terpenes having bioactive properties, are obtained from several natural sources. Other functional components are bioactive peptides which are defined as amino acid sequences of small length, from two to fifteen residues;being inactive inside the precursor protein, these sequences can be released upon hydrolysis of proteins by the action of gastrointestinal enzymes such as pepsin, trypsin and chymotrypsin, which allow obtaining these peptides in order to produce numerous beneficial effects on human organism[3].

Legumes such as fava beans (*Viciafaba*) have high protein content (24-32%); thereby, bioactive peptides with biological activity for the body may be obtained and act as functional foods. The aim of this study was to evaluate the hypolipidemicactivity of protein hydrolysates of *Viciafaba*.

Materials and methods

Fava bean seeds were purchased on the local market and the testa and cotyledon were manually separated, then, these portions were ground to powder and sieved to obtain a uniform particle size. The flour thus prepared was used for obtaining the protein concentrate, using the method of Pedroche*et al*. (2002)[4], whereas for hydrolysates, trypsin and chymotrypsin for 0.5, 1,1.5, 2, 2.5 and 3 h were used. The extent of hydrolysis was determined by the method of Kim *et al*. (1990)[5] and by electrophoresis (SDS-PAGE). Finally, antioxidant activity was measured by the method of DPPH and ABTS. In order to perform biological evaluation, the hydrolysate with the highest antioxidant activity by the both methods (trypsin 0.5h) was chosen. For the model, maleICR micewere used weighing 25-30g, fed with normal and

hyperlipidemic diets plus protein hydrolysate,intragastrically for 4 weeks. The hydrolysates were administered at a dose of 30 mg/kg. At the end of the administration period, blood samples were taken by retro-orbital puncture and subsequently centrifuged at 10000 rpm for 15 min. Serum was obtained and glucose, cholesterol, triglycerides, HDL and alkaline phosphatase activity were determined using an automated method (AutoanalyzerSelectraII Vitalab, Wiener Lab.); afterwards, the livers were extracted and analysed.

Results and Discussion

The bean flour used reported a protein content of 26.08 g/100 g sample (Table 1), which gave a yield of 22.48 g of lyophilized protein concentrate with a protein content of 74.25 g/100 g sample, obtaining a recovery efficiency of 60 %. Macarulla and Medina, (2001)[6], reported similar values obtaining 25.5 g/100 g sample for fava bean seed while for the isolate it was 77.3 g/100 g sample, so that they obtained a similar relationship to the values obtained here. The protein concentrate was obtained by alkaline dissolution and subsequent isoelectric precipitation, being the most common method to obtain protein isolate in the food industry. Among its advantages are its inexpensiveness and the modest equipment it requires, which makethis option advantageous compared to other processes such as separation of proteins by ultrafiltration with membranes (Sanchez-Vioque*et al.*, 1999)[7].

Table 1.Proximate chemical composition of flour and concentrate (g/100g sample)

Component	Flour *	Concentrate *
Moisture	7.45±0.210	4.88±0.0.07
Ash	3.82±0.011	3.40±0.05
Protein (N*6.25)	26.08±0.714	74.25±0.09
Lipids	2.33±0.05	1.57±0.03
Fibre	5.32±0.04	4.14±0.08
Carbohydrates**	55.03±0.01	11.76±0.02

* Values are the result of averaging three independent determinations ± SD.

** Determined by difference.

As can be observed, the moisture content of the seed was greater than in the protein concentrate, since values of 7.45 and 4.88% were respectively obtained. These values are similar to those from other studies with fava bean (Macarulla and Medina, 2001) with values of 10.9 and 3.2% for flour and concentrate, respectively.

Enzymatic hydrolysis and extent of hydrolysis (EH)

The hydrolysis of the protein concentrate was individually performed with three digestive enzymes: trypsin, chymotrypsin and pancreatin, with times of 0.5-3h. Trypsin had higher proteolytic effect compared with chymotrypsin and pancreatin, and remained constant after 2.5 h (Figure 1). Due to this hydrolysis, the molecular properties of the proteins change, resulting in the decrease of molecular weight, increase of electrical charge, and release of hydrophobic groups (Caessens, 1999)[8].

Trypsin is a specific enzyme that breaks the carboxyl side of lysine or arginine if they are not linked to proline. The proportion of these amino acids in the protein of the bean is approximately 13%; thereby, since trypsin acts over a greater proportion of amino acids and cleavage sites,the extent of hydrolysis is high. With chymotrypsin, hydrolysis peaks 1.5 h after the start of the process, and then stabilize. Chymotrypsin is a specific enzyme that breaks the carboxyl end of aromatic amino acids and methionine

if they are not linked to proline, the proportion of these amino acids in the protein bean is 7.2%, which is why there is a lower hydrolysis extent.The enzymatic hydrolysis is related to the degree of hydrolysis, which is the essential property of a hydrolyzate and is defined as the percentage of broken peptide bonds in relation to the original protein (Benitez, 2008)[9].

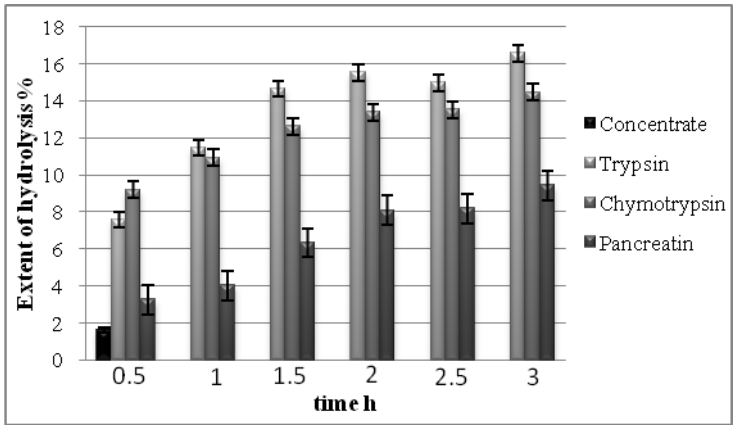

Figure1. Extent of hydrolysis of the protein concentrate and hydrolysatesobtained with trypsin, chymotrypsin and pancreatin. Values are the result of averaging three independent determinations ± SE analyzed by Two way-ANOVA. Comparisons between groups were performed by Student-Newman-Keuls (SNK) test.

Antioxidant activity (DPPH•)

The antioxidant activity (% inhibition), which corresponds to the amount of DPPH• radical neutralized by peptides with antioxidant activity was greatest for the hydrolysate obtained with trypsin (54.38%) at 0.5 h. The results were compared with vitamin E, which has the highest antioxidant activity (92%) compared with the samples studied. The antioxidant activity with chymotrypsin showed 19-31% inhibition, and showed significant difference compared with trypsin. This antioxidant activity may be attributed to the amino acid composition, the specificity of the cleavage sites of this enzyme to obtain bioactive peptides, as well as to the sequence, size and configuration of the peptides (Chen *et al*., 1996)[10].

Antioxidant activity (ABTS•+ method)

The antioxidant activity of trypsin hydrolysates by the method of radical ABTS•+ was 0.13 to 0.27 mMTrolox equivalents/mg protein, and the 0.5 h hydrolysate showed the highest activity. Also,the concentrate showed antioxidant activity (0.1917 mMTrolox equivalents / mg protein) compared to the hydrolysates and vitamin E reference. Given the trypsin hydrolysate showedthe greatest inhibition (3%), the radical ABTS•+ was blocked to a greater extent when compared with chymotrypsin.

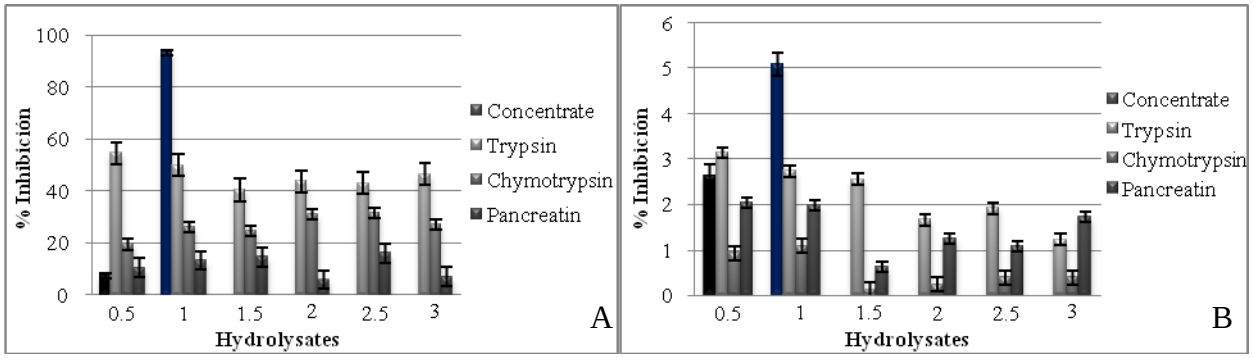

Figure2.Antioxidant activity by DPPH• (A) andABTS•+ (B) for protein concentrate and hydrolysates of Viciafaba.Values are the result of averaging three independent determinations ± SE.

Biologic evaluation

Table 2 summarises the effect of protein hydrolysates (30 mg/kg body weight) in the evaluation *in vivo* on lipid profile: cholesterol (total-col), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), atherogenic index (AI), and the activity of the enzyme alkaline phosphatase (ALP). As can be observed, in the control group that received normal diet for 40 days, a total-col serum concentration of 29.83 mmol/L was obtained, whereas in the group receiving the hypercholesterolemic diet during the same time, the values rose significantly to 67.16 mmol/L,i.e. an increase of 2.25 times. This confirms the effectiveness of the diet that was used in this experimental model. A number of studies suggest that soy protein may reduce cholesterol levels in blood in experimental animals andhumans (Potter, 1995). It has been reported that soy protein hydrolysate has a strong effect on the reduction of serum levels of cholesterol as compared to intact soy proteins in rats (Sugano, 1990)[11].

Table 2.Effect of hydrolysate of *Viciafaba* on lipidic profile

Group	Total cholesterol (mmol/L)	Triglycerides (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	AI	ALP (U/L)
N	29.83±1.92	0.83±0.04	11.4±0.32	16.74±1.35	1.33±0.16	214.66±18.2
N30	27±0.96	1.88±0.09	14.7±0.37	13.74±1.89	4.25±0.59	315.83±30.1
H	67.16±2.61	0.6±0.05	10.71±0.71	57.35±1.12	6.58±1.32	423±22.91
H30	60.33±2.32	0.7±0.08	11.65±0.73	52.51±1.83	4.22±0.57	364.5±12.72

N=(Normal diet); N30=(Normal diet +hydrolysate30 mg/kg); H=(Hyperlipidemic diet); H30=(Hyperlypidemic diet +hydrolysate30 mg/kg). Values are the result of averaging data from 6 mice ± SE, analyzed byOne way-ANOVAl. Comparisons between groups were performed by Student-Newman-Keuls (SNK) test.

On the other hand, the hypocholesterolemic effect has also been reported in a peptide fragment derived from soy: Leu-Pro-Tyr-Pro-Arg, which was found to decrease serum cholesterol levels in mice after oral administration at 50 mg/kg dose, where values were 25.4% and 30.6% total cholesterol and LDL-cholesterol, respectively (Yoshikawa, 2000)[12].

Regarding triglycerides, when comparing the control and hyperlipidemic groups, significant difference ($p < 0.05$) was observed and the values ranged from 0.83 to 0.6 mmol/L, respectively, which meant a reduction of 1.38 times, and the dose of 30 mg/kg decreases TG levels compared to the group N. In 2001, Macarulla reported a decrease in TG levels of hypercholesterolemicWistar rats fed with seeds and protein isolate of *Viciafaba* in comparison with the control group fed with casein; TG values obtained ranged between 0.73 and 1.21 mmol/L, respectively.

By doing a comparison between the control groups of both models, the level of HDL is similar; as well, there was significant difference ($p < 0.05$) compared to the group administered with 30 mg/kg in both models, in both cases there was a significant increase. Likewise, thehypercholesterolemic diet compared to N, increased serum levels of LDL from 16.74 to 57.35 mmol/L. However, the dose of 30 mg/kg decreased LDL levels in mice treated with both diets, which suggests that the bioactive peptides can help to reduce the occurrence of cardiovascular diseases.

The relationship between total cholesterol and HDL gives the atherogenic index (AI); significant difference ($p < 0.05$) was observed against the AI of the control group in both normolipidemic and hyperlipidemic mice where the values ranged from 1.33 to 6.58, respectively.
The obtained data show that there was an increase in alkaline phosphatase activity in mice fed with hypercholesterolemic diet, which was from 214.66 to 423.5 U/L compared to normolipidemic group. This behaviour may occur due to the increase in the lipid concentration,which increases metabolic activity of liver, thereby, the enzyme activity increases to counterbalance the metabolism of the organism, as has been shown in several studies when there is damage in the liver. Recently, it has been reported an increase in ALP in rats when there exist obstructive cholestasis induced by dysfunction of the bile ducts, and this can be due to deconjugation of bile salts by microorganisms which may lead to the formation of gallstones obstructing the biliary ducts, which explains the slight increase in the ALP (Accatino*et al*., 1996, Marteau and Rambaud, 1993)[13,14].

The results obtained in the present work allow observing that the protein hydrolysates from *Viciafaba* have important functional properties, showing hypolipidemic activity that makes them helpful against coronary diseases.

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Growth Kinetics Of Lactobacilli Using Agave Fructans As A Carbon Source

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Introduction

Prebiotics are non-digestible nutritional ingredients with beneficial effects due to their capacity to stimulate the growth and/or activity of probiotic bacteria in the colon, improving the health of the host. They are fermented by probiotics, which are living microorganisms that are beneficial for the host and increase the balance of the intestinal flora. Among the most common probiotics are those from the genuses *Lactobacillus, Streptococcus* and *Bifidobacterium.* Agave fructans are non-digestible, fermentable carbohydrates that are considered to be prebiotics because of their structure[1]. The extraction of fructans is an option for agave producers in Oaxaca. The current production of agave is 130,240 tons, of which only 27% is harvested, leaving raw material available for use. The object of this study was to evaluate the kinetics of four strains of *Lactobacilli* (*Lactobacillus acidophilus, L. casei, L. paracasei y L. rhamnosus)* using the fructans from *Agave angustifolia* as the carbon source.

Materials and methods

The fructans used were obtained from *Agave angustifolia* Haw., 8 years of age, from Tolapan, Tlacolula, Oaxaca. Four strains of *Lactobacillus* were used: (a) L. *acidophilus* LA-2 25302; (b) *L. casei* LCC-1 53103; (c) *L. paracasei* LPC-1 393; and (d) *L. rhamnosus* LR 4356. These strains were proven to be probiotics following the method reported by Gibson and Wang[2]. The experiment was established with a 4x5x2 factorial arrangement. The study factors were the following: (1) four strains of *Lactobacillus*; (2) five different sources of carbon: long-chain *A. angustifolia* fructans (A), short-chain (B), totals (C), and two inulin types from chicory, Raftiline (RN) and Raftilose (RS); (3) agave fructans were obtained using two methods: the conventional method (C), utilizating an ethanol-water solution and spray drying (100-80°C), and removal with water (H) clarified with active carbon and convection drying at 50°C. Silica gel plates were used for thin layer chromatography, and a developer for sugars was used to visualize the separation of compounds. 10 µL of each sample was applied to the silica gel plate using the standards of glucose, fructose, sucrose, 1-kestosa, 1,1-kestotetraose, 1,1,1-kestopentaose. The determination of direct sugars, totals, and fructose was done with colorimetric methods[3]. To obtain the growth kinetics, 1% of each source of carbon was used and dissolved with the MRS (Man-Rogosa-Sharpe) medium; the growth medium and carbon source were sterilized in a Market Forge-Sterilmatic autoclave; previously, 1% of the bacteria was inoculated and incubated at 37ºC in anaerobic conditions, measuring growth with optic density of 600 nm at different times: t=0, 2, 4, 6, 9,12, 15, 18, 24 and 30 hours.

Results and Discussion

Identification of fructooligosaccharides

Figure 1 shows the profile of one TLC of the *A. angustifolia* fructans that were used. It can be observed that in (AC) there was greater intensity in the stain at the base of the chromatograph, similar to the inulin syrup standard with an approximate DP of 20, than in the sample (AH), in which the presence of short chain fructans can also be observed or lower grade polymerization (DP) with greater fructose and glucose

intensity. This fact can be associated with greater hydrolysis in (AH), due to the method of extraction that was used and the convection drying, compared with (AC).

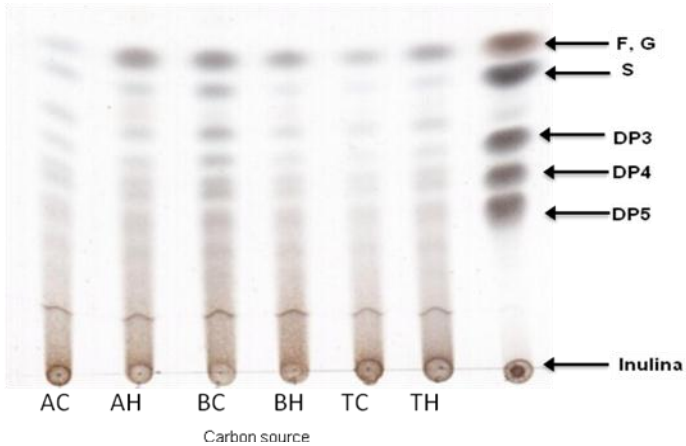


Figure 1. TLC of conventional chain fructans (AC) and water (AH), conventional short chain fructans(BC) and water (BH), conventional total fructans (TC) and water (TH). Standards: F= fructose, G = glucose, S = sucrose, 1-kestose (DP3), 1.1 kestotetraose (DP4) and 1,1,1, kestopentaose (DP5) and chicory inulin

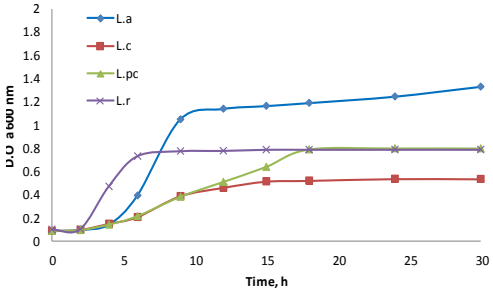
Table 1**.** Content of sugar in the dry *A. angustifolia* fructans

Fructans	Total carbohydrates mg/g	Reducing sugars mg/g	Fructose mg/g
AC	878.16	79.72	385
AH	726.21	135.25	280
BC	579.86	124.75	275
BH	511.01	90.79	310
TC	892.52	82.67	585

It can be observed that the production of free sugars was due to the hydrolysis of the short chain fructans during extraction (Table1). Moreover, the velocity of fructose formation during hydrolysis depends on the length of the chain in the fructose transformation

Growth kinetics

The growth curves were elaborated in agreement with the type of fructan evaluated for each lactobacillus, charting for each bacteria the number of generations (n), the velocity of multiplication of the number of generations for each unit of time (R), the duplication time (Td), the rate of specific growth (μ), and the coefficient of determination r^2. Figure 2 shows the growth kinetics of the four strains of *Lactobacillus* in long chain *A. angustifolia* fructans extracted by conventional form and spray dried in a temperature of 100-80 ºC (AC) and fructans extracted in water (AH). In this figure we can observe that each strain of *Lactobacillus* behaved differently with this substrate. *L. rhamnosus* had a shorter lag phase, with a greater specific growth rate (μ =0.3256 h^{-1}) and a lesser duplication time (2.1288 h) than the other *Lactobacillus*



Growth kinetics using AC

Lactobacillus	n	R	Td (h)	μ (h^{-1})	r^2
L. acidophilus	3.5112	0.3901	2.5631	0.2704	0.9809
L. casei	2.5003	0.1666	5.9992	0.1155	0.9530
L. paracasei	3.1007	0.1722	5.8051	0.1194	0.9984
L. rhamnosus	2.8184	0.4697	2.1288	0.3256	0.9906

Growth kinetics using AH

Lactobacillus	n	R	Td (h)	μ (h^{-1})	r^2
L. acidophilus	4.0799	0.3399	2.9412	0.2356	0.9753
L. casei	3.1134	0.2594	3.8543	0.1798	0.9520
L. paracasei	3.4877	0.2325	4.3008	0.1611	0.9851
L. rhamnosus	2.9273	0.4878	2.0496	0.3381	0.9824

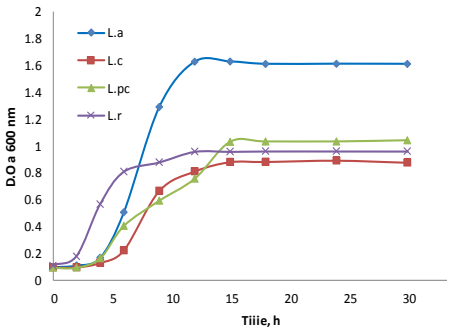
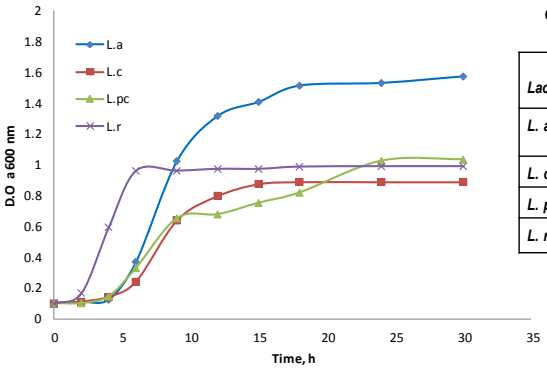


Figure 2. Growth kinetics of *L. acidophilus* (L.a); *L. casei* (L.c); *L. paracasei* (L.pc) and *L. rhamnosus* (L.r) using as a carbon source (AC and AH) respectively

Using the high DP fructans extracted in water (AH), the growth kinetics of the four strains of *Lactobacillus* can be observed, in which *L. acidophilus* had an exponential phase with a coefficient of determination of r^2=0.9752, which indicates that 97.52% of the growth variation of the lactobacilli was due to the linear relationship that exists between the incubation time and the source of carbon, which we can see was most efficient in *L. acidophilus*. With respect to *L. casei*, a r^2= 0.9520 was found, slightly less than that of *L. acidophilus* but very similar to that of *L. paracasei* and *L. rhamnosus*, which had a similar coefficient of determination.

Another substrate used for the growth kinectics was (BC). In Figure 3 it can be observed that greatest growth was obtained with the *L. acidophilus*, which can be related in this type of fructans to the greater presence of short DP fructans[4]. The respective table shows the results that were obtained from the growth curves for each lactobacillus, using (BC) as a substrate. The lowest velocity of multiplication is 0.1400, corresponding to *L. paracasei*, a bacteria with a duplication time of 7.1411 hours and a coefficient of determination from the exponential phase of r^2=0.8895, which is reflected in the growth curve, specifically in μ =0.0970 h^{-1}.

Figure 3 also demonstrates the behavior of the lactobacilli using (BH) as a substrate. The lag phase between *L. acidophilus* and *L. casei* was similar, in contrast with *L. rhamnosus*, which had a shorter lag phase than previous samples. In the case of *L. paracasei*, a longer lag phase was obtained than with the *L. rhamnosus*. In comparing the substrates (BC) and (BH) (Figure 3), the greatest growth of the bacteria was with (BC). Substrate (BH) did not have the greatest yield in terms of growth of lactobacilli, most likely due to the deficiency of the drying method and perhaps a caramelization of the sugars, which were then unusable.



Growth kinetics using BC

Lactobacillus	n	R	Td (h)	μ (h^{-1})	r^2
L. acidophilus	3.6996	0.3083	3.2435	0.2136	0.9765
L. casei	3.0231	0.2519	3.9694	0.746	0.9649
L. paracasei	3.3608	0.1400	7.1411	0.0970	0.8895
L. rhamnosus	3.2845	0.5474	1.8267	0.3794	0.9981

Growth kinetics using BH

Lactobacillus	n	R	Td (h)	μ (h^{-1})	r^2
L. acidophilus	3.5443	0.1969	5.0785	0.1364	0.9483
L. casei	2.7864	0.3096	3.2299	0.2145	0.9988
L. paracasei	2.9344	0.1956	5.1117	0.1355	0.976
L. rhamnosus	2.7904	0.4650	0.1502	0.3223	0.9980

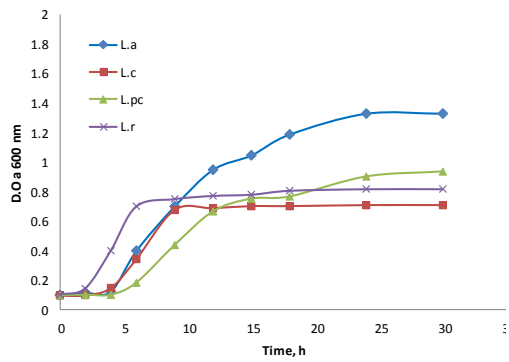


Figure 3. Lactobacillus growth curve *L. acidophilus* (La), *Lactobacillus casei* (Lc), *Lactobacillus paracasei* (L.pc) and *Lactobacillus rhamnosus* (Lr). fructans using low degree of polymerization (BC) and (BH).

Comparing the small fructans extracted through the two methods (conventional and in water) that were utilized as a carbon source for each strain of *Lactobacillus*, the greatest bacterial growth was obtained when substrate (BC) was added to the cultivation medium. Substrate (BH) did not yield the greatest growth in lactobacilli, probably due to the deficiency in the extraction method.

The behavior of the growth kinetics of the lactobacilli in the chicory inulin was very similar to that of the agave fructans. The results show that the specific growth rate and the duplication time for each strain of lactobacillus was similar, and also that *L. acidophilus* had the greatest growth in this substrate with a Td=3.3202 h and μ =0.2087 $h^{-1.}$ However, *L. casei* had lesser growth and its exponential phase had a Td=6.0916 h and μ =0.1137 h^{-1}. An exponential phase of μ = 0.0879 h^{-1} was obtained for *L. paracasei* and a duplication time of 7.8789 h. Makras [5] evaluated the growth of *L. paracasei* subsp. *paracasei* 8700:2 in Raftiline and Raftilose synergy 1 (inulin-type chicory fructans enriched with oligofructose), and growth was rapid and very similar between both prebiotics ($\mu = 0.55$ h^{-1} ; r^2 = 0.99). Another reference that was utilized as a comparison with the agave fructans was Raftilose (RS), which has a DP of 8-10. *L acidophilus* fermented this substrate better than the other strains of *Lactobacillus*, with a slightly better fermentation than the small agave fructans (BC and BH).

Conclusions

The extraction method and drying had a significant effect on the short chain fructans. From the growth kinetics, we can observe that duplication depends on the capacity of each lactobacillus. A greater content of short chain fructans increases the growth of the strains of lactobacilli.

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Expression Of Recombinant Bovine Lactoferrin In *Pichia pastoris*

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Introduction

Lactoferrin, a no hematic iron-binding glycoprotein belonging to the family of transferrins, wich molecular weight is approximately 80 kDa, is a protein with immunomodulatory and antimicrobial capabilities, manly, which makes their study has become a fundamental part of biotechnology in recent years. Some mammal species produce lactoferrin. Its expression is given by phagocytes of mammalian cell, some mucous secretions such as saliva, tears, semen and vaginal fluids, and a small part is synthesized by mature neutrophils, this is known as lactoferrin serum (5).

In normal milk, its concentration is low, but increases in response to inflammatory processes.

Some functions have been attributed to lactoferrin, these features are due to the structure of this protein has; its two regions known as N and C terminal lobes, in turn formed by the domains N1, N2, and C1, C2, respectively. In each domain C2 and N2, there is a place where lactoferrin, in the presence of carbonate ion is capable of chelating ions Fe^{+2} (8). The capability that we have considered most important is its ability to modulate the host immune response; such capacity has been tested *in vivo* and *in vitro*. Recent studies have been demonstrated in healthy human volunteers that bovine Lf reinforces the immune system and antioxidant status. Coupled with the immunomodulatory activity, the antimicrobial effects of Lf have been studied and demonstrated in vivo; across the parenteral administration in several experimental animals, above all mice, several studies in vitro have indicated that the structure of Lf plays an important role in the antimicrobial activity, for example, the interaction with viruses and bacterial toxins, through glycosylation sites of the protein, making it being exposed on its outer surface and maintain contact with these microorganisms. Antibacterial activity. Lactoferrin has direct activity with LPS of the bacteria and prevents the interaction between the endotoxin LPS binding protein (LBP) and CD14. In addition, this recognition that lactoferrin has with LPS, triggers a signaling cascade that results in the release pro-inflammatory mediators such as cytokines and chemokines, and small molecules, such as lipid mediators and reactive oxygen species. Furthermore, when lactoferrin was administered by oral via in *Staphylococcus aureus* infected models, there is a change in the host response by increasing the levels of TNF-α and decreased IL-5 and IL-1. Antiviral activity. Lactoferrin has activity against a broad spectrum of DNA and RNA virus, its major contribution to this antiviral defense involves binding to the cell membrane glycosaminoglycans (3). Antifungal activity.*Candida albicans* is one of the most commonly agents that causes vaginal infections. Their adhesion to the vaginal epithelium can be prevented by the action of lactoferrin due to their ability to sequester iron, in addition, lactoferrin has the ability to induce apoptosis in this yeast (4). Antiparasitic activity. The study of the mechanisms by which lactoferrin has shown antiparasitic activity is complex; Lactoferrin exerts activity *against Entamoebahistolytica, Trichomonasfaetus, Trypanosomacruzi, Trypanozomabrucei, Plasmoduim falciparum, Toxoplasma gondii and Eimeriastiedai* (5.6). The process by which such activity is exerted is not clear, however, it is believed that these antimicrobial properties are related to a direct action on microbes or activation of the immune response. The important role of lactoferrin in the host, has attracted the interest to produce a recombinant protein, which will be more biocompatible that the synthetic protein; expression systems of recombinant

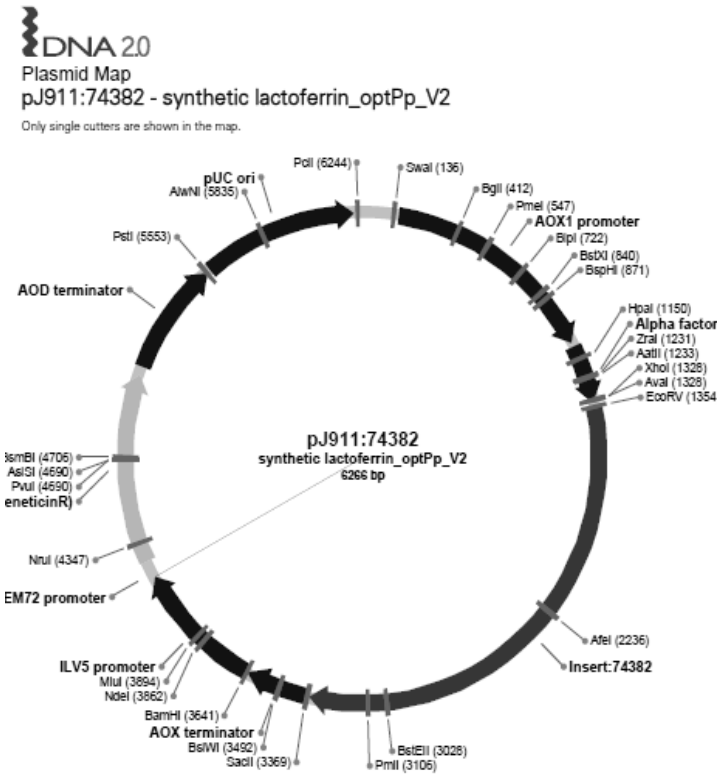
proteins based in yeast have demonstrated to be an efficient and economically viable source eukaryotic proteins. Yeasts provide a suitable environment for protein folding, besides which are capable to secret to the culture medium without need to lysing the cells. Its growth and handling is very simple due to its unicellular nature, although their eukaryotic organization allows them expression and maturation processes typical of animal cells (1). *Pichia pastoris* is a facultative methylotrophic yeast belonging to the group of ascomycetes, reproduced by budding and whose endowment genome homothallic remains, haploid, unless it is subjected to conditions of nitrogen limitation (5), also it has certain properties that justify the success of their increased use as eukaryotic expression system of heterologous proteins, as well as their high level of transcription, and the fact that native proteins are secreted into the extracellular medium, which makes the process recovery and purification of proteins easier (2). *Pichia pastoris* has been used as expression system in approximately 600 recombinant proteins (7), Jiang et al, in 2007, were able to express and produce 1200 mg / L of recombinant human lactoferrin in KM71-H strain of *Pichia pastoris*; Yong *et al* in 2006 expressed in this yeast, yak lactoferrin, where they obtained 40 mg / L. The background expression and production of lactoferrin in *Pichia pastoris*, have attracted our interest in expressing recombinant bovine lactoferrin in this system in order to use it as an alternative to the overuse of antibiotics, a situation that has led to the emergence of new microbial strains resistant to commonly used drugs, consumption of lactoferrin also mean strengthening the immune system in healthy people.

Material and methods

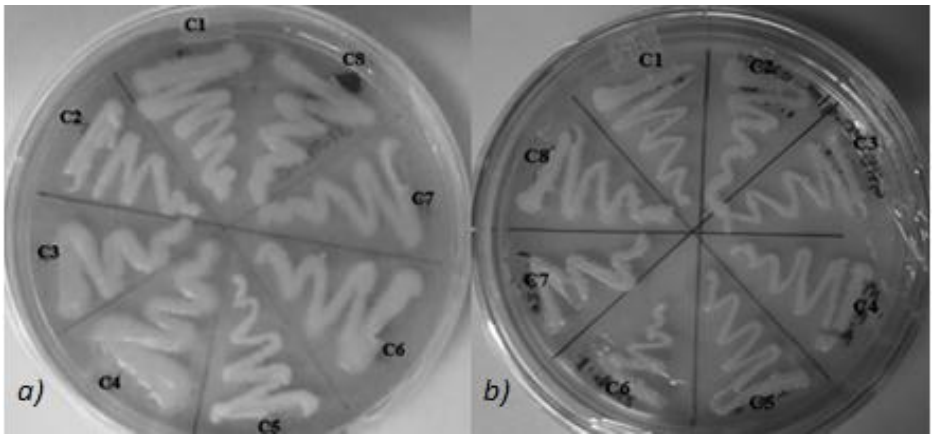
KM71-H and X33 strains of the methylotrophic yeast *Pichia pastoris* as used as expression model.Growth curve was performed to determine incubation time to prepare electro-competent yeast cells; the yeast were incubated in YPD liquid culture media (yeast extract, peptone, dextrose) in shaking at 30° C. Every 30 minutes the optical density was measure until to get the best absorbancy. Electro-competent cells were preparated with sorbitol and water method and yeast transformation was achieved by electroporation with the empty vector pJ911:74382 and expression vector containing synthetic lactoferrin pJ911ptPp_V2 of 6266 bp. After three days to incubation, we obtained transformed yeast clones, which were selected with G418. To assess lactoferrin expression, clones were induced with 0.5% methanol in intervals of 24 hours, during 72 hours in liquid culture medium. Protein extracts were recovered by precipitation of culture media and were analyzed by SDS-PAGE to search the best clones in order to express lactoferrin. Subsequently by western blot using anti-bLF HRP.

Results and discussion

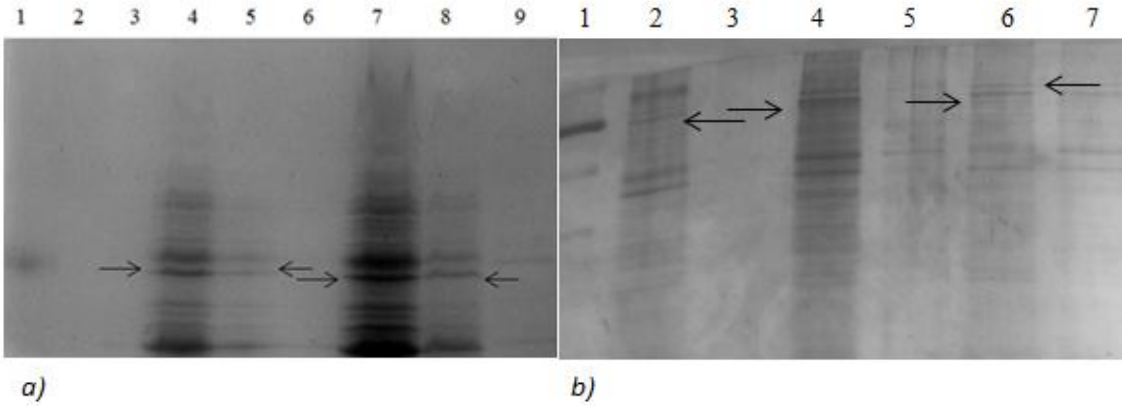
There was demonstrated the presence of recombinant bovine lactoferrin (rbLf) in the culture media due to externalization fusion peptide 5’ of lactoferrin synthetic gene. Expression and purification of rbLf will allow apply in food and feed industry, mainly as an alternative of use of antibiotic and as a nutraceutical supplement to improve the body's immune response.



PICTURE 1.Plasmid map pJ911:74382- synthetic lactoferrin_optPp_V2 Transformed cells containing the plasmid with the gene coding for the expression of bovine lactoferrin, which was inserted by electroporation.



PICTURE 2.*a)* KM71-H Transformed cells.*b)* X33 Transformed cells. The best 8 transformant cells were selected with G418 for each strain.



PICTURE 3. Proteins expression in SDS-PAGE 12%. a) KM71-H strain. ***1***. Positive control of commercial lactoferrin. **2**. C1 ***3***. C2 ***4***.C3 ***5***.C4 ***6***.C6 ***7***. C7 ***8***.C8 ***9.***Empty vector. The best expression of this strain were detected in C3. *b)* X33 strain. 1. Positive control of commercial lactoferrin. **2.** C2. 3. Empty vector. ***3***. C3***4***.C4***5***.C5***6***. C6 ***7***. C7. Arrows indicate the presence of recombinant bovine .lactoferrin.

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Nutritional Characterization of Muffins Elaborated With Agave Dietary Fiber and Jamaica (*Hibiscus sabdariffa*) Calyces

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Introduction

Dietary fiber (DF), is one of the most recognized functional ingredient because of their healthy properties in individuals.[1]A common strategy to increase their comsuption is throughout the addition or supplementation in foods with DF sources. The development andresearch of natural DF sources are being grown nowadays. The steam of *Agave tequilana Weber* plant exhibits an average of carbohydrates of 250 g/kg mostly fructans, and is usually used to obtain tequila. However, it is also used to obtain fructose syrup and agave fructans.[2] The last one is considered soluble DF like inuline type. Ground agave fiber is a by product obtained during extraction process and is consider as insoluble DF. Jamaica (*Hibiscus sabdariffa* L) is prepared generally as a beverage where the calyces are decoctedand the water obtained is extensively consumed because of the freshness sensation that is conveyed. The whole calyces are rich in DF and polyphenols.[3] Agave fructans (soluble DF), ground agave fiber (insoluble DF) and whole jamaica calyces, were used to elaborate muffins. The objective of this study was to evaluate the nutritional characterization of wheat-muffins susbtituted by this natural ingredients

Materials and methods

Ingredients preparation and muffins formulation

Agave fructans and ground agave fiber were obtained by Biogaves de la Costa S.A. de CV and were mixed in 1:3 proportion, similar to that recommended by international dietary guidelines.[4]This ingredient is referred henceforth as agave dietary fiber (ADF). Besides, whole jamaica calyces (JC) were adquired in Huajicori Nayarit by local producers, they were washed, dried in an oven at 60ºC, milled and sieved. The chemical composition of ADF and JC is shown in Table 1. The rest of the ingredients were purchased in a local supermarket. The muffins were prepared following a housemade formulation. Wheat from the original formulation were substituted in 30 % and 50 % by the ADF and a mixture of ADF and JC (1:1 w/w).

Table 1. Chemical composition in agave dietary fiber (ADF) and jamaica calyces (JC) (g/100g dry base)

	ADF[a]	JC[a]
Total dietary fiber[b]	82.03	49.89
Insoluble dietary fiber[b]	59.20 ± 1.89	41.86 ± 1.30
Soluble dietary fiber	22.83 ± 1.22	8.03 ± 0.35
Soluble carbohydrates	3.36 ± 0.09	5.50 ± 0.02
Protein	4.7 ± 0.41	12.92 ± 0.24
Fat	0.67 ± 1.7	0.89 ± 0.02
Ash	1.37 ± 1.7	3.50 ± 0.20
Extractable polyphenols	0.24 ± 0.08	2.06 ± 0.06
Hydrolysable tannins	nd[b]	0.63 ± 0.05
Condensed tannins	nd	1.85 ± 1.00

[a]Values are the mean ± DS ($n \geq 3$); [b]not detected

Sensory evaluation of the muffins

Two sensory tests were carried out, a pair comparison test to determine the maximun level of substitution of these ingredients in which the judges did not find differences between samples and a hedonic in the selected formulations.

Chemical composition analyses

Moisture, ash, protein and fat were analyzed according AOAC methods.[5] Extractable polyphenols by Montreu[6] method were evaluated in muffins.

Starch digestibility in muffins

Available and total starch were determined by Holm *et al*[7]and Goñi *et al*[8]methods. Also starch digestibility were evaluated by Holm *et al*[9] method.

Dietary fiber determination as Indigestible faction in muffins

A physiologic procedure that does not consider the use of thermo stable α-amylase was carried out to determine the indigestible fraction as described Saura Calixto et al.[10] Samples were incubated with digestive enzymes to simulate digestion in the small intestine.

Statistical analyses

One way ANOVA and LSD media comparison ($p>0.05$) were used to find differences between samples and in the pair comparison sensory test was used chi-square. All data was performed using the SAS system V 9.0.

Results and discussion

Sensory evaluation

In the paired comparision test, the judges did not detect discernible differences ($p>0.05$) on taste between muffins substituted with 30 or 50% by ADF and ADF-JC. Thus, we proceed to use the higher percentage of substitution (50%) of wheat from the original formula. Afterwards, an hedonic test was carried out where all samples were evaluated. Jamaica muffins were acepted with a similar score than control muffin, the highest score was reached by ADF muffins, however none statisfactory differences were observed, the lowest score was reached by ADF-JC muffins. This is remarkable because in the comments judges referred that the acid taste in jamaica calyces muffins as a different and a good taste.

Table 2. Hedonic test in muffins substituted with agave dietary fiber (ADF) jamaica calyces (JC) and ADF-JC.[a]

	Level of acceptance
Control muffin (CM)	7.4 ±2.7[a]
Jamaica calyces muffin (JCM)	7.1 ±2.3[a]
Agave dietary fiber muffin (ADFM)	8.1 ± 2.4[a]
Agave dietary fiber-jamaica calyces muffin (ADF-JCM)	4.6 ± 3.9[b]

[a] Data are the mean of 36 evaluations ± ES. Data with different letter are statistically different ($p<0.05$).

Chemical composition

The chemical compositon in the elaborated muffins is shown in Table 3. Ash content incresed with the addition of JC and ADF, because this ingredients have made an important contribution in the samples. Moisture was similar in the control muffin, JCM and other studied samples, showing that moisture was not affect by the agave ADF and JC added in the formulation. Usually, the addition of DF ingredients have the tendency to dry the products to which are added. Protein content was not statistically different ($p>0.05$) between studied samples, this means that the substitution with ADF and JC did not decrease the protein content. Only minor differences between fat content were found between samples. Total carbohydrates were the main component in muffins, were highlight their increase when ADF was added, this might be due to the mainly fructans component in the samples besides the simple carbohydrates that agave fructans contains[3]. The extractable polyphenols content was higher in the samples added with JC, this could be consider as a added value from a nutritional point of view. It is important to stress that DF is

consider as a carrier of antioxidants that may improve the antioxidant status[1] in foods and jamaica calyces has been demonstrated that posses healthy properties besides their polyphenol content.[3]

Table 3. Chemical composition in control (CM), jamaica calyces (JCM), agave dietary fiber (ADFM) and agave dietary fiber with jamaica calyces (ADF-JCM) muffins (g/100 g fresh base)[a]

Component	CM	JCM	ADFM	ADF-JCM
Ash	1.24±0.56[b]	2.77±0.50[a]	2.36±0.02[a]	2.23±0.06[a]
Moisture	20.46±0.72 [ab]	22.41±0.21 [a]	18.66±0.86 [b]	21.91±0.63 [a]
Fat	19.43±0.62[a]	21.44±0.20[a]	18.49±0.43[a]	18.70±0.88[a]
Protein[b]	11.04±0.91 [a]	10.78 ±0.74 [a]	9.87±0.76 [a]	11.19±0.12 [a]
TC[c]	42.13±0.94[a]	41.51±0.35[a]	49.86±0.58[b]	45.18±0.39[ab]
Extractable polyphenols[d]	0.28±0.02[a]	1.19±0.01[b]	0.56±0.02[c]	1.04±0.01[d]

[a]Values are given as mean (n≥ 3). The values with different letters are significantly different at (p≤0.05).[b]Factor 6.25. [c]TC: total carbohydrates. [d]mg GAE/100 g dry base.

Starch digestibility in muffins

The addition of DF sources decreased the starch content in the samples. The available starch , total starch content and starch digestibility in the studied samples are shown in Table 4. Regular muffin contains high amounts of digestible starch fractions present data for muffins suggest it as an alternative for products with reduced digestible starch contents. This reduced starch content improved their healthy properties.

Table 4. Total starch (TS) available starch (AS) (g/100 g fresh base) and starch digestibility (SD) in control (CM), jamaica calyces (JCM), agave dietary fiber (ADFM) and agave dietary fiber with jamaica calyces (ADF-JCM) muffins

Formulation	TS	AS	SD (%, min)				
			0	5	15	30	60
MC	45.1±0.7[a]	41.8±0.7[a]	3.2±1.4[a]	19.7±0.3[a]	64.5±1.8[ab]	66.8±1.7[a]	86.1±0.5[a]
MJC	41.5±1.1[ab]	38.5±1.1[ab]	8.0±2.5[a]	13.5±2.2[a]	69.3±1.2[ab]	70.8±0.8[ab]	86.2±1.3[a]
MADF	33.8±0.4[b]	31.2±0.4[b]	7.2±2.7[a]	11.4±1.4[a]	67.2±0.9[b]	69.5±2.0[a]	78.4±1.7[b]
MADF-JC	33.1±2.5[b]	30.0±2.5[b]	2.4±0.8[a]	7.2±1.4[b]	59.5±1.3[a]	66.8±2.3[ab]	73.3±1.1[b]

Values are given as mean (n ≥ 3). The values with different letters are significantly different at (p≤0.05).

Dietary fiber determination as Indigestible faction in muffins

The indigestible fraction in the muffins is shown in the Table 5. It is important to stress that in samples added with ADF the soluble indigestible fraction increased two fold regard the other samples.Scientific evidence that the primary characteristics of DF assigned to non-starch polysaccharides and lignin (resistance to digestion and absorption in the small intestine and fermentation in the large intestine) can be extended to other indigestible food constituents. As a whole, it has been defined as food indigestible fraction.

Table 5. Indigestible soluble fraction (ISF), indigestible insoluble fraction (IIF) and total indigestible fraction in control (CM), jamaica calyces (JCM), agave dietary fiber (ADFM) and agave dietary fiber with jamaica calyces (ADF-JCM) muffins (g/100 g dry base)[a]

Formulation	ISF	IIF	TIF [1]
CM	4.91±0.42[c]	24.46±3.47[a]	29.14±3.08
JCM	3.36±0.15[d]	24.23±0.35[a]	27.83±0.46
ADFM	9.31±0.30[a]	17.77±1.18[b]	27.08±1.34
ADF-JCM	7.72±0.30[b]	17.31±2.70[b]	25.03±2.39

[a]Values are given as mean (n ≥ 3). The values with different letters are significantly different at (p≤0.05). [1] Total insoluble fraction is the sum of ISF and IIF.

Conclusions

The substitution of DF from natural ingredients such as *Agave tequilana* and *Hibiscus sabdariffa* calyces in the studied samples makes possible to obtain a bakery product which besides have an acceptable sensory properties, reduced starch content, contains extractable polyphenols and mainly a soluble dietary fiber source with the concomminat healthy properties.

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Identification Of Polyphenolic Compounds And Evaluation Of Antioxidant Activity In Crude Extracts And Their Fractions of Mexican Pecan Nut (*Carya illinoinensis*)

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Introduction

C. illinoinensis is a nut native of North America, that grown in southern and central USA and northern Mexico, known as pecan (1). Pecans are one of the most consumed nuts around the world that in the last years have gained scientific interest due to its high phytochemical content (2) as well as potential positive health effect on their consumers, especially in avoiding chronic degenerative disease such as cataracts, Parkinson and different types of cancer (3-6).

Polyphenolic compounds are widely distributed in the plant kingdom and help to prevent chronic degenerative diseases due to their antioxidant properties (7,8). Recently the FDA suggested that eating about 42 g a day of certain nuts like pecans, could help to prevent cardiovascular diseases (9). There are around 8000 phenolic compounds that can be classified in two major groups: flavonoids and non-flavonoids, the first group shares a common skeleton, consisting in a 2 aromatic rings and one oxygen heterocyclic, while non-flavonoids present a phenolic group in their structure (10). Both types of phenolic compounds can form complex structures known as condensed (flavonoids) or hydrolizable (non-flavonoids) tannins (11). Their abundance in plant, function and biological activity depends on their structure, and its variability is very influenced by external conditions (soil nutriments, water availability, pathogen attack, etc) (12). Some authors highlight that tannins showed higher antioxidant capacity, compared to their monomeric units (13).

Chihuahua is one of the major pecan producers in Mexico, with an annual production of 79 ton per year (14). Actually the pecan industry calculates that 50% of the total weigh of pecans is lost in the worthless shell. It would be interesting to explore the use of the shells as a source of antioxidants with diverse applications. The aim of this study was to quantified total phenols (TP), flavonoids (FC) and tannins (CT) in *C. illinoinensis* kernels and shells growth in Chihuahua, and determinate, monomeric and polymeric tannin fractions in both parts of the nut (kernel and shell). Finally, identify by high performance liquid chromatography (HPLC) important polyphenols like catechin, epicatechin and ellagic acid currently suggested as alternative cancer treatments. These results provide a new view on the benefits of consuming Chihuahua's pecan nut (*C. illinoinensis*), and suggest a new use of an industrial byproduct that is now a waste product.

Methods and materials

Sample preparation. Pecan nuts were donated by three producers from different regions of the state of Chihuahua, México: Flores Magón, Delicias and Jiménez. The nuts were manually cracked and the kernels were separated and storage at 4 °C until analysis. The kernels were defatted by blending with hexane (1:10, w/v) 3 times for 3 min. The defatted powder and shell (4.5 g previously grounded) were extracted for phenolic compounds. Extraction consisted in sonicated the sample with 80% acetone (45ml) in ultrasound equipment for 30 min at room temperature. After that, the extract was centrifugated for 10 min at 3000 g at 4ºC. The pellet was re-extracted, and the solvent removed under vacuum. The polyphenolic extracts were lyophilized and storage at -80°C in freeze vacuum plastic bags.

Determination of total phenolic content (TP). Was determinate by Folin-Ciocalteu technique, according to reference (3). Results were expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE/gr sample)
Determination of total flavonoid content (FC). This was carried out by adapted technique from Zhishen (15). Results were expressed as milligrams of catechin equivalents per gram of sample (mg CE/gr sample).
Determination of condensed tannins (CT). Determinate by the vanillin assay according to the method of Chavan (3) Results were expressed in milligrams of catechin equivalents per gram of sample (mg CE/gr sample).
Fractionation of crude extracts. Separation of crude extracts in mono and polymeric fractions was carried out by a chromatographic column packed with sephadex LH-20. Monomeric phenolic compounds were eluted form the column using ethanol 95%. Polymeric compounds (tannins) were washed from the column with acetone 50% (v/v).
Identification of phenolic compounds by HPLC. Phenolic compounds were analyzed using a C_{18} column in a HPLC system. Formic acid 1% in water (A) and acetonitrile 5% in methanol (B) were used as mobile phase, at a 1mL/min rate. The detection was monitored at 280 and 320 nm. The retention times were compared with commercial standards.
Antioxidant activity. The antioxidant activity of kernels and shells (both crude extract and its fractions) were determinated by three different colorimetric techniques. DPPH and ABTS as radical scavenging techniques and FRAP as ferric reducing antioxidant power: according to the methodologies proposed by de la Rosa (3) and Álvarez-Parrilla (16). Results were reported as μmol of trolox equivalents per gram of sample (μmol TE/g sample).

Results and discussion

No difference in phenolic compounds were observed between kernels from the three regions. The amount of the three types of polyphenolic compounds in crude extracts from shell was dependent on the growing area (Table 1). Jiménez showed the highest levels for the three quantified compounds, followed by Delicias and Flores Magón. These values are consistent with those found by de la Rosa in 2011, and Villarreal in 2007 for Chihuahua and New Mexico, respectively. The minor differences between our results and those reported previously can be explained due to differences in the extraction method used by the first reference and the climatic condition in second. It is well known that external factors and genetics influence the abundance, structure and type of polyphenolic compounds in plants (12,6).

Sample	TP (mgGAE/g shell)	FC (mgCE/g shell)	CT (mgCE/g shell)
Flores Magón	24.73 ± 1.58 [c]	16.27 ± 0.34 [a]	282.29 ± 19.14 [c]
Delicias	34.50 ± 1.87 [b]	19.62 ± 1.53 [a]	426.32 ± 29.86 [b]
Jiménez	54.29 ± 0.93 [a]	32.86 ± 1.9 [b]	700.17± 93.10 [a]
Kernel	9.5 ± 0.9	3.4 ± 0.3	30.3 ± 3.09

Table 1. Quantification of three different type of polyphenolic compounds in kernel (average between regions) and shell crude extracts of Mexican *C. illinoinensis***.**

The same behavior was observed in antioxidant activity, the shell from Jimenez showed the highest antioxidant capacity measured by all the techniques followed by Delicias and Flores Magón. In general terms, phenolic quantification and antioxidant activity was 5-20 folds higher in shells compared to kernels. Kernels didn't show differences on phenolic compounds and antioxidant activity between areas.

Sample	DPPH (µmolTE/g shell)	ABTS (µmolTE/g shell)	FRAP (µmolTE/g shell)
Flores Magón	336.39 ± 28.71 [c]	640.76 ± 66.30 [c]	475.92 ± 25.44 [c]
Delicias	476.90 ± 47.23 [b]	922.50 ± 91.91 [b]	644.04 ± 93.26 [b]
Jiménez	837.08 ± 77.06 [a]	1385.22 ± 138.43 [a]	965.11 ± 54.58 [a]
Kernel	93.5 ± 9.8	143.3 ± 13.5	101.54 ± 4.9

Table 2. Antioxidant activity in kernel and shell crude extracts of Mexican C. *illinoinensis*.

A positive correlation was established between the three types of phenolic compounds in shell and the three techniques for antioxidant activity (**Fig 1**).

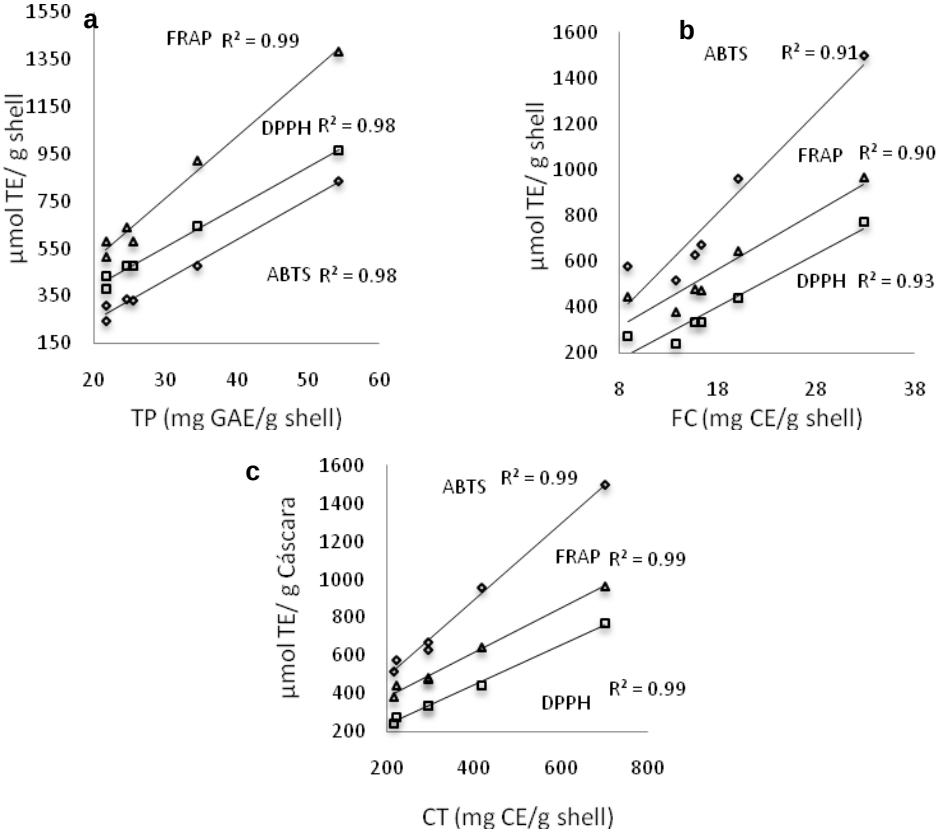

Figure1. Correlation between shell antioxidant capacity measured by DPPH, ABTS and FRAP and total phenolic content (a), flavonoid content (b) and condensed tannins (c).

Based on these results, we suggest an extra value for shell that represents an important source of antioxidant compounds with possible applications in agricultural and/or nutraceutic industry (7).

Ellagic acid, catequin and epicatequin were identified by HPLC (retention times of 10.7, 7.5 and 8.1 min respectively). They were only identified in the monomeric fraction of shell and kernel (**Fig 2**). None of these compounds were identified in polymeric fraction. It is difficult to found free phenolic compound in nature, they're easy conjugated with polysaccharides and proteins, and they're difficult to isolated without going through various methods of isolation and purification (17,18).

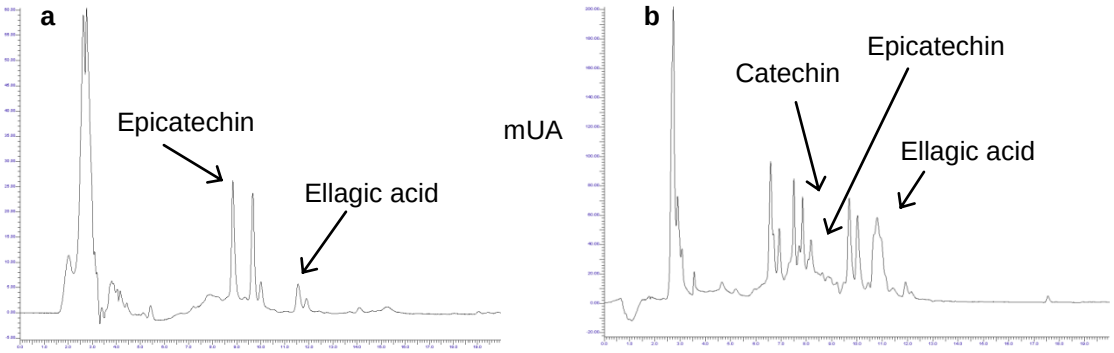

Figure 2. HPLC chromatograms with UV detection at 280 nm of monomeric fraction in shell (a) and kernel (b).

The antioxidant capacity was measured in monomeric and polymeric fractions of *C. illinoinensis* shell and kernel. In shell, monomeric fraction showed more antioxidant activity than polymeric by both techniques. In the case of kernel, polymeric fraction had the highest antioxidant activity, studied by DPPH method (**Table 3**). This is in agreement with some authors that explain that the high antioxidant activity in kernels could be because they're protecting polyunsaturated fats against oxidation (19).

	Method	**Monomeric µmol TE/g sample**	**Polymeric µmol TE/g sample**
Shell	ABTS•	54.81 ± 23.4[a]	35.11 ± 1.22[b]
	DPPH•	172.79 ± 2.0[a]	83.89 ± 10.19[a]
Kernel	ABTS•	18049.72 ± 1577.5[b]	63.81 ± 1.1[a]
	DPPH•	3778.76 ± 700.5[a]	60911.60 ± 5006.9[b]

Table 3. Antioxidant capacity of kernel and shell fractions.

Acknowledgments. Financial support from CONACYT (CB-2011-164167) and PROMEP (Red Uso de Subproductos) is gratefully acknowledged. A.A.V.F. Thanks CONACYT for a master in science scholarship.

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Optimization Of The Extraction Of Mangiferin And Lupeol High Hydrostatic Pressure In Shells Mango (*Mangiferaindica* L.) Variety Ataulfo

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Introduction

The growing interest in natural bioactive compounds as aids in disease prevention has increased considerably in recent years. Bioactive compounds from fruits and vegetables are preferred over synthetic, due to safety. During processing of Mango fruit, peel of the fruit is the main product is not considered important for commercial purposes; it is discarded and becomes a source of contamination. However, it was reported that the shells contain bioactive compounds such as Mangiferin and Lupeol, with antioxidant properties, so that might be considered as aimportant source of these compounds with bioactivity.
The extraction is an important step in the bioactives compounds isolation. Severals extraction techniques can be applied for obtaining these compounds. The extraction by conventional methods has demostrateto be productive, however some of them have a minimum impact on the environment.The unconventional methods, emerging interest to assess its efficiency compared to conventional methods in order to obtain a higher productivity extraction of bioactive substances.
Whereas the state of Nayarit is one of the leading producers of Mango, which has great economic importance, with the interests of research methods for efficient and favorable for the extraction of bioactive substances, raising overall objective is to optimize extraction of Mangiferin and Lupeol in the peel of mango (*Mangiferaindica* L.) variety Ataulfo high hydrostatic pressure (HHP) with three different pressure levels (150, 200 and 250 MPa) and three times (10, 15 and 20 min), in order to generate technical and scientific information is established as a starting point for application at industrial level.

Materials and methods

Raw Material. Mango fruits were taken from the community on 5 de Mayo, Tepic, Nayarit, an area noted for high production of this fruit, the fruit is cut by handinan index of physiological maturity and consumption, based on visual quality and advice from the locals, were transported in cardboard boxes, the nunderwent a facelift and selection. Obtaining the pulp and the shells to be frozen at -80°C, followed by lyophilizationat -50°C and 0.12 mb arusingalyophilizer FreeZone4.5 and spray, this to avoid loss of bioactive compounds of interest.

Extraction with high hydrostatic pressure. For extraction of Mangiferin and Lupeolas extraction solvents ethanol- watermixture (8:2) tohexane to Mangiferin and Lupeol, in a ratio 1:10 (g sample: solvent ml) were used. Vacuum packedin high- density polyethylene bags using a computer model Multivac C100. Then the high hydrostatic pressure as a method of extraction we applied. The application of high hydrostatic pressure was carried out on a computer model CIP42260 isostatic pressure (Avure Autoclave Systems, EriPA USA) equipped with apressurizing chamber 101.6mm internal diameter and 584.2 mm in length, which operates ata up to 300MP aprovided with a compressor Sullair brand with a top speed of 125psiair. Aratio of5:1 (water: anti-corrosive lubricant) as pressurizing fluid at 25°C, with pressures of 150, 200 and 250MP a and 10 times, 15 and 20 min was used in this research.

Quantification. Once the extraction method employed was generated two products, the solid absent of bioactive compounds of interest andt he extract, which was vacuum filtered with Whatman No. 1 filter paper and concentrated using a Buchi Rotavapor brand, model R-205, at a temperature of 40°C. The extract obtained was analyzed quantitatively by Biospectrophotometer CARY 50 to a wave length of 254 nm. Using external standards for identification and quantification (Sigma-Aldrich 95%).

Analysis of results. In the statistical analysis used a factorial design 3^2 with anα= 0.05, making a comparison of means (LSD) with the statistical program SAS (Statistical Analyst System, version 9.0 for Windows) as response variables having the Mangiferin and Lupeol concentration on the AtaulfoMango peel variety. Performing in triplicate.

Optimizationof extraction. There sponse surface methodology using a second degree polynomial model, setting the optimal process model with the statistical package STATGRAPHICS5.1 PLUS was used.

Results and discussion

In recent year's extraction techniques to reduce environmental impact have been developed. In these cases it is necessary to employ higher pressures to facilitate recovery of the compounds of interest; therefore, work is being directed toward techniques HHP [1]. Asassessed by HH Pextraction for obtaining Mangiferin and Lupeol with three levels of pressure and time, the results obtained are shownintable 1.

The results obtained show that the HHP is efficient for obtaining bioactive compounds,because the pressure attains the solvent diffusion through the cell walland washing of the cellular contents related to solventextraction[2].

Considering that the isostatic principle states that pressure is instantaneously and uniformly transmitted through the sample is obtained to increase the solubility of the materials and the mass transferrate [3]. Separating compounds based on their solubility by immiscible liquids.

The high pressure treatment can increase the mass transferrate; improve solvent penetration into the cells by disrupting cell walls and hydrophobic bonds in the cell membrane which may lead to a high-permeability [4]. HHP can also cause deprotonation of charged groups and hydrophobic bonds, resulting inconformational changes and denaturationof proteins, achieving high availability of the phenolic compounds for the extraction to equilibrium [5]. Based on the the oryofphase behavior, the solubility of these compounds increased with the increase of pressure [6].

The variance analyses shown asignificant difference between the pressure and timefor the extraction of compounds with bioactivity, so the next step is heoptimize the extraction process.

Table 1. Extraction of Mangiferin and Lupeol by HHP.

Pressure (MPa)	Time (min)	Average concentration (mg/gr sample on dry basis) ± standard deviation	
		Lupeol	Mangiferin
250	10	0.1587 ± 0.15^{aa}	13.9514 ± 2.72^{ab}
	15	0.3233 ± 0.27^{ab}	19.9280 ± 1.69^{aa}
	20	0.2580 ± 0.22^{ab}	20.7042 ± 3.54^{aa}
200	10	0.1077 ± 0.13^{aa}	20.4727 ± 3.37^{ab}
	15	0.4736 ± 0.24^{ab}	22.8579 ± 4.15^{aa}
	20	0.8205 ± 0.21^{ab}	15.7568 ± 2.18^{aa}
150	10	0.3318 ± 0.23^{ba}	6.1556 ± 2.56^{bb}
	15	0.3070 ± 0.12^{bb}	16.4260 ± 1.96^{ba}
	20	0.9076 ± 0.19^{bb}	13.8112 ± 2.76^{ba}

(Means with unequal letter are significantly different with a P value <0.05 where the first letter corresponds to pressure and the second time).

Optimization of HHP extraction process.

The response surface methodology is a set of mathematical and statistical techniques used to model and analyze problems in which the variable of interest is influenced by others, the objective is to optimize the variable of interest. This is done to determine optimum operating conditions [7].
The optimizationto minimizecosts,maximize profits andreduce the use ofingredients [8]. Theresponse surfacesare representedmathematically byregression functions(Figure 1and 2).However,how you arefunctionsmay be unknownor verycomplex, it is necessary approximated bysimpleempirical functionssuchas polynomialsof secondorder,which describesthe response variables.

Adjustingthe modelwith r^2=0.85 andr^2=0.83 forMangiferin andLupeolrespectively, the coefficients for the prediction of Mangiferin and Lupeol were determinatethe concentrationratiosshownin Table 2.
.

Table 2. Regression coefficients for the prediction of Mangiferin and Lupeol.

	Mangiferin	**Lupeol**
Intercept	-110.8093385	-1.97434324
Pressure (P)	0.799468223	0.018249811
Time (T)	6.018028534	0.06621557
P*T	-0.000902724	-0.000476473
P^2	-0.00181323	-3.44765E-05
T^2	-0.18381323	0.002511488

This study analyzed two response variables, so you got two polynomial models, which are presented in equations 1 and 2 forMangiferin and Lupeol respectively.

Mangiferin concentration = (-110.809)+(0.799468)(P)+ (6.01803)(T)-(0.00181323)(P^2)-(0.000902724)(P)(T) (0.183813)(T^2) **Equation 1**
Lupeol concentration = (-1.97434)+(0.0182498)(P)+ (0.0662156) (T)–(0.0000344765)(P^2)-(0.000476473)(P)(T)+ (0.00251149) (T^2) **Equation 2**

The equations 1 and 2 permit to predict the MangiferinandLupeolconcentrationsin the HHP extraction process, at different conditions of time and pressure.The response surface analysis permit to obtain the optimal values for Mangiferin and Lupeol and the effect of the analyzed factors in the extraction process mediated by HHP (Fig. 1 and Fig. 2). Finally, it was possible to obtain the optimal conditions (Table 3) to maximize the obtaining of bioactive compounds of interest in this study.

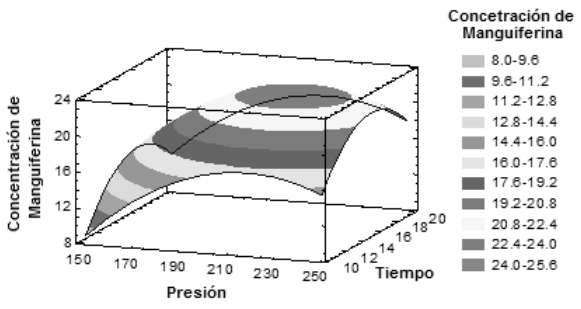

Figure 1. Estimated response surface for obtaining Mangiferin by HHP.

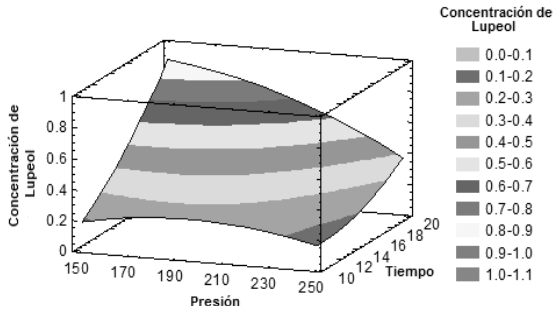

Figure 2. Estimated response surface for obtaining Lupeol by HHP.

Table 3. Optimal values for the extraction of Lupeol and Mangiferin.

Factor	Low	High	Lupeol optimal	Mangiferin optimal
Pressure (MPa)	150	250	150	217
Time (min)	10	20	20	16

The use ofHHPcan be consideredas afavorable methodfor obtainingthe fruitMangiferin andLupeol fromMango.

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Extraction And Identification Of Mangiferin And Lupeol In Leaf And Bark From Mango Tree *(Mangifera Indica)*

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Introduction

Natural antioxidants in food and in the bodies of some vascular plants have attracted considerable interest because of their potential therapeutic value.

In recent years, much of the human population,has faced chronic diseases associated with age, such as cardiovascular, neuro degenerative, type II diabetes, as well as several types of cancer, common in elderly, however incurrently it is observed that at earlier ages these diseases predominate. The bioactive compounds, due to their antioxidant activity, exert some beneficial effects on human health, particularly on these diseases.

Several studies have indicated that the mango has a high antioxidant potential, for diversity of bioactive compounds that are present in its bark, leaves, pulp and peel; these compounds with functional and technological properties, have gained great importance in the markets food, pharmaceutical and cosmetic. There are conventional methods used most frequently in the extraction of bioactive compounds and that turn out to be productive; as maceration and heating, however has emerged the interest for the study of new methods of extraction,such assonication, microwave, non-conventionalmethods, this in orderto checkits efficiencyoverconventional methods, with the vision to expanding its use in industry, since the interest is to find methods that allow greater productivity.

So that intended by this project is evaluate four extraction methods for two important compounds found in bark and leaves of mango: mangiferin and lupeol in two varieties of the state of Nayarit: ataulfoand criollo, in order to determine the extraction method of more efficient to obtain the compounds of interest.

Methods andmaterials

Raw material

Was usedas raw materialleaves andbark ofmangovarieties ataulfo and criollo, samples were taken from theMunicipality ofSantiagoIxcuintla, community Puerta de Mangos. Samples were collectedmanually, choosing thelarger leavesandgreen, the bark was takenfrom the trunkand branchesof the tree. Were transportedin cardboard boxesstored in the laboratory of food engineering of the technological Institute of Tepic; later performed them a wash and a selection based that do not submit physical and microbiological damage.

Preparation of raw material

Samplesofleaves andbarkchoppedof the two varietiesofmango, were subjected tofreeze-80^{0}C, subsequentlylyophilizationto -50^{0}Cand 0.12mbarusing aFreezone 4.5lyophilizermodel7750020, in order to avoid the loss of the compounds of interest.Subsequently ahomogenizationof the dried samples, using a Homogenizer brand Braun commercial. Finally, powdered samples were stored in jars, to avoid contamination.

Extraction of bioactive compounds

The extractionof bioactive compoundswascarried out bytwoconventional methods,maceration andheating, andtwonon-conventional, sonication andmicrowave. Thesolventsusedwereethanol-water (8:2)for extractionofmangiferinand hexane tolupeol.

Maceration

Was conductedcoldmaceration, 10 g ofsample iscontactedwiththesolvent in anErlenmeyerflask, in agitation for24 hours, in order to increasethesolubilityofthematerial andthemasstransfer rate[1], sothattheseparationwill be based onthesolubilityofthe compounds.

Heating

Was carried out continuous in a soxhelt extraction. In a ball 250 ml flaskwere placed200 mlofsolventandin extraction chamber10g sample.The extraction was carriedout in atimeof8 hours[2].

Sonication

We used aBransonsonicatormodel1510, by applying a constantfrequencyof42 kHzto10 gofsample into contactwith the solvent,this for30minat room temperature,in order to remove searched compound of the plant material. The solid and liquid particles vibrate and are accelerated by ultrasonic action; as a result the solute passes quickly from the solid phase to the solvent [1].

Microwave

This method was applied for 10 sec, using a homemade microwave 600 watts of power, 10 g of the sample in contact with the solvent, causing of irradiation of microwave,movement in molecules by ions migration and rotation of dipoles which contribute to rapid transfer of energy to the solvent and plant material [1].

Concentrationof the extract

Once employed the extraction method were generated two products, absent solid of the bioactive compounds of interest and the extract, which was filtered and concentrated using a Buchirotavapor, model R-205, at a temperature of 40 °C to 150 rpm.

Qualitative determination ofbioactive compounds

The qualitative determination the presence of mangiferin and lupeol in the extract from the leaves and bark of mango, obtained from different extraction methodswas performed on thin layer chromatography (TLC) using silica gel plates 60 F_{254}, as eluent phase: ethyl acetate, methanol and water (8:2:1) to mangiferin and a developer 2,2-diphenyl-1-picrylhydrazyl DPPH; for lupeol as eluent phase: hexane and ethyl acetate (9:1) and as developer anisaldehyde, using mangiferin and lupeol (Sigma-Aldrich,95%) as standard [3].

Statistical Analysis

Was carried out 4x2x2 a factorial design(extraction methods, variety and source), and analysis of results was performed by comparison of means (LSD) with $\alpha = 0.05$, using the SAS statistical program (Statistical Analyst System, version 9.0 for Windows), taking as response variables the amount of extract obtained from the bark and leaves of mango varieties ataulfo and Criollo. Determinations were performed in duplicate.

Results and discussion

Extraction of mangiferin by conventional and non-conventional methods in leaf and bark of mango, the varieties of ataulfo and criollo.

The extractions were carried out by the four methods proposed using as solvent a mixture of ethanol-water (8: 2), obtaining the following results shown in the following table (Table 1).

Table 1. Extract obtained inleaf andbarkof varieties ataulfoand criollo, by fourdifferent extractionmethods, using as solventethanol-water (8:2).

SOURCE	VARIETY	EXTRACTION METHODS			
		Maceration	Heating	Sonication	Microwave
		obtained extractg/ 10gsample			
LEAF	Ataulfo	2.2±0.14 ^{aaa}	3.35±0.07 ^{aab}	3.5±0.14 ^{aab}	2.25±0.07 ^{aaa}
	Criollo	2.1±0.28 ^{aba}	2.75±0.21^{abb}	2.65±0.07 ^{abb}	1.8±0.14 ^{aba}
BARK	Ataulfo	1.7±0.14 ^{baa}	1.8±0.14 ^{bab}	1.9±0.28 ^{bab}	1.75±0.07 ^{baa}
	Criollo	1.7±0.28 ^{bba}	1.95±0.07^{bbb}	1.8±0.14 ^{bbb}	1.75±0.0 ^{bba}

Is well known compounds such as flavonoids, tannins, chlorophylls, among others, are soluble compounds, in polar solvents [4]. Therefore, it is considered that these compounds may be present in the extracts.

The statistical analysis was performed of the results obtained, finding difference from 3 variables involved (source, variety and extraction method). Regarding the methods of extraction was obtained that maceration and microwave are statistically equal, as between warming and sonication.Demonstrating that greater quantity of extract is obtained by the methods of heating and sonication, both in variety and source. If we make a comparison between these two methods, taking into account other variables,such as amount solvent and extraction time,it can be said that method by sonication is more efficient as it required a time of 30 min and 100 ml of solvent, in contraposition to the method by heating was required a time for 8 hr and 200 ml of solvent.

In 2005, a study was conducted for the extraction of flavonoids in a "valuable" and traditional Chinese herb with the name of Saussurea Medusa Maxim, demonstrating that the ultrasonic assisted extraction has advantages of efficiency and simplicity over traditional methods such as solvent extraction at room temperature, thermal and soxhlet reflux. According to this study, the ultrasound has an efficiency 70 times greater than the solvent extraction, 11 times the extraction by distillation and 35 times greater than the soxhlet extraction [1].

Extraction of lupeol by conventional and non-conventional methods in leaf and bark of mango, the varieties of ataulfo and criollo.

Table 2 shows the results of the extractions using hexane as a solvent by four methods of extraction, two conventional methods and two non-conventional.

Table 2. Extract obtained inleaf andbarkof varieties ataulfoand criollo, by fourdifferent extractionmethods, using as solventhexane.

SOURCE	VARIETY	EXTRACTION METHODS			
		Maceration	Heating	Sonication	Microwave
		obtained extractg/ 10gsample			
LEAF	Ataulfo	0.15 ± 0.07^{aaa}	0.2 ± 0.0^{aaa}	0.15 ± 0.07^{aaa}	0.15 ± 0.07^{aaa}
	Criollo	0.2 ± 0.0^{aaa}	0.1 ± 0.0^{aaa}	0.25 ± 0.07^{aaa}	0.25 ± 0.07^{aaa}
BARK	Ataulfo	0.1 ± 0.0^{baa}	0.075 ± 0.03^{baa}	0.1 ± 0.0^{baa}	0.075 ± 0.03^{baa}
	Criollo	0.1 ± 0.0^{baa}	0.05 ± 0.0^{baa}	0.1 ± 0.0^{baa}	0.1 ± 0.0^{baa}

In conducting the analysis of results was obtained differences for the source variable and found no difference between varieties and extraction methods, although no differences were obtained between conventional extraction methods and non-conventional, you might consider using non-conventional methods to carry out extractions (sonication and microwave) because they require less extraction time.

Also the results were obtained of most extract by non-conventional methods (sonication and microwave) on the leaves of Criollo variety, this can give us guidelines to propose, which continues to demonstrate that new methods of extraction, respect to traditional methods have advantages, especially in time, thus making them more efficient [5].

Identification of mangiferin by thin layer chromatography (TLC), in leaf and bark of mango, the varieties of ataulfo and criollo.

In figures 1 and 2 it can be seen that was qualitatively identified, the presence of mangiferin by the four extraction methods employed,notappreciatingdifferences between each of the methods, nor in the two sources and varieties, since the intensity is very similar in all samples,this can be attributed to the concentration of mangiferin in leaf and bark of the two varieties is similar.

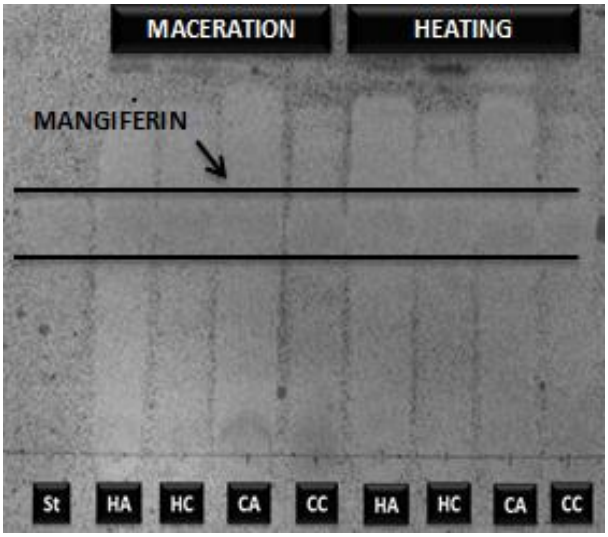

Figure 1. Plate obtained for the identification of manguiferina in leaf and bark of mango ataulfo and criollo by conventional methods of maceration and heating.

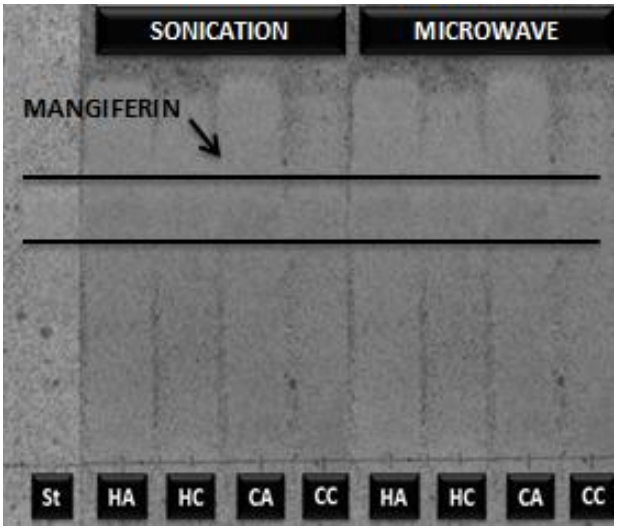

Figure 2. Plate obtained for the identification of manguiferina in leaf and bark of mango ataulfo and criollo by non-conventional methods of sonication and microwave.

The method of revealed by DPPH is based on the ability of the stable free radical 1,1-diphenyl-2-picrilhidrazilo (DPPH) to react with donors of H and when this happens capture antioxidants, generating a colorless product [(6)] ; in the samples to be revealed this effect is observed, appreciating in figures 1 and 2 that there is the presence of other compounds which increase the antioxidant capacity of the leaves and bark of varieties ataulfo and criollo. Some of these compounds presentmay be among other, chlorophylls, polyphenols, flavonoids [(7).]

Identification of lupeol by thin layer chromatography (TLC), in leaf and bark of mango, the varieties of ataulfo and criollo.

In identifying lupeol for the two sources can be seen that the intensity of purple coloration provided by the developer anisaldehyde, is greater for ataulfo bark, compared with the two leaves varieties and criollo bark (Figure 3). This may be due to different factors influencing the presence of these compounds as growth season, species, environmental conditions, age, among other[(8)].

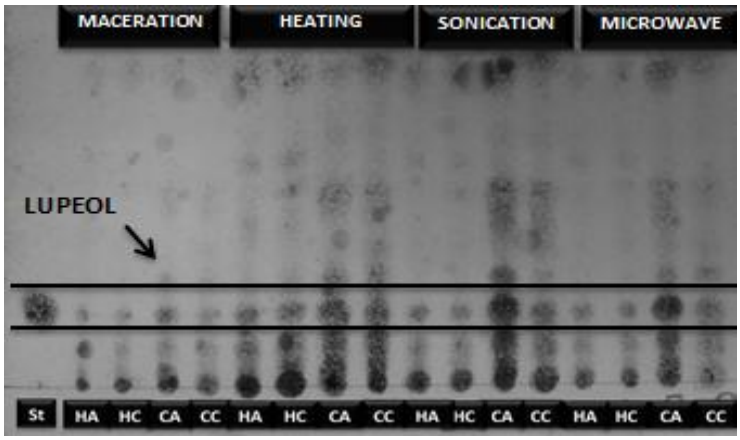

Figure 3. Plate obtained for the identification of lupeol in leaf and bark of mango ataulfo and criollo by conventional methods (maceration and heating) and non-conventional (sonication and microwave).

With respect to methods of extraction, non-conventional methods (sonication and microwave) show better results since intensity of coloration to lupeol is greater than in the case of conventional methods (heating and steeping) (Figure 3).

Agree some authors, the non- conventional extraction methods are based on improving the efficiency of traditional methods, this by physical action on the means [(1)].

In the extraction by the method of heating, for the coloration intensity, both leaf and bark of both varieties, shows a variety of compounds that increase antioxidant capacity of these.

Conclusion

When making extractions by means of the four proposed methods, differences were statistically significant, greater amount of extract found with non-conventional methods, showing that the new methods of extraction, relative to traditional methods have advantages, especially in time.In the qualitative part was possible to detect the presence of mangiferin and lupeol in the two varieties and sources, for the four extraction methods. In the case of manguiferina could not appreciate differences on any of the three variables that are taken into account and lupeol was obtained that the intensity of the coloration is larger for ataulfo bark, this could be due to the different factors influencing the presence of these compounds as: season of growth, environmental conditions, species and age, among others. Regarding the methods, was more intense coloration with sonication and microwaves, which demonstrates that non- conventional extraction methods are more efficient than by the physical action on the environment.Therefore it is considered that the non-conventional methods, could replace the conventional methods, for its efficiency in time and productivity.

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Use Of Powder From Bagasse Of Carrot In a Bakery Product

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Abstract

The carrot bagasse is a residue obtained by informal industries and businesses from to extract the juice of the carrot which contains nutraceutical components (1.2% fiber and 70 to 115µg / g of β-carotene) that can be used by the population incorporating bagasse carrot powder into a food highly consumed, such as bread (annual per capita consumption of 32.5kg, INEGI, 2010). For biscuit formulation was replaced wheat flour by bagasse carrot powder in 10% and incorporated a modified starch (corn dextrin) as a substitute for 20% added fat; the texture, water activity (aw) and color during shelf life were monitored (8 and 30 days respectively); baking quality and proximate analysis were made to characterize each batch of bread made and finally sensory analysis was performed on both samples using an affective test with five-point hedonic scale which was analyzed with "t" student method (confidence interval of 95%). Bread was becoming stiff and the water activity decreased during the shelf life because of the fiber added in the bagasse carrot powder and starch retrogradation process. The color of the crumb and crust of the biscuit were constant for 30 days, indicating the presence of the carotenes in bagasse carrot powder. Carotenoids are retained in the bread even after preparation and baking, 11.3 times more carotenoids quantified with twice the antioxidant capacity in the biscuit with carrot powder than control. The fiber in the bread increased 30% when adding carrot powder. The sanitary quality of the products fulfilled to the specifications of the NOM-147-SSA1-199. Finally, the biscuit supplemented with carrot powder was significantly preferred color, flavor and aroma by consumers regarding the biscuit control. Therefore, it was possible to obtain a highly foodstuff consumed by the population contains more antioxidants and fiber than a common one, which can help to prevent chronic degenerative diseases.

Introduction

The carrot (*Daucus cariotta*), is native to the Near East and western Asia, but is grown in many parts of the world. The root belongs to the family of *Umbelliferae* and to the *Plantae* kingdom. The carrot is wide at the top and it thin, of orange color, white or a combination of red and white with a crisp texture when it is fresh. The carrot is 10 to 30 cm in of length (FAO, 1983). The production in 2007 was 385 metric tons. The nutriemental content of the carrot is: 86% humidity, 10.7% carbohydrates, 0.1% lipid components, 0.9% protein, 1.1% ash and 1.2% fiber (FAO, 2008). The fiber is part of the nutraceutical components of the carrot, together with the antioxidant content (115 mg of carotenoids per gram of fresh carrot (Amaya Rodriguez, 2001)) are active chemicals or biological compounds which dose administered over the existing in food causes a favorable effect on health, which has more normal food conditions favoring healthy and serving as an assistant in the care and maintenance of health as well as disease prevention (of chronic degenerative diseases such as heart attacks, stroke, hypertension, diabetes, hormone-dependent cancers (mammary gland, prostate, thyroid, etc.)) (Pérez, 2006). The bagasse is obtained as a result of the extraction of carrot juice, which is usually discarded by industries or used as feed for cattle. The bagasse is composed of filaments that form part of the organic tissue of carrot non-absorbable constituted by polysaccharides and lignin from the thick cell wall of plants (Martin, 2006). Because of this and the relatively high temperature exposure during the shelf time, fresh bagasse inevitably undergoes a process of fermentation, which depreciate the quality of the fiber and prevents its consumption, therefore preserved by a drying process, by applying heat under controlled operating conditions, with the aim of reducing the weight, volume and increase the life of the bagasse (Camarilla, 2002). In Mexico, the per capita consumption of bread in ranges 32.5kg/año (Canainpa, 2010), reason because of the carrot bagasse powder was added to a biscuit, in order to increase the nutritional value compared to conventional one. To decrease the amount of added fat, modified starch were added for crosslinking to replace by 20% butter.

Background

There is evidence dating back several years in which we have studied the mechanism and kinetics of carrot drying to constant geometry with hot air, in order to provide necessary information for kinetic modeling in the design of dryers mechanical. In this research, it was found that the optimum temperature range for drying carrot, is between 50 ° C and 70 ° C for densities of 10 and 20 kg/m2 (Mullet et al., 1983).

In 2007, Gamboa and cols., presented a publication about the nutritional value and sensory pancakes made from flour of wheat (*Triticum aestivum*) and carrot (*Daucus cariotta*), which are proposed in different proportions of both flours (50 % HT, 50% HZ and 25% HT, 75% Z) to be added to pancake, noting that it

produces an increase in levels of fiber, ash (minerals), fat, carbohydrates and pigments, from which the β-carotene is the most characteristic. In addition to the incorporation of carrot cupcakes wheat flour (25% HT, 75% Z) substantially increases calcium levels. The pancakes made with carrot flour 50% and 50% of wheat flour showed high acceptance in terms of sensory attributes: taste, color, smell and texture. Finally, the incorporation of carrot cupcakes is a condition of high feasibility to improve the nutritional value of the final product (Gamboa and cols, 2007).

In 2009, it published a research conducted in the School of Chamborazo in Ecuador about the quality control of Strawberries (*Fragaria vesca*) dried by microwave method to three powers, in which nutritional assessment was performed and Nutraceutical of strawberry (Fragaria vesca.) fresh and dried, taking as an indicator of efficiency of conventional dehydration process, the amount of anthocyanins and vitamin C for the purpose of minimizing the loss of nutrients. In the nutritional parameters obtained is a marked difference between the two methods, in the dehydrated water content decreases to 11.32%, thus ensuring optimum product preservation. The ash content increases to 0.48% in the fresh sample to 3.67% in the dehydrated sample. In the case of fiber, the concentration of solutes in the dehydrated is greater than in the sample hydrated, that is, 7.86%. The strawberry was dried at 300W for 40 minutes showing a concentration of 16.79 and 261.16 mg/100g mg/100g anthocyanin vitamin C. Given the fact that the fresh strawberries contains 20 277 354.95 mg/100g mg/100g anthocyanins and vitamin C, it follows that at 300 W power loss of anthocyanins and vitamin C is lower. We applied the method to dehydrate fruit trays reporting a anthocyanins loss of 10.63 g/100g and 2.99 mg/100g Vit C and there is a greater loss than the microwave method (Sagñay, 2010).

Objectives

1. Harnessing nutraceutical components of bagasse carrot powder in a bread product.
2. To condition the carrot dry bagasse for use as flour into a biscuit.
3. Replace in different proportions the wheat flour for carrot bagasse powder in the formulation to develop a biscuit.
4. Evaluate the quality of the baker substituted in proportions of 0, 10, 15 and 20g of carrot bagasse powder per 100g wheat flour.
5. Evaluate nutraceutical substances (carotenoids and crude fiber) of carrot bagasse powder and added to the powder biscuit.
6. Assessing health quality (mesophilic aerobes, total coliforms and fungi and yeasts) carrot bagasse, bagasse carrot powder and finished product.
7. Sensorially evaluate the biscuit with the best percentage of substitution of wheat flour by carrot bagasse powder.

Justification

Mexico is a developing country, inhabited by more than 112 million people, of which nearly 9 million (INEGI, 2010) are concentrated in the Federal District, consequently resulting in problems in the food and nutrition situation in the population by lack of nutrients while obesity, malnutrition and protein deficiency energies, plus vitamin deficiency affecting mainly young children under five years. Overcrowding in the City, it also brings environmental problems, especially in terms of waste, both inorganic and organic (bagasse fruits and vegetables).

Considering that annually produce 2692 tons of bagasse in the industrial area, regardless of what is thrown away by restaurants and shops and vegetable juices, it is important to note the need for an alternative use for human consumption. Therefore, due to the need to reduce the high amount of waste generated in Mexico City, took out a technology development project baker (consumption per cápita32.5kg/año, Canainpa, 2010) to use the carrot bagasse vector as a food fiber source and nutraceutical components are still present, because this residue is intended to produce cattle feed, but a high proportion is discarded as trash and hence as a source of environmental pollution.

Materials and methods

a. Sampling bagasse carrot used were collected from stores in Mexico City where they make this vegetable juices. Specifically from stand that is in front of the National School of Biological Sciences, located in prolongation of Carpio and Plan de Ayala s / n, Colonia Santo Tomas, Miguel Hidalgo, D. F.

b. Formulation and development of biscuit. For product development we used a formulation and method provided by the National Baking Industry (Canainpa).

c. Baking quality. Baking quality of each batch of bread produced was determined by measuring the following parameters: mass (m) of each piece of biscuit gravimetrically, volume (v) using the displacement method rapeseed and density by calculation.

d. Colour. Was measured at the center and periphery of the biscuit crumb and crust, and eight readings were obtained luminosity (L) with Team Color Reader DR-10 Konica Minolta company, whose interpretation corresponded to the color scale of Hunter based on the theory of opposite colors.

e. Determination of texture. The bread texture is very important because it is a parameter that determines the quality of a bread product to consumers, so that was studied over a period of 8 days shelf. Was measured by inserting a rod of 0.95 cm 2 in area to a height of 7 mm in both the central and peripheral scone bark and 8 readings were obtained texture (kgf) using the penetrometer equipment Fruit Pressure Tester FT 327 (3-27 Lbs.) (Apple and pears) and 0-13 kg force.

f. Water activity (a_w). Water activity (aw) is a critical factor that determined the life of bakery product. This parameter established limits for the development of microorganisms, while other parameters such as temperature, pH or sugar content, generally influenced the growth rate. The sampling was made from the scone of the central part of the crumb, Rotronic equipment using the Palm Hygro Bassersdorf 8303 Series CE No. 60545639 characteristics: Power: 3.3-5.0 VDC; Op range: -40 - +60 ° C; out: aw 0-1 (0-100% rh) range: 5-50 ° C. The determination was performed in triplicate for each case studied during a period of eight days.

g. Chemical characterization. The chemistry characterization and control batch of bagasse added with carrot powder was conducted by means of a proximate analysis. Was determined moisture (drying method AACC 40-15), total protein (Kjeldahl method AACC 46-12), lipids (Modified Soxhlet method, AACC 30-10), ash by calcination method (AACC 08 -03), crude fiber following the NMX-F-090-S-1978 and nitrogen-free extract by calculation.

h. Total carotenoids. For the extraction of carotenoids methodology followed Amaya Rodriguez, 1999, using acetone extraction caring for the parameters of temperature, light and oxygen concentration to prevent degradation of antioxidant compounds.

i. Antioxidant capacity. Methodology was followed ABTS (2,2 'azinobis-(etilbencotiazolin-6-sulfonic acid)). The percent inhibition values were obtained by multiplying the sample values of ΔA 100. Standard curve was constructed percent inhibition of 6-hydroxy-2 ,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) in a concentration of 0-16 mM. The antioxidant atividades samples were calculated using the equivalent of Trolox standard curve.

j. Sanitary quality. Were determined: mesophilic aerobic plate by the method of NOM-092-SSA1-1994, total coliform by the methodology outlined in NOM-113-SSA1-1994 and finally, fungi and yeasts in the methodology indicated in NOM-111-SSA1-1994.

k. Sensory evaluation. Sensory evaluation was performed using a 5-point hedonic test batch is prepared using fresh bread, with the participation of 50 judges randomly selected consumers who evaluated the product on different days.

l. Statistical Analysis. Statistical analysis was used to "t" and one student of variance ANOVA for multiple comparison of data from each of the relevant determinations, using a confidence interval of 95%.

RESULTS AND DISCUSSION

a. ***Baking quality and chemical characterization***

The biscuit was replaced with 10g of powdered carrot bagasse per 100g wheat flour employed in the formulation, which certain characteristics imparted to this product with respect to controlling, a bread prepared simultaneously. The addition of bagasse carrot powder, increased 30% crude fiber content of bread, in turn increasing the density by 14% and the texture in 54% over scone control, because the

employee carrot bagasse consists of fibers for the characteristic of hydrophilicity and its negative influence on the formation of gluten, produced a tougher scone but with an added value. However, not modify the rest of their chemical composition.

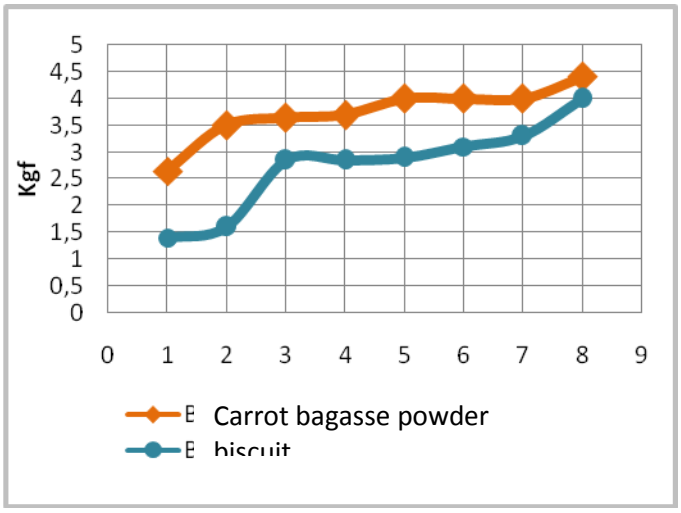


Figure 1. **Monitoring texture of biscuits during eight days**

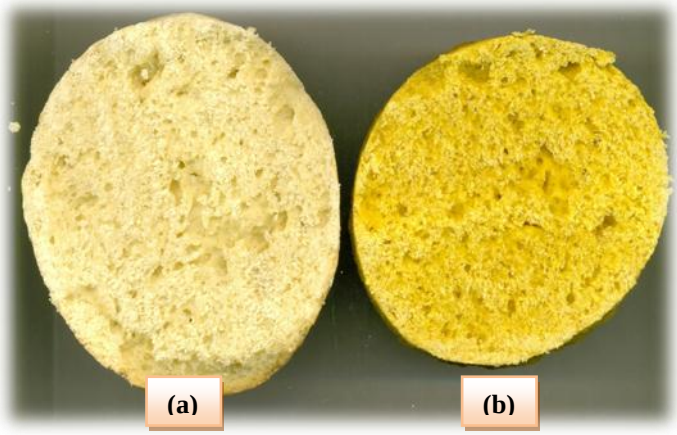


Figure 2. Comparison of color (a) Control biscuit and (b) Carrot bagasse powder biscuit

b. Determination of texture

Three parameters were monitored in each scone important for shelf life: change texture, color variation and modification of water activity (a_w). The figure 1 shows the variation in texture over a period of eight days, which shows an increase in hardness of the two samples of bread due to retrogradation undergoes normal starch.

c.Colour, total carotenoides and antioxidant capacity

The addition of powdered carrot bagasse also changed hue of bread (figure 2), owing to the characteristic color of the carrot carotene imparted primarily by the present, therefore indirectly to this parameter may be regarded as indicative of the presence or absence of these pigments. There was a 650% increase in shades of red and yellow shades twice both in the cortex and in the crumb carrot powder biscuit with respect to control over periods of 8 and 30 days respectively.

The presence of carotenoids in the bread was confirmed by quantification, obtaining 15.38±2.6 mg / g of sample with antioxidant capacity 3.04±0.5 µmol Trolox / g of sample, it means 11.3 times more carotenes and twice the antioxidant activity than in a biscuit control. This confirms the presence and functionality of pigments even after the baking process and product development, imparting functionality to the product.

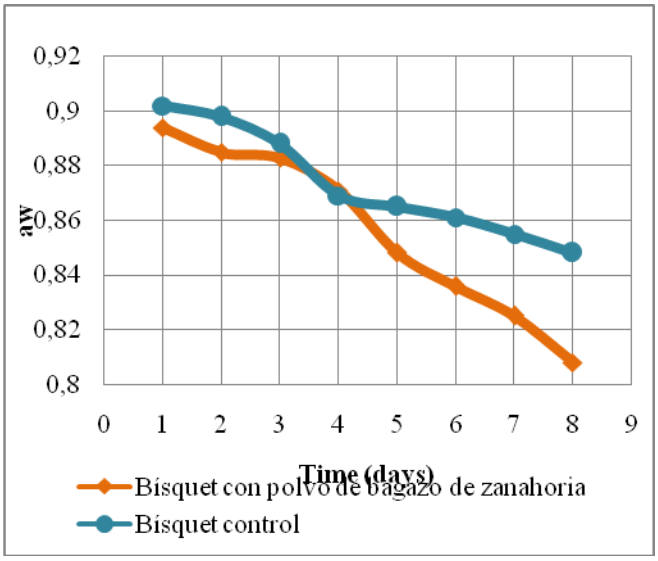


Figure 3. **Monitoring of water activity in biscuits during eight days**

d. Water activity (a_w)

Another important factor was modified by adding carrot bagasse powder was water activity, which decreased at a rate of 10% at 24.7 ° C (figure 3), however, the pan control always presented higher values, which was observed for fungal growth on the samples after eight days of storage. The carrot powder bread had not contamination even after 30 days of storage, which is because of the color analysis was stopped after 8 and 30 days respectively.

e. Sanitary quality

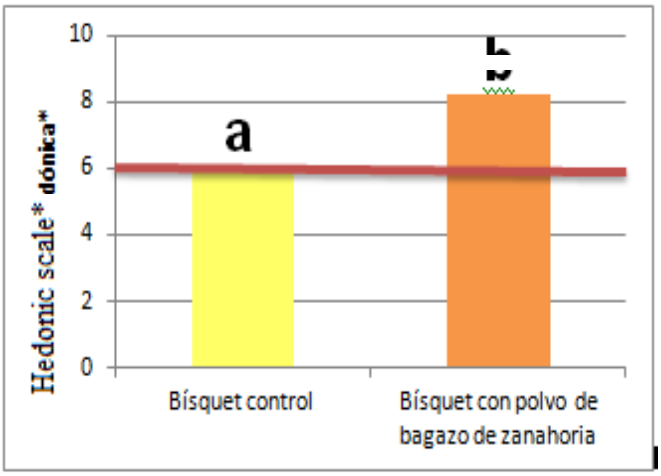
Also conducted to determine the sanitary quality (mesophilic aerobic plate count of total coliforms and yeasts and molds) of the raw material and finished product, which was due to the specifications of the NOM-093-SSA1-1994 sanitation and hygiene practices in food preparation offered in fixed and NOM-147-SSA1-1996 for cereals and cereal products. Provisions and health and nutritional specifications.

f. Sensory evaluation

The figure 4 expresses the significant difference in the level of acceptance among both samples. The slope was due to the color was more attractive, improved aroma and taste was sweeter than the control.

CONCLUSION

It was possible to develop a formulation bakery product (biscuit) carrot bagasse added in powder form for human food source leveraging it as nutraceutical components (fiber and carotenes) as an alternative to use of bagasse carrot discarded as trash by the food industry and currently used for cattle feed and / or as a source of environmental pollution.



Carrot bagasse

* Hedonic scale: 2, I dislike a lot; 4, dislike, 6, or I like, nor dislike; 8, like, 10, I love it. Statistical analysis using Student t method with a significance level of 5%, a confidence interval of 95% and 49 df (degrees of freedom).

Figure 4. Histogram of the degree of liking of biscuit bagasse supplemented with carrot powder compared to Control one.

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ROS Scavenging Capacityof Lyophilized Aqueous Extract Of Avocado (*Persea Americana*) Seed And Peel

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Introduction

Avocado (*Perseaamericana*) is a tropical fruit native to Mexico, which is the main consumer and producer of this fruitin the world (1). The consumption and relevance of this fruit are increasing because the evidences of its nutritional value and health benefits(2). Avocadois rich in unsaturated fatty acids, fiber, vitamins B and E, and other important nutrients in the edible portion(1, 3).Although the consumption of this fruit generates unused residues such as seeds and peels, both by-products contain antioxidant molecules as polyphenols and carotenoids which can be used against some diseases where oxidative/nitrosative stress is involved or to extend the shelf life of a food product. There are few applications of these by-products, such as the use of the seed as a natural colorant (4) and the treatment of mycotic and parasitic infections in the aztec and mayan cultures (4).It is well established that avocado seed and peel extracts present antioxidant activity (5) in its different varieties (6), including hass variety, the most common worldwide.However, there is no evidence about the specific scavenging activity of these extracts against reactive oxygen species (ROS) and reactive nitrogen species. The aim of this study was to analyze the *in vitro*scavenging ability of avocado seed and peel lyophilized aqueous extracts against the following ROS: peroxynitriteanion ($ONOO^-$), hydroxyl radical($OH^\bullet$), superoxide anion ($O_2^{\bullet-}$),peroxylradical($ROO^\bullet$), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) by established spectrophotometric and fluorescent methods.

Methods and materials

1.1 Materials

1.1.1 *Fruit*: Ripened avocado (*Persea Americana*, Hass variety) were purchased on several separate occasions from local markets in Mexico City.

1.1.2 *Chemicals*: Xanthine, xanthine oxidase, nitrobluetetrazolium (NBT), diethylenetriaminepentaacetic acid (DTPA), diphenylisobenzofuran (DPBF), terephthalic acid, iron(III) chloride, peroxidase from horseradish(HRP), amplex red, 2,2′-azobis(2-methylpropionamidine) dihydrochloride(AAPH), fluorescein sodium salt, dimethylthiourea (DMTU), nordihydroguaiaretic acid (NDGA), penicillamine, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), Folin-Ciocalteu phenol reagent, gallic acid (GAE), quercetin, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ)and other reagents were purchased from SigmaAldrich Co. (St. Louis, MO, USA). H_2O_2, ethylenediaminetetraaceticacid (disodiumsalt) (EDTA)and sodium hypochlorite (NaOCl) were from JT Baker (Xalostoc,Edo.Mexico,Mexico).2,7-dichlorodihydrofluorescein diacetate (DCDHFDA) was from Cayman Chemical Company (Ann Arbor, MI, USA).

1.2 Methods

1.2.1 *Sample preparation*: Avocado was maintained at room temperature until full maturity, which was identified by subjectively assessing the softening of the avocado pulp and by instrumentally measuring the color of skin (L: 32.69, a*:-1.52, b*:9.71) according to Rodríguez-Carpena et al.(7). Fully ripened fruits were manually separated into seed, pulp, and peel, measured for theirweight, and then stored at -80°C.

Seed and peel were finely ground in a blender and dried at 70°C for 12 hours. To prepare the lyophilized water extracts, 50 g of dry avocado seeds or peels were added to 300 mL of distilled water. This mixture was stirred by a magnetic stirrer for 1 day at 4°C. The extract was filtered with filter paper (Whatman No.4). The resultant peels or seeds were extracted again with the same procedure described above. Both filtrates were combined, frozen and lyophilized (Labconco, Freezone 2.5) at 5 mmHg at -50°C. The lyophilized powder was stored at -20°C until using for analysis (8, 9). Before each assay, the extracts were dissolved in distilled water.

1.2.2 *Total phenolic content*:Phenolics were measured using the Folin-Ciocalteu's phenolreagent (9). The absorbance was measured at 760nm using a spectrofluorometer Synergy HT (Biotek Instruments Inc., Winooski, VT, USA). Gallic acid was used as a standard.

1.2.3 *Total flavonoid content:* Flavonoids were determined according to the method Zhizhen et al. (10) with slight modification, using 1N NaOH, 10% $AlCl_3$ and 5% $NaNO_2$. The absorbance at 510 nm was measured. Quercetin was used as a standard.

1.2.4 *Tannins content:* These compounds were determined according to Zilic et al.(11) and also by the Folin-Ciocalteu method. The difference between total and non-absorbed phenolics was considered as tannins content.

1.2.5 $O_2^{\bullet -}$ *scavenging capacity*:This radical was produced by the xanthine-xanthine oxidase system. The activity of xanthine oxidase wasmeasuredby NBT reduction at 560 nm and uric acid production at 295 nm(12). The reaction mixture contains: 0.116mMxanthine,20mMNa_2CO_3, 29mMNBT,50mMphosphatebuffer(pH7.0) and xanthineoxidase(168U/L).Scavengingpercentwascalculatedfromtheoptical densitiesat 560nm only if xanthine oxidase activity was not affected by the extracts. NDGA was used as a reference compound.

1.2.6 $OH^{\bullet}$ *scavenging capacity*: This oxidant speciewas produced by the Fenton reaction, and its generation was monitoring by the increase in fluorescence, product of the reaction of $OH^{\bullet}$ with terephthalate (13)**.** The reaction mixture contains: 0.2mM ascorbic acid, 0.2mM $FeCl_3$, 0.2mM EDTA, 1.1mM H_2O_2, 1.5 mM terephthalate and 50 mM phosphate buffer (pH 7.4). The increase of fluorescence was followed during 30 minat 326 nm of excitation and 432 nm of emission. DMTU was used as a reference compound.

1.2.7 $^1O_2$*scavenging capacity:* $^1O_2$ was produced by the reaction of H_2O_2 and NaOCl, itsproduction was determinedby the increase in fluorescence producedbyits reaction with DPBF(14)**.** The reaction mixture contains: 1.1 mM H_2O_2, 0.033mM NaOCl, 1.1 μM DPBF dissolved inethanol. The fluorescence was measuredat 410 nm of excitation and 455 nm of emission. NDGA was used as a reference compound.

1.2.8 $ONOO^-$ *scavenging capacity:*This reactive nitrogen specie was synthesized as previously described (15) and its concentration was determined at 302 nm using a molar extinction coefficientof 1,670 M^{-1} cm^{-1}. $ONOO^-$reacts with DCDHFDA and producesa fluorescent product (16). The reaction mixture contains: 0.037mM DTPA, 0.023mM $ONOO^-$,and 0.13mM DCDHFDA. The reaction was followed during 30 minutes using a spectrofluorometer at 488 nm of excitation and 525 nm of emission. Penicillamine was used as reference compound.

1.2.9 H_2O_2 *scavenging capacity*: The Amplex Red/horseradishperoxidase (ARHP)reagent was used to determinate the H_2O_2 concentration(17).The reaction mixture contains: 0.012 mM H_2O_2 and ARHP reagent (1mMamplex red, 0.2 U/mL HRP and 50 mMphosphate buffer, pH 7.4). The fluorescence was

measuredat 550 nm of excitation and 590 nm of emission. The concentration of H_2O_2 was calculated using a standard curve.Pyruvate was used as a reference compound.

1.2.10 *Oxygen Radical Absorbance Capacity (ORAC):* Peroxyl radicals,generated from the radical initiator AAPH, oxidizes fluorescein and decreases its fluorescence. The presence of an antioxidant prevents the decrease in the fluorescence (18). The reaction mixture contains: 153 mM AAPH and 4 nM fluorescein. The reaction was monitored for 1.5 hours at 485 nm of excitation and 526 nm of emission. The area under the curve and equivalents of trolox were calculated by Gen5™ software (Biotek Instruments)USING A standard curve of Trolox.

1.2.11 *Ferric reducing antioxidant potential (FRAP):*At low pH and in presence of electron-donating antioxidants thecompound Fe^{3+}-trpyridyltriazine, a colorless ferric complex is reduced to Fe^{2+}-tripyridyltriazine, a blue-colored ferrous complex. This change can be monitored at 593 nm. The FRAP reagent was prepared minutes before the assay by mixing 10 mMTPTZ (dissolved in 40 mMHCl), 20 mM $FeCl_3$ and 300 mMacetate buffer, pH 3.6(18). The reaction was incubated at 37°C andmeasured at 593 nm.

1.2.12 *Statistical analysis*: Samples were analyzed in triplicates and data were expressed as mean ± standard deviation(S.D.). One-way ANOVA and Tukey multiple comparisons test were conducted using software Prism 5.0 (GraphPad, San Diego, CA, USA). A p-value < 0.05 was considered statistically significant.

Results

The average weight of avocado fruits was 153.89±16.06 g; 15.22±4.7% corresponds to seed and 16.12±0.3% to peel. The moisture percentage of seed and peel was 55.4±2.3% and 74.99±3.2%, respectively. The yields of extracts were 8% and 13.2% in the peel and seed, respectively.
Thecontent of phenols, flavonoids and tannins as well asthe antioxidant capacities of each extract are shown in Table 1. In all cases the peel extract contains more compounds with greater antioxidant capacities than the seed extract.

Table 1.Antioxidant capacities and total phenols, flavonoids and tannins content.

	Seed extract	Peel extract
Total phenols (mg GAE/g extract)	5.7±0.2 [a]	19.7±1 [b]
Total flavonoids (mg QER/g extract)	2.8±0.8 [a]	10.9±1.03 [b]
Tannins content (mg GAE/g extract)	0.09±0.001 [a]	0.4±0.001 [b]
ORAC (mgtrolox equivalents/g extract)	1.6±0.09[a]	216.8±2.5[b]
FRAP (mg Trolox/g extract)	9.5±1.6 [a]	23.1±5.9[b]

Data are expressed as the mean±S.D. N= 3 independent experiments. Values with different letters (a-b) within a row are significantly different (p <0.05). GAE= Gallic acid, QER= Quercetin.
Table 2shows the scavenging ability of both extracts and reference compounds for different ROS expressed as IC_{50} values (the amount of compound that scavenges 50% of total oxidant species). Seed and peel extracts were able to scavenge in a concentration-dependent way (data not shown) all the ROS

described in this study. In the case of $O_2^{\bullet -}$scavenging assay, the extracts and the reference compound do not interfere with the xanthine oxidase activity.For $O_2^{\bullet -}$ and H_2O_2 there were no statistical difference between extract. The IC_{50} values for $^1O_2$,$OH^\bullet$and $ONOO^-$ were different forboth extracts and forreference compound. the fact that in all cases the peel extract IC_{50} values were lower than those of seed extract suggests that peel extract is more effective to scavenge ROS.

Table 2. ROS scavenging capacities of extracts and reference compounds (IC_{50}).

Scavenging capacity	Seed extract (µg/mL)	Peel extract (µg/mL)	Reference compound (µg/mL)
$O_2^{\bullet -}$	3.1±1	1.4±0.9	0.44±0.07 [c]
$OH^\bullet$	430±86[a]	26.5±1.0[b]	0.49±0.04[c]
$^1O_2$	2,854±72[a]	1,741±3[b]	2.9±0.6[c]
$ONOO^-$	612±184[a]	198±44[b]	3.0±1.4[c]
H_2O_2	0.8±0.03	0.6±0.09	11.0±0.2 [c]

Data are expressed as the mean±S.D. N= 3 independent experiments. Values with different letters (a-c) within a row are significantly different ($p < 0.05$).

Discussion

The morphometric results like seed and peel weightas well as moisture percentage are similar with the results reported previously by Rodríguez-Carpena and cols.(5). Also these data show that avocado Hass variety has a high pulp percentage (around 75.9%), 11.1% of peel and 13% of seed, we report similar results.

However the type of solvent extract was used in this study not permit to obtain similar results reported previously by other authors, about the total phenolic content and antioxidant activity. For example, a methanol extract from seed has 3,511 mg GAE/100g dry matter, in our case we obtain 74.1 mg GAE/100 g dry mater (5), or 0.41 mg GAE/g fresh matter compared with 51.6 mg GAE/g fresh matter obtained with a acetone/water/acetic acid extract(19). The type of solvent is important to obtain a better yield in the extraction of polyphenols and antioxidant compounds.

There are reports that describe that the seed extracts have better antioxidant capacities compared with the peel extract and pulp extract (5, 19, 20)measured by different methods such as 2,2-Diphenyl-1-picrylhydrazyl, 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), and FRAPassays.

However, in our study the peel extract shows a better antioxidant capacity than seed extracts, measured by ORAC, FRAP and the specific ROS-scavenging capacity assays.Similar results obtained by Kosinska et al.(6)showthat total phenolic content and antioxidant activity from peel extracts of avocado Hass and avocado Shepard is higher than thosefrom seeds extracts.

Particularly the$O_2^{\bullet -}$, $OH^\bullet$, $^1O_2$,and $ONOO^-$scavenging capacity of peel extract was statistically different ($p<0.05$) than those of seed extract.This would be consequence of type of solvent used in the extraction processsince the compounds obtained in the extracts may be different due to properties as polarity and solubility of phenols, flavonoids and tannins.

The avocado extracts rich in polyphenols block the oxidative reaction that deteriorate the appearance and nutrimental quality (7), therefore they are a good alternative to be used in food industry such as porcine patties.

The lyophilized aqueous extracts of seed and peel, which shows ROS scavenging activity, may be used in food industry to prevent deterioration reactions or oxidative stress-associated diseases.

The deterioration reactions in food industry andoxidative stress-associated diseases can be prevented by the ROS scavenging activityof lyophilized aqueous extracts of seed and peel.

Acknowledgements: Thanks to Instituto de Ciencia y Tecnología del Distrito Federal (ICyTDF) and Universidad AutónomaMetropolitana (UAM),for the support to participate in this Congress. Also this study was support by SEP-CONACYT science basic 2008-105870.

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Extraction And Characterization Of Dyes From *Bouganvillea glabra* For Potentially Use As A Food Additive

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Introduction

Nowadays the appearance and color of a food are factors that determine the product acceptance by consumers; the color is considered an important attribute of a food, because it increases its appeal and commonly it is associated with food quality. Most food additives including colorants are generally of synthetic origin. However, given the relatively high doses of these compounds that can be ingested by consumers, it has begun to pay attention to their health effects. Several toxic and allergic reactions in humans has been associated with the consumption of synthetic dyes (Vankar *et al*., 2006), being in some countries banned those containing azo chromophore group (Vankar *et al.*, 2005). As a result, the FDA in USA has withdrawn the authorization of use of a large number of compounds (Azeredo, 2009). For these reasons, it has become necessary to research on the feasibility of the use of natural dyes. Moreover, people now tend to avoid the consumption of synthetic dyes, preferring natural pigments, which are considered harmless or even healthy. The nature produces a variety of compounds that can be suitably used as food colorings, such as carminic acid, anthocyanins, betalains, carotenoids and chlorophylls (Tafoya and García, 1993). Nevertheless, in the case of the betalains due to the relative scarcity in the nature, few studies have been conducted with the aim of elucidating its function as bioactive compounds, but some interesting results in this sense have demonstrated their potential as antioxidant compounds. These findings have highlighted the suitability to use betalains as food dyes (García, 2007).

Moreover, the trend today in the food industry not only seeks to develop products whose appearance is the determining factor of acceptance by the consumer, but also to enrich the compounds capable of conferring beneficial effects to health (probiotic). Phenolic compounds are important components in the human diet; the average consumption of phenols in European countries is estimated at 23 mg / day. Phenols are a large group of compounds widely distributed in plants, have a chemical structure particularly suitable to show a probiotic action as they exert an antioxidant action through neutralizing harmful free radical reactive oxygen species, or to function as chelators of metal ions. In addition, several reports in the literature have shown the positive effect of phenolic compounds against some diseases such as certain cancers and heart disorders arising from its powerful antioxidant activity, thus fostering a growing interest in its implementation in food (UNAM, 2011).

The *Bougainvillea glabra* Choisy (also called orange bougainvillea, purple bougainvillea and bugambilia, camellia) is originally from Brazil, living in almost any weather, at altitude from sea level to 2700 meters above sea level and is mainly cultivated in orchards family. The bracts of flowers develops magenta and red colors and are the most widely part of the plant used for healing purposes by preparing infusion administered orally. This plant is used across the country to treat conditions such as cough, asthma, bronchitis and dysentery and to relieve snoring, lung pain and pertussis. Phytochemical studies in the bracts of *Bougainvillea glabra* have detected the production of betalains and also two indole alkaloids type: diglucosyl bethanidine rutinoside and iso-bethanidine, plus 16 heterocyclic alkaloid: the bougainvilleinas I to XVI, while in the leaves have been detected benzenoid gentisic acid (Heuer *et al*., 1994; Sheeja *et al*., 2005). Although no reports exist about phenol detection in *B. glabra*, those compounds have been determined from *B. spectabilis* (Rathod and Saxena, 2007). This paper studies the extraction of betalains from the bracts of flowers of *Bougainvillea glabra*, stability of extracts and

determination of the total phenol content and their antioxidant capacity as an approach to use the bugambilia extracts as a natural source of colorants for the food industry.

Methods and materials

Extraction. Follow the trend of green chemistry, the extraction of the pigments from bugambilia flowers was performed with water and ethanol, and mixtures thereof in proportion 1:1, 1:3 and 3:1, respectively, so for every prepared mixture, fresh biomass of *Bougainvillea glabra* bracts of flowers (1g per 100 ml of solution) was added, subsequently placed into a water bath at 50 ° C (Azeredo, 2009) for 30 min and finally vacuum-filtered through Whatman No. 1 paper. Once the extraction was carried out, the extracts were analyzed by UV-Vis spectrophotometer to determine which obtained mixture was more suitability to extract the pigments, thus the one showing the highest absorbance at 546nm was selected for further experiments.

Pigment stability at different pH. Different solutions were prepared in order to assess the effect of several pH values (2-12) over the pigment stability of bugambilia selected extract. For basic pH, 2 ml of the acid solution were mixed with 3 ml of distilled water and this resulting solution was adjusted to the desired pH (range 8 to 12) with sodium hydroxide solution (1 M) (the volume added depend of the value pH desired), then distilled water was added until completed 10 ml of final volume, followed of adding 5 ml of the bugambilia extract. For acid pH, 2ml of the Briton-Robinson buffer solution (Rojas-Hernández *et al*., 2010) were diluted with distilled water to complete 10 ml. The obtained solution was adjusted to the desired pH (range 2 to 7) with hydrochloric acid solution (2M) and, finally 5 ml of extract bugambilia were added. All the beforehand solutions were bubbled with nitrogen for a period of 5 seconds, and were spectrophotometrically analyzed for determining the pH effect on the stability of the pigment after 24 hrs and 28 days.

Spectrophotometric characterization. The extracts generated under the mixtures of water-ethanol (ratio 1:1, 1:3 and 3:1, respectively) and those that were derived for selected extract in pH test were analyzed by UV-Visible spectrophotometer through measuring the corresponding absorption spectra from 200 to 900 nm of wavelength (λ).

Determination of total phenol content. The total phenol content (TPC) was assessed by Folin-Ciocalteau method (Gutiérrez *et al*., 2008) from the extract of bugambilia flowers, the results were expressed as mg gallic acid equivalents per g of extract (EAG mg g^{-1}), from a respective standard curve (10 - 150 µM). Absorbance measurements were made at 760 nm in a spectrophotometer.

Determination of antioxidant capacity. Antioxidant activity was determined by the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method (Gutiérrez *et al*., 2008; Ramos *et al*., 2008) with minor modifications, the results were expressed in mM Trolox (TEAC). To perform this analysis, stock solutions of DDPH (0.2mM) and Trolox (1 mM) were prepared. The Trolox stock solution was diluted to construct a standard curve (0.1-1.0 mM). The test consisted of mixing 0.5 ml of Trolox solution or bugambilia selected extract with 3.5 ml of DDPH solution, followed of 15 minutes of incubation under darkness at temperature room and finally absorbance measurements were carried out in a spectrophotometer at 515nm.

Microencapsulation. Formulations of maldodextrin-extract at 20%, 40% and 60% w/w were performed to assess what mixture was more suitability in accordance with final qualitative properties (color and morphology). The corresponding amounts of maltodextrin (encapsulate agent) were weighted and combined with extract to give a solution of 250 g of total weight. Each solution was homogenized by kept it in agitation and heating during two minutes.

Spray Drying. All the maldodextrin-extract formulations were fed by means of a dispenser to a spray drying equipment (Nichols / Niro Turbo Spray). The operating conditions were: an air inlet temperature of 100 ± 5 ° C, an outlet temperature of the air of 80 ± 5 ° C and injecting compressed air at 4 bar pressure. According to the final qualitative properties, such as color and morphology, the formulation that showed better characteristics was selected to test as food additive. So, once drying process was completed, the product was diluted in distilled water (8g of dry extract in 40 ml of water) to determinate the degradation in the color through analyzing absorbance measurement at 546 nm in a spectrophotometer.

Application of microencapsulated dry dye as food additive. The microencapsulated dry pigment (MDP) of bugambilia was applied in natural yogurt and gelatin. For the yogurt (10g), 2 g of MDP was directly added and mixed. Meanwhile for gelatin, 20 ml of water (at 65 ° C) was mixed with 10g of gelatin, maintaining it in rest for 15 minutes at 65 °C to get hydration. After that time, 10g of mixture were added to aqueous extract (20 g of MDP per 100ml) whilst other 10g of mixture were added to distilled water (100 ml) as a control. Finally, both were let to cool and put in refrigeration.

Assessment of the color stability into foods. The gelatin and yogurt formulated with the bugambilia dye were kept refrigerated at 4 °C for 15 days, period in which color measurement of foods was done with a colorimeter (Konika Minolta VCR-400) on alternate days.

Results and discussion

Extraction and characterization of pigments. Water-ethanol extracts were obtained in ratio 1:1, 1:3 y 3:1, respectively, showing all of them the maximum absorption at a wavelength of 546 nm, parameters characteristic related to betacyanins and betalains (534 to 544 nm). However, the better betalain extraction was carried out with water-ethanol in ratio 1:3 as it showed the highest value for absorbance (Fig. 1). Also, it could be that betalains from bugambilia extracts were acylated as it is known that betalains acylated also exhibit an absorption maximum between 260 and 320 nm (Ayala and Beltrán, 2007), agreeing with the absorption spectra presented in this work (Fig. 1). Then, the extract derived from water-ethanol in ratio 1:3 was selected to carry out succeeding experiments pigments.

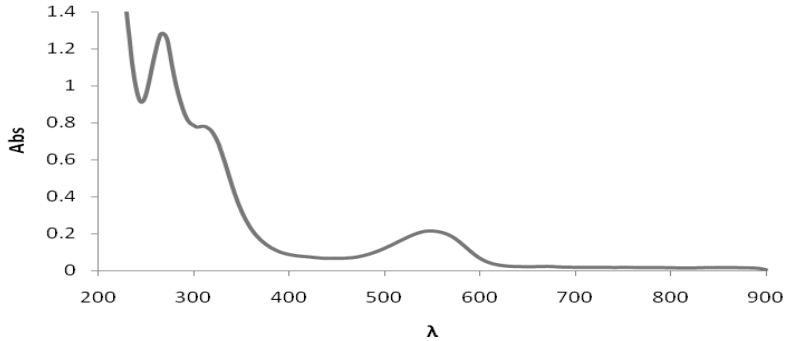
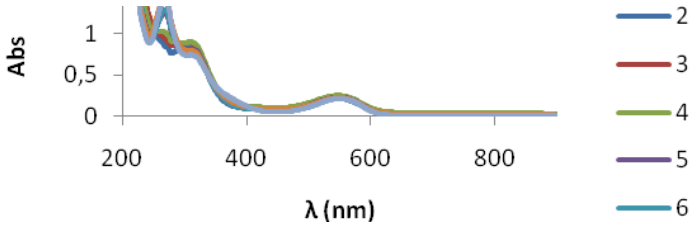


Fig. 1. Absorption spectra of *Bougainvillea glabra* Choysi extract (water-ethanol, ratio 1:3).

Stability of pigment mainly at acidic pH. All water-ethanol extracts (in ratio 1:1, 1:3 y 3:1, respectively) from *Bougainvillea glabra* showed stability at pH ranging from 4 to 8, physicochemical parameters related to betalains. Notwithstanding, as the better betalain extraction was carried out with water-ethanol in ratio 1:3, so it was analyzed in detail regarding the time. After 24 hr of extraction the dye showed an stable absorption spectra at pH values between 2 and 8 (Fig. 2A) and it began to change from 9 to 12 (Fig. 2B). Whereas the color was better preserved at pH between 4 and 8 values (Fig. 2C), opposite to it, a progressive degradation occurred in the other pH values (Fig. 2D) after 28 days. The found results confirmed the presence of betalains (Ayala and Beltrán, 2007).



A

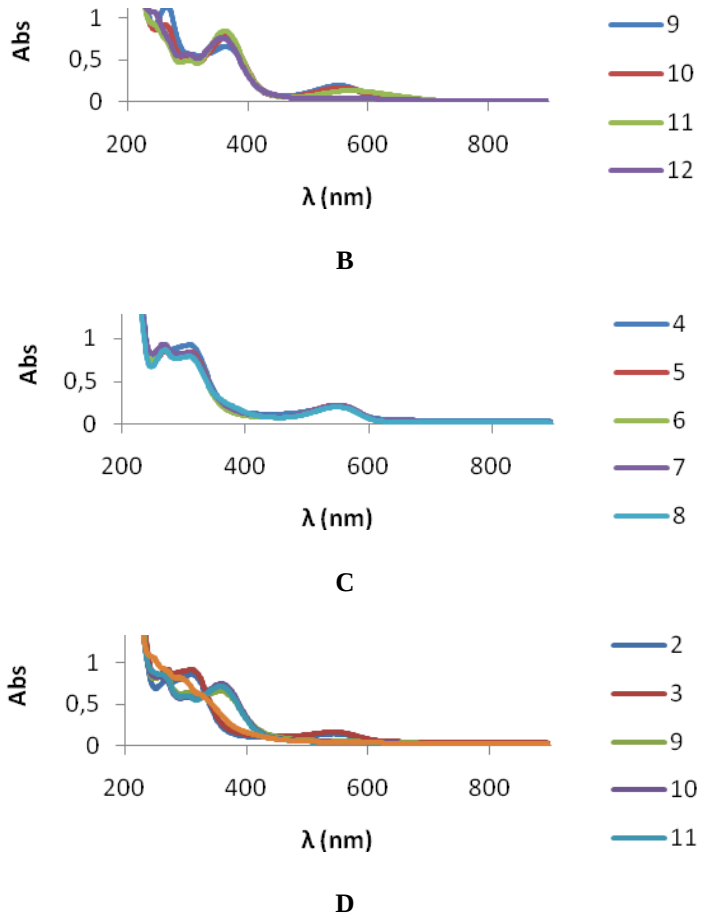


Fig. 2. Effect of different pH on the stability of the extract obtained from *Bougainvillea glabra* through time. pH values **A** between 2 and 8, and **B** between 9 and 12 after 24 hr, **C** between 4 and 8, and **D** 2,3, 9 to 12 after 28 days.

Suitability of bugambilia extracts as food additives. Phenolic compounds were detected in the selected bugambilia extract and also antioxidant activity was determined whose results were 126 mg EAG g^{-1} for TPC and 0.481 mM TEAC, respectively. Then, maybe the bugambilia extract can exert a probiotic action when used as food additive. By another hand, the microencapsulation of extract with maltodextrin was success in all the developed formulations and their drying showed no problems. However, the drying process affected the qualitative properties of those formulations. The better color and morphology (hard and uniform) characteristics were appreciated in the formulation at 20% w/w maltodextrin- extract. Also, the highest drying yield (16%) was obtained with the formulation containing maltodextrin at 20%. However, for that formulation a color reduction (18.85%) could be noticed once dryness was reached, probably due to high temperature established in the equipment; the absorbance assessed before drying was 0.504 and became to 0.409 after drying. Furthermore, the application of microencapsulated dye in yogurt was success as it has any change in properties including color after 15 days, while in gelatin was not suitable because prevented the gelation process, although no changes were observed in color after 15 days. Concluding, *Bougainvillea glabra* flowers may represent a natural source of dyes for food coloring not only contributing to acceptable appearance, but also providing phenolic compounds exerting antioxidant activity.

Acknowledgements

The authors thank to the Secretaría de Investigación y Estudios Avanzados of the Universidad Autónoma del Estado de México for supporting this work through the project “3087/2011”.

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Spaghetti Added With Unripe Banana Flour Modified By Acid Treatment: Chemical Composition, Cooking Quality And Starch Digestibility

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Introduction.

Changes in food habits and consumption of rapidly digestible foods high indigestible carbohydrates and a decreased intake of indigestible carbohydrate intake as dietary fiber (DF) and resistant starch (RS) has been related with the increment on the prevalence of degenerative diseases such as obesity and diabetes. Studies suggest an inverse relationship between dietary fiber intake and the risk of diseases like colon cancer [(1)] and coronary disease.[(2)] Thus it has been recommend the consumption of foods with high content of indigestible carbohydrates, as they provide health benefits.
Unripe banana flour (UBF) presents considerable amounts of (RS) and other non-starch polysaccharides present in DF, this flour can be an alternative to increase the amount of indigestible carbohydrates in foods; however, the indigestible carbohydrates in UBF was increased by acid treatment. [(3)]
Spaghetti is widely accepted food; this food is traditionally made with durum wheat semolina, water and some alternative ingredients [(4)]; for this reason, DF content is low. The aim of this study was to prepare spaghetti added with unripe acid-modified banana flour and evaluate chemical composition, cooking quality and digestibility of starch.

Materials and methods

Raw material.

Unripe banana (*Musa paradisiaca* L.) was purchased from a local market in Cuautla Morelos. Bananas were washed, cut into 0.5 cm slices and dried at 50 +1°C, ground to pass a US 40 sieve (0.038 mm) to obtain unripe banana flour (UBF). Durum wheat flour was obtained from Harinera San Blas, Puebla, Mexico.

Acid Modification of UBF (lintnerization).

UBF lintnerization was performed as follows: 100 g UBF was weighed in around flask of 1 liter of capacity and 400 mL of 1.6M HCl were added. The solution reacts at 38°C for11 days with continuous stirring at 200 rpm. After hydrolysis time reaction was neutralized with 1.6M NaOH thus acid treated unripe banana flour (ATUBF) was obtained.

Proximate analysis.

Moisture (44-19), lipids (30-25), protein (46-13) and ash (08-01) were determined according to the official methods of the AACC (2000) [(5)]. Total dietary fiber by AACC method 32.05.

Elaboration of samples:

Control spaghetti (CS), unripe banana flour spaghetti (UBFS) and acid treatment unripe banana flour spaghetti (ATUBFS).
CS was prepared with wheat semolina, which was mixed with water (50% based on the dry weight of the sample) in a Kitchen Aid mixer (Model KPRA, St. Joseph, MI. USA) by 5 min to obtain a homogeneous paste. For unripe banana flour spaghetti (UBFS) and acid treatment unripe banana floor spaghetti (ATUBFS), spaghetti were prepared by mixing 50% plantain flour in each case, with 50% wheat semolina, 50% of water was added (based on the dry weight of the sample), mixed 5 min to obtain the pasta. Each sample was allowed to stand 30 min before lamination. The pasta was laminated and cut with a Kitchen Aid metallic mark (Model KPRA, St. Joseph, MI. USA), roller cutter obtaining spaghetti shape. Finally, the samples were dried at 45 ± 1 ° C.

Optimal cooking time and cooking loss

Optimal cooking time was determined with AACC 66-50 (2000). Method of the cooking loss calculation was performed using AACC method 66-50 (2000).

Sample preparation

Spaghetti was cooked and frozen in liquid nitrogen for 1 min. Frozen sample was lyophilized, ground to pass a US 50 sieve (0.028 mm), and stored in plastic containers at room temperature for further study.

Total dietary fiber (TDF) content (AACC 32-05).

Which is based on a combination of enzymes used α-amylase, protease and amyloglucosidase to remove undigested protein and starch and, being non-digestible material corresponding to the fiber, which is filtered and weighed.

Rate of starch digestion in vitro (Holm and others., 1985). [6]

Based on quantification of the enzyme hydrolyzed starch α-amylase. With incubation at 37 °C with constant agitation and intermediate time sof 15, 30, 45, 60, 75 and 90 minutes, quantifying the reducing sugar content with DNS. Absorbance were read at 530 nm in parallel with a standard curve which was prepared maltose to calculate the reducing sugars which are reported as maltose equivalents and obtain the percentage (%) of the percentage of hydrolysis as maltose liberated during the reaction.

Results and discussion

Chemical Composition of spaghettis.

No differences were found in moisture content (Table 1) when both UBFS native and modified as compare, but they had lower moisture content than control spaghetti (CS)[7,8]. The substitution of semolina with UBF decreased the protein content of spaghetti, which produced a weaker protein network; Gallegos-Infante and others [8] reported similar response, thus producing greater water loss during the drying step. Regarding ash value an increase in the spaghetti made with both plantain flour (native and modified) was found; as it know banana contains minerals that remain in the spaghetti. A similar pattern was observed in samples of spaghetti with different levels of banana flour [7]. No difference with respect to lipid content was found, similar value was obtained in samples of spaghetti containing light buckwheat and dark buckwheat [9].

Table 1. Proximate chemical analysis of experimental spaghetti.

Sample	Moisture (g/100 g)	Ash (g/100 g)	Proteins (g/100 g)	Lipids (g/100 g)
CS	11.15±0.44[d]	0.76±0.05[b]	11.57±0.23[c]	0.53±0.12[b]
UBFS	7.90±0.25[c]	2.36±0.03[d]	7.24±0.04[b]	0.53±0.01[b]
ATUBFS	8.04±0.35[c]	3.72±0.04[f]	7.69±0.05[b]	0.38±0.09[a]

• **CS** (Spaghetti Control, 100% wheat semolina)
•**UBFS** (Unripe banana flour spaghetti)
•**ATUBFS** (Acid treatment unripe banana flour spaghetti)
•Different letters indicate a significant difference with a significance level of α= 0.05.

Optimal cooking time and cooking loss

The optimal cooking time values obtained for the spaghetti were 11.33, 8.33 and 6.66 min for CS, spaghetti with unripe banana flour, and spaghetti with acid treatment banana flour, respectively (Table 2). This decrease could be due to a lack of protein matrix in spaghetti that weaken the structure of the spaghetti, producing that the temperature penetrate easily and cook the spaghetti in short time. This is related with the cooking loss values, because the weak protein network facilitates that starch chains and other solids may leaching out from the spaghetti matrix, increasing cooking loss. Similar results were reported by Gallegos-Infante and others (2010), [(8)] with a range of 9.9% to 21.56% of cooking loss for spaghetti with common bean flour. However, the quality of the spaghetti with both UBF is considered good due to that the cooking loss were lower than 10%[(10)].

Table 2. Quality assessment of the spaghetti by cooking analyzing*

Sample	Optimal cooking time (min)	Cooking loss (g/100g)
CS	11.33±0.57[a]	4.60±0.25[a]
UBFS	8.33±0.57[b]	5.59±0.19[b]
ATUBFS	6.66±0.57[c]	7.44±0.04[c]

CS = (Spaghetti Control ,100% wheat semolina)
UBFS = (Unripe banana flour spaghetti)
ATUBFS = (Acid treatment unripe banana flour spaghetti)
*Different letters indicate a significant difference with a significance level of α= 0.05.

Total dietary fiber (TDF).

Total dietary fiber content in uncooked and cooked spaghetti is shown in Table 3. CS had similar TDF content than the uncooked and cooked samples, showing that the cooking process did not affect this component. Spaghetti with UBF and ATUBF presented a slight decrease in the TDF content after cooking, although the network present in these spaghettis is weak, the non-starch polysaccharides remaining in the food matrix, and only small amount is leaching out. However, it is worth mention that ATUBFS had a double TDF content than UBFS. The chemical modification to the UBF increased the TDF content and this is maintained in the food products added with this flour.

Table 3. Total dietary fiber in raw and cooked spaghetti*

Sample	TDF (g/100g)	
	Uncooked	**Cooked**
CS	6.68±0.31 [a]	6.73±0.0.38 [a]
UBFS	11.04±0.07 [c]	9.64±0.016 [b]
ATUBFS	21.31±1.29 [f]	18.46±1.20 [f]

CS = (Spaghetti Control, 100% wheat semolina)
UBFS = (Unripe banana flour spaghetti)
ATUBFS = (Acid treatment unripe banana flour spaghetti)
* Different letters indicate a significant difference with a significance level of α= 0.05.

***In Vitro* digestion rate (Holm and others., 1985)**.
Addition of both banana flours (UBF and ATUBF) produced a considerable decrease in the hydrolysis rate (Figure 1), which means that digestion of the glycemic carbohydrates present in banana flours is lower compared with the Control. At the end of the test, control spaghetti shows around 52 % of hydrolysis, while the spaghetti with UBF showed a 36.75%. ATUBFS presented the lowest rate of hydrolysis that is in agreement the high TDF content The low hydrolysis percentage and the hold liberation of glucose in the ATUBFS, is very important due to the moderate postprandial glycemic response in vivo[(6)], Nowaday, is an important factor in the dietary management of altered metabolic conditions, such as diabetes [(11,12)].

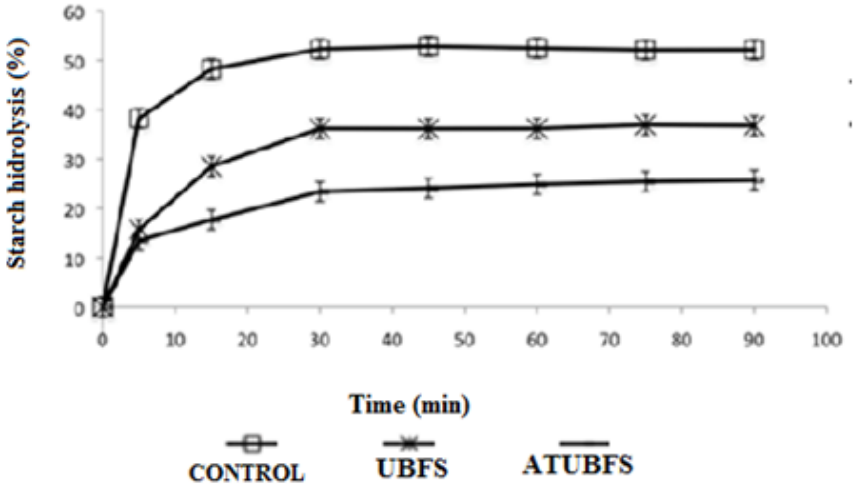

Figure 1. Hydrolysis rate of spaghetti.

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Renealmia alpinia A Plant With Potential Anticholesterolemic

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Abstract

Renealmia alpinia is a plant belong to the family of Zinginbereceae, grows wild in the States of Veracruz and Puebla, Mexico, and its fruits can be used for obtain active compounds. The main goal of this work was the identification and quantification of phytosterols in *Renealmia alpinia* fruits by gas chromatography. Fruits were dried and conditioned for analysis. The seeds were washed and milled (mesh # 40) for processing to obtain the ether extract. For efficiency of the extraction process the samples suffered several extractions with constant agitation maceration. The oil was characterized by physical parameters of quality control (relative density, refractive index, humidity, iodine value, acidity and saponification value). Subsequently, the oil samples were analyzed in a gas chromatograph. The operating conditions were: Column temperature, flow rate, temperature sensing and sample injection. The *Renealmia alpinia* seed oil evidenced the presence of β-sitosterol, stigmasterol and campestrol, corresponding to phytosterols family, which have also been identified in olive and soy oils.

Keywords: *Renealmia alpinia*, phytosterols, gas chromatography.

Introduction

Renealmia plant is perennial, aromatic, spicy, with varying sizes, with oil-secreting cells and have been reported in other species the presence of terpenoids and phenyl propanoides compounds. Many vegetal species have ethnobotanical value as spices (including ginger, Zingiber officinale, turmeric, Curcuma longa and cardamom, *Eleffaria cardamomum*) and ornamental (Alpinia and Hedychium R.) (Simpson, 2005). The vegetative and reproductive periods are linked to the rainy season, its flowering stage is in the months from April to July and fructification occurs in a period which includes the months of September and October. The plant grows to about 2 m, the fruit is a capsule lobulated, has 100 to 200 seeds in each fruit representing the 70% of total weight of the fruit. In the maturity, the fruit has a purple (Maas, 1977). For the Alpinia speciosa volatile oil have been identified more than 35 constituents (gas chromatography and GC / MS) including β-pinene (34.0%), α-pinene (14.8%) and β-caryophyllene (10.8 %) as the main compounds (Nguy, et al 2011). Leaves of Renealmia chrysotrycha (Zingiberaceae) was isolated for the sesquiterpene alcohol, the spectral data suggest that the structure is a 10-aromadendranol. In a thorough investigation of NMR, it was established its relative stereochemistry of ledol. Three compounds were isolated: aromadendrene, cis-γ palustrol calamenene (Kaplan et al, 2000). In leaves fractions, flowers and rhizomes of Zingiberaceae family members, there are an appreciable amount of monoterpenes and related sesquiterpernes with antimicrobial activity (Habsah et al, 2000; Oonmetta-Aree et al, 2006). In the oil of Alpinia zerumbet Pers. leaves was obtained the presence of a high content of oxygenated monoterpenes (52.5%), terpinen-4-ol, 1.8 cineole and γ-terpinene as major constituents (Victorio et al, 2009). Phytosterols are plant sterols with chemical structure similar to cholesterol, but differ from this by the presence of methyl or ethyl groups in the main chain of the molecule (Redd and Kattan 2004). It is estimated that a daily intake of phytosterols present in legumes in a range of 160 to 500 mg/day, the intake of these compounds can help to reduce the blood cholesterol levels. There are around 25 phytosterols chemically identified, but there are three that are in higher proportion in plant extracts like α-sitosterol, campesterol and stigmasterol, which constitute 95 to 98% of the total sterols (Smith, 2004).

Materials and methods

The extraction of phytosterols was determined according to the method proposed by Fernandez and Cabral (2007), to 1 g of *Renealmia alpinia* seed oil, it was added 0.5 N potassium hydroxide and heated by 30

min. The mixture was placed in a separatory funnel with distilled water, sodium chloride and 10% petroleum ether. The ether phase was evaporated and then a required volume of petroleum ether was added until dissolution. The water phase was filtered and the identification of phytosterols was performed in a gas chromatograph (Perkin-Elmer, USA). The working conditions were: column temperature 300° to 250°C, flow rate, temperature and sample volume (3 µl) detection and column CB 35 m x 0.32 m.

Results y discussion

It can be observed in Figures 1 and 2 the presence of three major phytosterols in the oil samples of *Renealmia alpinia* identifed as stigmasterol, α-sitosterol and lanosterol, by gas chromatography. Figure 1 shows the identification of the phytosterols from problem samples and Figure 2 shows the standards used, in order to identify by reference in oil samples of *Renealmia alpinia*.

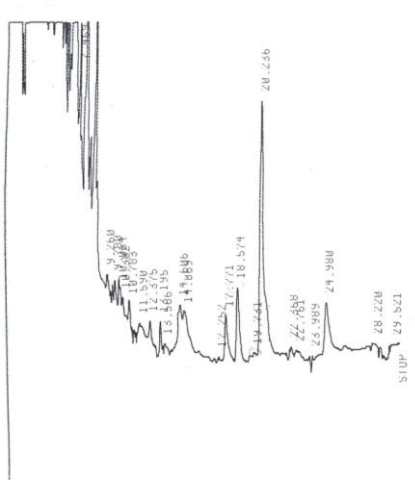

Figure 1. Chromatogram of phytosterols obtained in seed oil from *R. alpinia*.

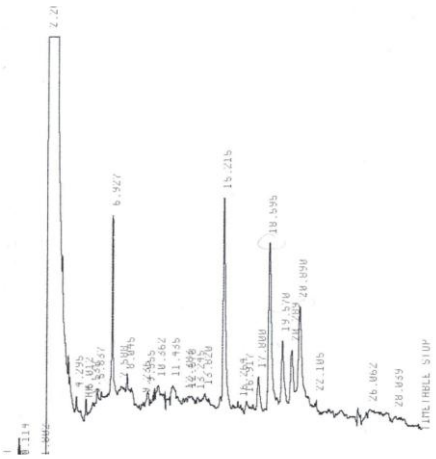

Figure 2. Chromatogram of standards phytosterols (β-sitosterol, stigmasterol and campestrol).

The *R. alpinia* phytosterols identified, there was a greater proportion of lanosterol, which is one of the major compounds which derive all steroids, besides being the first product of the cyclization of squalene (Devlin, 2004), found in minor proportion α -sitosterol and stigmasterol, this last compound has also been reported for soybean oil and legumes oil, mainly beans (Alton, 1984).

Fernandez and Cabral (2007) note that in vegetal species there are more than hundred different phytosterols, being the most abundant β-sitosterol, stigmasterol and campestrol, among others. This group of compounds have not yet identified in several plant species.

Conclusion

It was possible to identify the presence of phytosterols as stigmaesterol, α-sitosterol, lanosterol in the seed oil of *Renealmia Alpinia*, therefore can be considered as a potential use for food grade and with possible application for nutraceutical use. The phytosterols of R. alpinia could help to reduce levels of low density cholesterol in the blood.

Acknowledgments: The authors acknowledge the support of CONACYT scholarship num.170132 to JLEE and technical support to Gas Chromatography QFB E. Rios Leal and Mr. C. Rojas Chavez.

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Ion Chelating Activities of *Brassica napus* Protein Isolate and Hydrolysates

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Introduction

Canola/Rapeseed (*Brassica napus*) is an important oilseed crop in many countries and is considered to be the second most abundant source of edible oil in the world (1). Canola is the rapeseed variety which was developed in Canada characterized by low erucic acid (<2%) and glucosinolate (<30 lmol/g) contents (2).
Canola seeds contain approximately 40% oil and 17-26% protein, processing of this seed for oil production generates an industrial by-product which can be used as an important protein source (˜34%). Due to its balanced amino acid profile and considering that during the past decade research encompassing bioactive peptides has significantly increased, this protein has been suggested as an alternative source for bioactive protein peptides production(3).
The interest in such bio-molecules lies in the bio-activities they are able to exert in the body systems, beyond their nutritional and metabolic function as proteins (4). Unique intrinsic properties of bioactive peptides, make them attractive therapeutic agents; being able to promote health and reduce the risk of diseases, if regularly included in balanced diets, besides being useful to maintain nutritional deficiencies (5).
In general, bioactive peptides are amino acid residues encrypted within the sequence of the parent protein andwhilst not active within the primary structure of their source proteins, peptides that possess specific amino acid sequences known to exhibit biological activity (6) can be released through enzyme-mediated hydrolysis, during gastrointestinal digestion, microbial fermentation, proteolysis by enzymes *in vitro* and/or during food processing (7).
A number of different therapeutic activities like, antihypertensive, anticancer, antimicrobial, anti-obesity, hypocholesterolemic, anti-thrombotic, opioid, opioid antagonist, cytomodulatory, antioxidant activity and immunomodulatory effects have been demonstrated through *in vitro* and *in vivo* assays. Moreover, some of these peptides may exhibit multifunctional properties (8).
Among them chelation properties of some bioactive secquencies has been found effective to prevent oxidative damage because metal chelation appears to be at least partly responsible for the antioxidant activity that has been found in several amino acids,including Tyr, Met, His, Lys, Arg, and Trp. Furthermore, antioxidant peptides containing Pro and His have a synergistic effect in the presence of other antioxidants such as polyphenols. In addition, chelation by His, Glu, Asp, and Cys results in enhanced calcium and iron absorption, and can also result in reduction of ferric to ferrous ion (9)
Therefore value-adding to canola meal is possible via generation of bioactive peptides for eventual use as dietary supplements if considering that sequences with biological activities have been identified in both main storage proteins in canola, napin and cruciferin (10).

Methods and Materials

Materials

Canola seeds (*Brassica napus*varHyola) grown in 2010 were obtain from Mexico state and store at 4°C.Alcalase® 2.4L (E.C. Number 232-752-2), Flavourzyme® 500L were provided by Novozymes (Bagsvaerd, Denmark) Pepsin A (E.C.3.4.23.1, PP-77163, 800–2500 units/mg protein,from hog stomach), pancreatin (P-1750, 4XUSP, from porcine pancreas), $FeCl_2$, ferrozine, pyrocatechol violet, EDTA (ethylenediaminetetraacetic acid), were purchased from Sigma–Aldrich (Sigma Chemical Co.,St. Louis, MO, USA). All other chemicals were of analytical gradeprovided by JT Baker (Phillipsburg, NJ, USA), Merck (Darmstadt,Germany) or Bio-Rad (Bio-Rad Laboratories Inc., Hercules, CA,USA).

Protein isolated production

Whole seeds were ground to powder and passed through a 0.2 mm-mesh sieve. The resulting flour was defatted by extraction with hexane in a soxhlet equipment for 9h.

Conditions for isolate preparation were optimized by RSM methodology. Briefly proteins were extracted by suspending the flour in distilled water (1:10, w/v) adjusted to pHs according to the treatment of the Box-Behnkendesing (Table 1) with 1 N NaOH, and agitation for 60 min at room temperature. The supernatant resulting from centrifugation at 6000 gfor 30 min was adjusted to the first pH (p.I.1) using 1 N HCl and after centrifugation under the same conditions described above the supernatant was adjust to a second pH value (p.I.2) in order to precipitate most of the protein. The protein precipitate was recovered by centrifugation at 6,000 g for 30 min and lyophilized.

Experimental design, modeling and optimization

The effects of three processing factors protein extraction pH (pHs)and two isoelectric pH points, were studied upon %Nitrogen in the concentrate determined by a micro-Kjeldahlequipement; with regardto obtain the higher protein content of the corresponding hydrolysates using a centralcomposite design with 28 independent experiments withfourcentrepoints. All experiments were run in random order. The associatedmatrix of experimental design is shown in Table 1.The quadratic polynomial model proposed for the responsevariable, were estimatedby multiple linear regression using Design expert® V8 (Stat-Ease, Inc)

Table 1.Box-Benhken experimental desing

Run	Levels				**Value**			**Run**	**Level**				**Value**		
	pH s	PI 1	PI 2		pH s	PI 1	PI 2		pH s	PI 1	PI 2		pH s	PI 1	PI 2
1	5	-1	-1	0	10	3	5	**15**	13	0	0	0	11	3.5	5
2	15	1	-1	0	12	3	5	**16**	1	0	0	0	11	3.5	5
3	6	-1	1	0	10	4	5	**17**	5	-1	-1	0	10	3	5
4	3	1	1	0	12	4	5	**18**	24	1	-1	0	12	3	5
5	2	-1	0	-1	10	3.5	4.5	**19**	21	-1	1	0	10	4	5
6	12	1	0	-1	12	3.5	4.5	**20**	19	1	1	0	12	4	5
7	17	-1	0	1	10	3.5	5.5	**21**	18	-1	0	-1	10	3.5	4.5
8	8	1	0	1	12	3.5	5.5	**22**	12	1	0	-1	12	3.5	4.5
9	10	0	-1	-1	11	3	4.5	**23**	17	-1	0	1	10	3.5	5.5
10	9	0	1	-1	11	4	4.5	**24**	22	1	0	1	12	3.5	5.5
11	11	0	-1	1	11	3	5.5	**25**	10	0	-1	-1	11	3	4.5
12	4	0	1	1	11	4	5.5	**26**	23	0	1	-1	11	4	4.5
13	16	0	0	0	11	3.5	5	**27**	11	0	-1	1	11	3	5.5
14	7	0	0	0	11	3.5	5	**28**	20	0	1	1	11	4	5.5

Electrophoresis (SDS–PAGE)

SDS–PAGE was carried out according to Laemmli (1970) (11) using a Minin-Protean 3 Gel electrophoresis Unit (BioRad). Gels consisted of a 13% polyacrylamide resolving gel (pH 8.8) and a 5% stacking gel (pH 6.8). Samples were dissolved in sample buffer (0.1 M Tris–HCl, pH 6.8, 2% SDS, 5% b-mercaptoethanol and 0.02% bromophenolblue) and loaded onto the gels (15 ll, 75 lg protein/well).Gels were stained using 0.125% (w/v) Coomassie brilliant blue R- 250 in 7% acetic acid and 40% methanol (v/v) solution and destained in 7% acetic acid and a 30% ethanol (v/v) solution.

Enzymatic protein hydrolysis

Isolate prepared under optimized conditions was then hydrolyzed by treatment with Alcalase-Flavourzyme (AF) and Pepsin-Pancreatin (PP) proteolitic systems in order to investigate the possible release of peptides with metal chelating properties.

Protein fractions were hydrolyzed by treatment with Alcalase or Pepsin for 120 min followed by treatment with Flavourzyme or Pancreatin for another 120 min according to Megías et al. (2008) (12). Protein suspension in water (4%,w/v) were adjusted to pH 8.0 for Alcalase and pH 2.0 for Pepsin using HCl.Alcalase at 0.3 AU g-1 enzyme to substrate ratio or Pepsin at a 1:20 (w/w) enzyme to substrate ratio was added at time 0, and Pancreatin 1:20 (w/w) or Flavorzyme 50LAPU g-1 enzyme to substrate ratio, was added 120 min later after adjusting to pH 7.0 for Flavourxymeand 8.0 for Pancratin respectively .

Temperature was kept at 50° for both Alcalase and Flavourzyme and 37 °C for Pepsin-Pancreatin throughout the whole process, Aliquots of the reaction mixtures were withdrawn at regular intervals and heated at 90° C for 10 min in order to inactivate enzymes. Aliquots at time 0 represent non- hydrolyzed protein. Hydrolysates were clarified by centrifugation at 10,000 g for 15 min Lyophilisedand kept at -20°C.

Metal chelating activity

Cooper, iron and calcium chelating activities were determined for 13 different hydrolysates mixtures from each proteolitic system.

Iron chelating activity

Fe^{2+}-chelating activity was determined by measuring the formation of the Fe^{2+}-ferrozinecomplex .Samples(100 lg) were mixed with 250 ll 100 mM Na acetate bufferpH 4.9, and 30 ll FeCl2 (0.01%, w/v). Ferrozine (12.5 ll, 40 mM)was added after incubation for 30 min at room temperature. EDTAwas used as a positive control. Binding of Fe(II) ions to ferrozinegenerates a coloured complex that was measured at 562 nm usinga microplate reader (Multiskan Spectrum, ThermoLab Systems,MA, USA).

Iron chelating activity was calculated using the followingequation:

$$\%\ \text{Chelating Activity} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Copper chelating activity

Cu^{2+}-chelating activity was determined according to Saiga et al.(2003). Na acetate buffer, pH 6 (290 ll, 50 mM), 6 ll 4 mMpyrocatechol violet prepared in the same buffer, and $CuSO_4.5H2O$ (1 µg) were added to the hydrolysates (100 µg). Absorbance at632 nm was measured using a microplate reader (Multiskan Spectrum, ThermoLab Systems, MA, USA).

Copper chelating activity was calculated as described above foriron.

Statistical analysis

Results were expressed as mean ± standard error. The meanscomparison was subjected to ANOVA and differences amongmeans were determined using Fisher's least significant difference(LSD) at $p < 0.05$ by using version 1.0.

Results and discussion

Optimization of protein isolate production

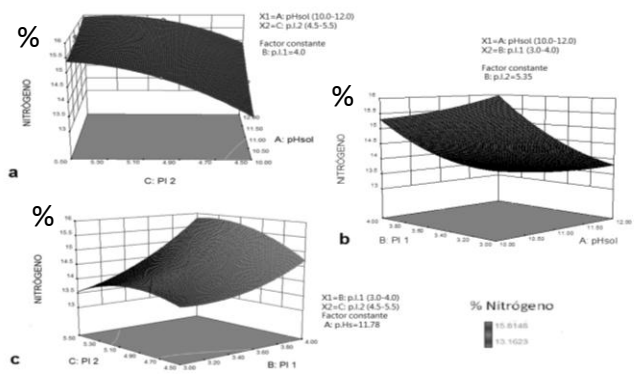

Fig. 1. Influence ofpHs, p.I.1 and p.I.2 factors on Nitrogen content (%N) of canola concentrates.

A second order polynomial model were generated from the respond surface methodology (RSM) results to describe the influence of each factor and their interactions with the Nitrogen content, factors pHs, p.I.1, p.I.2, pHs^2,$p.I.1^2$, $p.I.2^2$, pHs.p.I.1, pHs.p.I.2 y p.I.1.p.I2 were significant to the model accordingly to the equation:

$$\%\ Nitrogen = 0.25373\ pHs + 0.61902\ p.I.1 + 0.38718\ p.I.2 + .22665\ pHs.p.I.1 + 0.22102\ pHs.p.I.2 + 0.32869\ p.I.1.p.I.2 + 0.1$$

Influence of solubilization pH value (pHs) and precipitation pH values (p.I.1 and p.I.2) are presented in the surface respond graphs (figure 1). With the increase of the solubilization pH value the Nitrogen

content tent to a maximum when the extracted protein is precipitated with a p.I.2 value near to pH=4. Numerical optimization suggested a maximum nitrogen content at solubilization and precipitation values of pH=11.78, 5.36 and 4 respectively. Experimental results for Nitrogen content with optimized condition was 15.5916%.

Electrophoresis (SDS–PAGE)

The electrophoretic profiles of all treatments in the Box Benhkendesing are showed in figure 2. Characteristic bands of the two major storage proteins from *Brassica napus* were found present in all the isolates obtained.

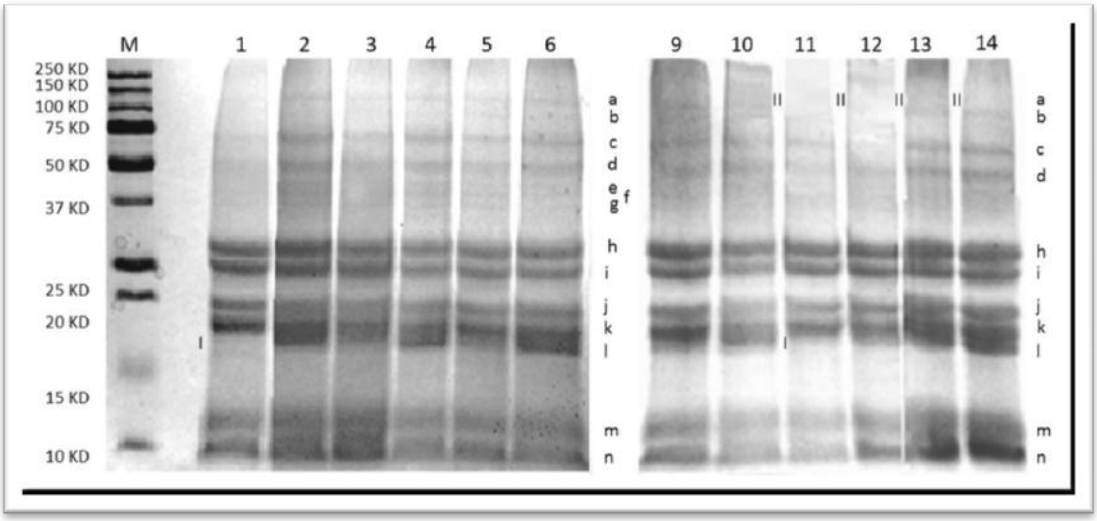

Fig. 2 SDS-PAGE of canola meal and concentrates. M= molecular weight marker; 1= defatted canola meal, 2= optimized conditions isolate, 3-12 Box-Benhken treatments desingisolate and concentrates, 13 and14 designcentre points.

Ion chelatingactivity

Table 1. Cooper, Iron and Calcium Chelating Activities

Sample		**Hydrolysis Time**	**Cu++ ChelatingActivity (%)**		**Fe++ Chelating Activity (%)**		**Ca++ QuelatingActivity (%)**	
Isolate		16.47 0.44^{a}		19.68 0.29		55.91 0.40$_{a}$		
--		AF	PP	AF	PP	AF	PP	
Hydrolysate	0	71.55 0.40$_{b}$	17.87 0.58$_{a}$	5.05 0.23$_{b}$	23.96 0.94$_{l}$	18.70 0.60$_{b}$	2.58 0.07$_{m}$	
10	63.11 0.62$_{c}$	16.60 0.45$_{a}$	5.61 0.20$_{b,c}$	23.14 0.71$_{l}$	19.50 1.14$_{b}$	23.67 1.50$_{fh,n}$		
20	36.15 0.31$_{d}$	27.57 1.00$_{i}$	8.87 0.03$_{d}$	11.84 0.47$_{g,m}$	12.37 0.24$_{d}$	11.75 0.07$_{d}$		
30	52.56 0.21$_{e}$	12.45 0.11$_{j}$	7.37 0.37$_{e}$	9.76 0.38$_{d}$	8.91 0.50$_{e}$	25.25 1.84$_{f,h,n}$		
45	64.19 0.68$_{c}$	21.10 1.20$_{k}$	9.30 0.36$_{d,f}$	19.67 0.18$_{a,n}$	10.79 0.21$_{d}$	18.67 0.41$_{b,c,i}$		
60	78.12 0.62$_{f}$	30.95 0.40$_{l}$	9.79 0.25$_{d,f}$	18.01 0.51$_{i}$	25.00 0.39$_{f}$	25.08 0.07$_{f,h}$		
90	77.49 0.10$_{f}$	14.43 0.59^{a,j}	12.28 0.27$_{g}$	18.90 0.79$_{a,i,n}$	16.46 0.21$_{g}$	25.17 0.41$_{f,h,n}$		
120	72.40 0.10$_{b}$	26.88 0.80$_{i}$	12.65 0.55$_{g}$	16.42 0.61$_{o}$	24.37 1.07$_{f}$	24.58 1.84$_{f,n}$		
135	78.37 0.79$_{f}$	25.88 0.18$_{i}$	14.28 0.09$_{h}$	7.21 0.35$_{e}$	18.77 0.37$_{b,c,f}$	31.08 0.20$_{f}$		
150	79.87 0.19$_{f}$	33.67 2.79$_{m}$	16.84 0.43i	6.72 0.27$_{c,e}$	6.31 0.35$_{h,i}$	29.58 2.11$_{o}$		
180	82.94 0.49$_{g}$	34.67 0.45$_{d,m}$	13.11 0.29$_{g,h,j}$	12.41 0.49$_{g,j,m,p}$	5.19 0.23$_{i,j,k}$	23.83 0.68$_{h,n,p}$		
210	85.66 0.07$_{h}$	33.88 1.78$_{d,m}$	29.05 0.37$_{k}$	15.29 0.25$_{h,o}$	14.97 0.38$_{k}$	21.39 0.36$_{p}$		
240	81.42 0.55$_{g}$	18.47 0.14^{a}	17.71 0.18	11.51 0.38	14.97 0.54$_{g,l}$	15.67 0.54$_{g,l}$		
EDTA		88.21 0.56$_{n}$		94.23 0.31$_{n}$		55.91 0.40$_{q}$		

*Brassica napus*hydrolysates100 µg protein/well; positive control EDTA: 5 µg/well,respectively. Values represent the mean ±standard deviation of three determinations.

Chelating activity of isolate and protein hydrolysateswas studied since divalent ions are often involved in prooxidantreaccions within biological systems. Metals ions react with the hydrogen atom from unsaturated fatty acids affecting both speed of autooxidation and redirecting hidroperoxide decomposition to volatile compounds (13). From all different hydrolysates those corresponding to 210min of Alcalase-Flavourzymeproteolytic system had the higher cooper quenching activity (85.66 %) with a concentration of 50 µg of protein. Both Alcalase-Flavourzyme and Pepsin-Pancreatin systems were found succesfull in

releasing peptides with chelating properties, the chelating activity for cooper and iron ions was significantly enhanced (α=0.95) with the hydrolysis process with the best result for the 210 min AF hydrolysate (50µg for cooper and 100µg for iron assay) where the % of chelating activity obtain was 85.66± 0.07 and 29.05± 0.37% respectively, in contrast calcium chelating activity was better in the isolate (55.91%) than in the hydrolysates where the highest % of chelating activity (31.08± 0.20%)corresponded with the 135min PP hydrolysate. Thus, experimental data indicates that metal ion chelating peptides are abundant in *brassica napus* enzyme produced hydrolysates, where the ion chelating affinity maybe related to specific characteristics of the sequences produced with a selected enzyme. The evaluation of canola meal as a source of bioactive compounds will generate useful information for production of bioactive ingredients and their further application in health-promoting foods.

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Antioxidant Activity Of *Phaseolus vulgaris* L. Peptides By The Chemiluminescence Method

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Abstract

A large number of food protein-derived peptides have been shown to possess antioxidant properties. Peptides derived from bean (*Phaseolus vulgaris*L.) varieties could offer antioxidant activity.Antioxidant compounds had been analysed by several methods such as $DPPH^+$, $ABTS^+$, DMPD but chemiluminescence is not widely applied in this kind of test. The aim of this study was to evaluate the antioxidant activity as luminol oxidative reaction of peptides obtained from proteins of three bean varieties. The methodology to obtain the peptides included the protein extraction by isoelectric precipitation, enzymatic hydrolysis andmolecular weight separation by ultrafiltration. The chemiluminescence test was realized by triplicatein a luminometer 1250 BioOrbit with PBS, luminol, H_2O_2, HRP and the sample in a final volume of 1mL. Trolox 1.2mM were used as positive control. As result, it was observed thatall the hydrolysates and their fractions presented inhibition of the free radical generated by luminol, but the highest inhibition percentage was presented when low molecular weight peptides were testedprincipally in the fraction between 1-3kDa in the three bean varieties.This method could be extensively used to probe more compounds which may act as natural antioxidants and be used as nutraceutics.

Keywords: Peptides, Antioxidant, Chemiluminescence, *Phaseolus vulgaris*.

Introduction

The common bean (*Phaseolus vulgaris* L.) originated in Central and South Americaand their seeds are of great importance because they represent the main legume grain used as human food in the world. Brazil is the largest producer of common bean since 2002, and its culture is socially important because it is farmed as a subsistence culture and is the main income for small farmers. Mexico is situated in the fifth place (1).Nevertheless, the Mexican diet is based on maize and common bean, consumption per capita is located in high levels turning these seeds increase in value. Proteins are vital to nutrition since they provide the nitrogen, aminoacids and energy required for normal body functioning. Enzymatic hydrolysis of proteins can improve their chemical, functional and nutritional properties. Some proteins and peptides have shown biological activity such as antihypertensive, antimicrobial, antioxidant, anticarcinogenic, antiobesity, cholesterol lowering, immunomodulatory, mineral sequestering and regulation of nervous system. The most of them coming from casein, whey, ovalbumin and fish muscle but some have vegetable origin like pea, chickpea, soy, potato and lupins hydrolysates (2). A large number of food protein derived peptides have been shown to possess antioxidant properties. Peptides derived from bean (*Phaseolus vulgaris* L.) varieties could offer antioxidant activity. Antioxidant compounds had been analysed by several methods such as $DPPH^+$, $ABTS^+$, DMPD but chemiluminescence is not widely applied in this kind of test. Recent studies have shown the wide use of the chemiluminometric method using the horseradish peroxidase-luminol-hydrogen peroxide system (HRP-luminol-H_2O_2) as a sensitive assay for monitoring free radicals and reactive metabolites from cell-free, enzyme, cell, or organ systems and for screening antioxidant activity.The peroxidase catalyzed chemiluminescent oxidation of luminol involves the formation of a complex between oxidant (H_2O_2) and peroxidase to produce a luminol radical. Luminol radicals then undergo further reaction resulting in the formation of an endoperoxide, which decomposes to yield an electronically excited 3-aminophthalate dianion emitting light on return to its ground state (3).

The aim of this study was toevaluate the antioxidant activity employing the luminol oxidative reaction of peptides obtained from proteins of three bean varieties.

Methods and materials

Drugs and Chemicals

Luminol and horse-radish peroxidase (HRP) were purchased from Sigma (St Louis, MO). Hydrogen peroxide 36% was purchased from Calbiochem EMD Biosciences Inc. (San Diego, CA). All other chemicals were of reagent grade and were used without further purification. (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (TROLOX) and Alcalase® from *Bacillus licheniformis* ≥2.4 U/g were purchased from Sigma-Aldrich (USA).

Samples Preparation

Bean seeds were ground until passing through a 80-mesh screen, then fat were removed by hexane washes finally protein isoelectric precipitation was carried out as Torruco-Uco *et al*. (2009) reports. Protein concentrate hydrolysis was done using the method of Pedroche *et al*. (2002) with Alcalase. Hydrolysis reaction time was 120 min, pH 8.0 and the response variable measure was degree of hydrolysis (%DH). The hydrolysatesobtained were ultra-filtrated to obtain peptide fractions. Before the chemiluminiscence test was carried out protein in the samples were determined by Bradford (1976) method.

Chemiluminescence in the Presence of HRP

Samples of 10 µL of each test compound were mixed with 0.1 M phosphate buffer, and luminol solution (2 mg/mL in DMSO) was added to yield a final concentration of 1.13×10^{-4}M. H_2O_2 was then added to a final concentration of 5×10^{-5}M. The reaction was initiated by adding HRP at a final concentration of 0.2 IU/mL, yielding a final volume of 1.0 mL. Trolox 1.2 mM was used as antioxidant reference. Chemiluminescence was measured for 10 minutes at 25°C with a luminometer 1250 (BioOrbit, Finland).Based on the measurement of the area under the time-course of the chemiluminescence curves for trolox and peptides, the relative inhibitory activity at different concentrations was tested by triplicate. The inhibition ratio (%) of each sample was calculated as:

$$Inhibition\ (\%) = \frac{100 \times AUC_1}{AUC_0}$$

Where AUC_0 and AUC_1 represent the area under the curve observed for the control and in the presence of the sample solution, respectively (4).

Statistical analysis

The means comparison data were analysed by Duncan's test considered significantly different when $p<0.05$. The analysis and graphics were performed using the GraphPad Prism software (USA).

Results and discussion

In order to have a standard antioxidant control trolox 1.2 mM was used. This compound presented an inhibition percentage of 37.79± 1.93 %. This value was taken as reference for later results comparison. In a preliminary test the protein concentrate, total hydrolysate and all fractions were analysed in black bean.Antioxidant activity was observed in all cases. When protein was not hydrolysated the inhibition was 36.07 ± 1.78 % but when fraction peptideswere analysed the inhibition increase from 76.32 ± 0.91 % until 92.68 ± 3.07 %being the 1-3 kDa fraction which presented the higher activity comparing to Trolox (Fig 1).

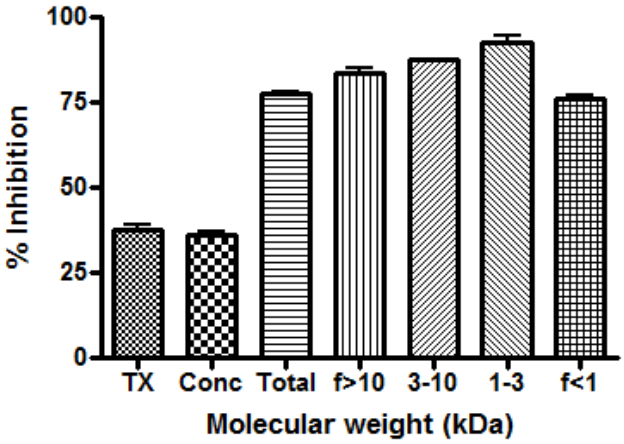

Fig 1. Inhibition presentedfor the chemiluminescent effect in black bean.
Where: TX = Trolox 1.2 mM, Conc = protein concentrate, Total = Total hydrolysate, f = fraction.

Then three common bean (*Phaseolus vulgaris*) varieties were analyzed in fraction 1-3 kDa: black bean (FNP), sulfur bean (FAH)and spotted bean (FPS), where the antioxidant effect was not significant different (Duncan, $p<0.05$) among varieties obtaining values of 91.65 ± 1.55, 94.02 ± 1.09 and 93.83 ± 0.83 respectively. These results indicate that chemiluminescent activity in *P. vulgaris* is not dependent on bean variety but is dependent on peptide molecular weight (Fig 2).

Since chemiluminescence is not a widely used method to determine antioxidant activity is not possible to compare previous works in peptides from seeds however, some works had been published using plant extracts.

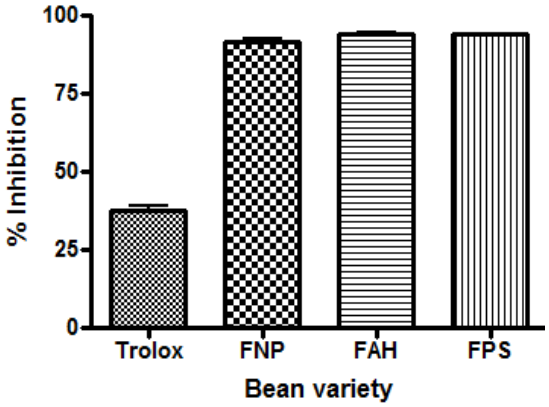

Fig 2. Chemiluminescent effectpresented as percentage between bean varieties.
Where: FNP = black bean, FAH = sulfur bean and FPS = spotted bean.

The dried ripe fruit of *Terminalia chebula* is a native plant in India and used in Indian system of medicines. The antioxidant ability by unfermented extracts and fermented products against both vitamin C and trolox was determined by Chang and Lin (2010) and their results in order of activity were trolox > methanol extract > vitamin C > 95 % ethanol extract > water extract > fermented products > supercritical fluid extraction (SFE) extract/SFE modified with 5 % ethanol extract. In this case, all extractions presented less antioxidant activity than trolox which means that *P. vulgaris* peptides had presented better results than *T. chebula*.

Lychnophora species are popularly known in Brazil as “arnica” and their hydroalcoholic preparations, obtained from the leaves, roots and inflorescences, are often used in folk medicine to treat inflammatory disorders.Crude extracts or isolated compounds from these species have been reported to exhibit other pharmacological effects, such as antitumoral, molluscicidal, trypanocidal, anticonvulsant and analgesic activities Grael *et al*. (2010) estudied the effects of phenolic compounds (0.01-1.0 mM/L) isolated from *Lychnophora* species on reactive oxygen species generation by HRP-H_2O_2-luminol chemiluminescence system. All isolated compounds shown activity but cathecol group was less active than flavonoids group.

Georgetti *et al*. (2003) studied the ability of red clover extract to inhibit free radicals using the chemiluminescence method using quercetin as control reference. Results presented shown that red clover in concentration 80 µg/mL can inhibit free radicals in 100 %. They presented chemiluminescence as a quick and sensitive method to measure antioxidant activity of several compounds. Nevertheless, since it follows several reaction steps, and the vegetal extracts are mixtures of different compounds, antioxidant activity measurements by chemiluminescence should be confirmed by other suitable methodologies.

Conclusion

Antioxidant activity as luminol oxidative reaction of peptides obtained from proteins of three bean varieties was determined. The results indicated that chemiluminescent activity in *Phaseolus vulgaris* is not dependent on bean variety but is dependent on peptide molecular weight. Low molecular weight increased the antioxidant activity percentage.

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Evaluation Of The Antioxidant Activity Of *Phaseolus vulgaris* L. Peptides by DPPH

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Abstract
The objective of this work was to determine the antioxidant activity (AA) of *Phaseolus vulgaris*hydrolysates and their peptides analyzed using 1,1-diphenyl-2-picrylhydrazyl ($DPPH^+$) free radical-scavenging assay.Protein concentrates from three *Phaseolus vulgaris*seeds varieties were obtained by isoelectric precipitation. The P. vulgaris L. protein was hydrolysed using Alcalase® and their peptide fractions prepared by ultrafitration (UF) using membranes with molecular weight cut off of 10, 3 and 1 kDa. The results showed that all peptides fractions had antioxidant activity and itincreased when the molecular weight peptides decreased. Highest antioxidant activity was found in peptides from fractions between 1-3 kDa (Duncan, $p<0.5$) in the three bean varieties studied.
Keywords: Peptides, Antioxidant, Enzymatic hydrolysis, *Phaseolus vulgaris*, Antioxidant activity.

Introduction
Enzymatic hydrolysis of food proteins is an efficient way to recover potent bioactive peptides. Protein hydrolysates are used to obtain protein fractions and bioactive peptides, which are short peptide chains with 2 to 15 amino acid residues. Plant protein hydrolysates may be a source of short chains peptides, with biological properties that include antioxidant, immunomodulatory, antimicrobial, antithrombotic, antihypertensive and protease inhibitor effects, as well as growth promoting activities of mammalian cells. The peptide molecular size is sometimes reported as a potential parameter affecting cell behaviour (1). In recent years many scientist have been interested in utilizing natural antioxidants, this had led to new investigations into assessing the antioxidant potential of bioactive peptides from protein such soy bean protein, peanut protein and corn protein (2). Protein of several sources such as grains and seeds had been reportedrecently due to the increasing interest in finding nutraceutic compounds from natural sources, which until now have been unexploited; it is the case of the common bean (*Phaseolus vulgaris*), a legume whose grain is an important source of protein for Mexican population. Common bean contains high digestibility protein (up to 22 g/100 g seed) making this a good candidate to explore as a source of bioactive peptides (3). *P. vulgaris* has been widely utilized as food in Mexico and Latinamerican for hundred years, however, could be exist a potential antioxidant activity in the peptides obtained of different *Phaseolus*species. The objective of this work was to obtain peptides from *Phaseolus* by enzymatic hydrolysis and to evaluate the potential antioxidant from *Phaseolus vulgaris* L. protein hydrolysates.

Methods and materials
Drugs and Chemicals
1,1-diphenyl-2-picrylhydrazyl ($DPPH^+$) free radical scavenging, (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (TROLOX) and Alcalase® from *Bacillus licheniformis* ≥2.4 U/g were purchased from Sigma-Aldrich (USA).
Samples Preparation
Bean seeds(*Phaseolus vulgaris* L.) were ground until passing through a 80-mesh screen, then fat were removed by hexane washes finally protein isoelectric precipitation was carried out as Torruco-Uco *et al*. (2009) reports. Protein concentrate hydrolysis was obtained by thePedroche*et al*. (2002) method. The protein hydrolysates were obtained using Alcalase® for 120 min at pH 8.0. The hydrolysis degree (%HD) was evatuated like response variable. The hydrolysateswere obtained by ultrafiltration with Millipore membranes. Protein in the samples was determined by Bradford (1976) method.

DPPH^{+} free scavenging analysis

DPPH was prepared in 100 mM/L methanol; 2.9 ml of DPPH solution were mixed with 0.1 ml samples and left for 30 and 60 min in the dark at room temperature. Trolox concentrations were 0 – 1.2 mM and peptides were analyzed in 200 µg protein function. The absorbance was determined at 515 nm. Scavenging activity of the samples was expressed as the percentage of free radical-scavenging effect following the equation:

$$Scavenging\ effect\ (\%) = \frac{Abs\ Blank\ - Abs\ Sample}{Abs\ Blank} \times 100$$

Additional, the antioxidant activity obtained was plotted as a function of antioxidant Trolox concentrations in the standard reference data. The Trolox equivalent antioxidant coefficient was calculated by dividing the absorbance percentage inhibition absorbance versus antioxidant concentration slope by the Trolox plot slope (2).

Statistical analysis

The means comparison data were analysed by Duncan's test considered significantly different when $p<0.05$. The analyses were performed using the SAS System Software for Windows 9.0 (USA). Graphics were performed using the GraphPad Prism Software (USA).

Results and discussion

In order to determine differences in 30 and 60 min time-reaction in the protein concentrate and inhibition of DPPH free radicalsamples of 25 to 200 µg of protein were tested. Table 1 shows the results for200 µg of protein from black bean (FNP), sulphur bean (FAH) and spotted bean (FPS). In the analysis was observed that the percentage of scavenging effect increases as protein does to. Also, in this test significant difference was presented neither between bean varieties nor between reaction time.

Table 1. Antioxidant activity presented by the protein concentrates.

Reaction time (min)	**% AA in each *Phaseolus vulgaris* variety**		
	FNP	**FAH**	**FPS**
30	$23.92 \pm 2.60^{A,a}$	$23.16 \pm 0.50^{A,a}$	$15.45 \pm 3.01^{B,a}$
60	$26.15 \pm 1.46^{A,a}$	$24.16 \pm 0.70^{A,a}$	$17.59 \pm 3.94^{B,a}$

^{A,B}. Means in the same line with same letter have not significant difference (Duncan, $p<0.05$)
^{a,b}. Means in the same column with same letter have not significant difference (Duncan, $p<0.05$)

In Table 2 the results for the test in peptide fractions are presented. When each bean variety was test in all fractions the results had showed that the antioxidant effect in FNP and FPS differ from FAH in which variety the scavenging effect is higher. Opposite to previews results when fractions were tested reaction time was significant to increase DPPH free radical inhibition being higher the measurement at 60 min. The lower values were presented by peptides with molecular weight higher than 10,000 Da. Secondarily peptides between 3,000 to 10,000 Da and peptides with molecular weight lower than 1,000 Da. But the higher effect was observed when the fraction from 1,000 to 3,000 Da was tested, in which case values until 40.19 ± 3.83 % of scavenging effect were obtained.

Table 2. Antioxidant activity presentedby the fractions ofprotein hydrolysates.

Bean variety	Time (min)	% AA presented by each fraction of peptides			
		f>10 kDa	f 3-10 kDa	f 1-3 kDa	f<1 kDa
FNP[2]	30	12.96 ± 0.76 [C,b]	29.89 ± 3.90 [B,b]	36.35 ± 3.33 [A,b]	24.27 ± 0.46 [B,b]
	60	15.83 ± 4.64 [C,a]	34.46 ± 4.65 [B,a]	40.19 ± 3.83 [A,a]	26.09 ± 0.97 [B,a]
FAH[1]	30	14.10 ± 2.36 [C,b]	34.51 ± 0.70 [B,b]	35.24 ± 1.21 [A,b]	33.79 ± 0.58 [B,b]
	60	18.02 ± 1.92 [C,a]	35.50 ± 0.63 [B,a]	36.91 ± 0.94 [A,a]	35.31 ± 0.71 [B,a]
FPS[2]	30	14.86 ± 4.46 [C,b]	25.58 ± 7.19 [B,b]	28.61 ± 1.92 [A,b]	34.02 ± 1.68 [B,b]
	60	20.31 ± 3.18 [C,a]	30.18 ± 7.97 [B,a]	32.30 ± 1.80 [A,a]	37.71 ± 1.63 [B,a]

[A,B,C]. Means in the same line with same letter have not significant difference by peptide fraction (Duncan, $p<0.05$)
[a,b]. Means in the same column with same letter have not significant difference by reaction time (Duncan, $p<0.05$)
[1,2]. Means in the same column with same number have not significant difference by bean variety (Duncan, $p<0.05$)

Trolox 1.2 mM was tested in order to compare the antioxidant activity in the most active fraction with a standard reference (Fig 1). Although values presented in Table 2 are considerable, when were compared to a standard antioxidant did not seem potent since they were only the half of active. Peptides were significant different to trolox. But when the comparison was between them FNP and FAH presented higher AA percentage than FPS.

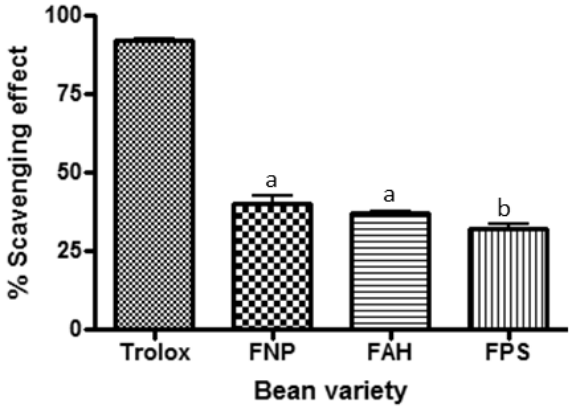

Figure 1. Scavenging effect presented as percentage between bean varieties.
Where: FNP = black bean, FAH = sulfur bean and FPS = spotted bean.
[a,b]. Means in the same block with same letter have not significant difference (Duncan, $p<0.05$)

Antioxidant activity by $ABTS^+$ technique in relation to ACE-I was tested by Torruco-Uco *et al*. (2009) in *Phaseolus lunatus* and *P. vulgaris*. The hydrolysis conditions were Alcalase in 90 min of reaction time. They presented values for antioxidant activity of 58.94 % and 60.59 % respectively, such values are approximately 20% higher than theirs presented in this work, nevertheless they did not found a relation between AA and ACE-I in their research concluding that such activities are independents. These superior values compared with which are presented in Table 2 coulddiffer because of the method.

Valdez-Ortiz *et al*. (2012) determined AA by $DPPH^+$ in three sulphur bean varieties the conditions were Alcalase as enzyme, reaction time 120 min, pH 8.0 and sample concentration 1 mg/mL of hydrolysate. They obtained values among 33.59 % to 44.05 % of inhibition which are comparable to results presented in Fig 1.

Conclusion

The hydrolysatesshowed good antioxidative capacity with values up to 40.19%. These results indicated that antioxidant activity in *Phaseolus vulgaris*depends on bean variety, also are dependent on peptide molecular weight and reaction time. In this work the higher antioxidant activity percentage was presented when the conditions were FAH in the fraction from 1,000 to 3,000 Da after 60 min of reaction time.

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Partial Characterization Of Proteins From The Fruit Of Mistletoe (*Cladocolealoniceroides*)

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Introduction

Mistletoe is a tree parasitic plant which takes salts and water from host throw haustoriums, causing slow death and susceptibility to other infections (1). This situation cause the lost of tree and affect fruit and wood national production.*Cladocolealoniceroides*is an endemic species of Mexican mistletoe spread through the faeces of birds moving from tree to tree throughout the country.

There is no efficient chemical control to stop the plague and the only way to prevent the infestation is cutting it from trees, preferably before fructification. As well, there is no final disposition of mistletoe rests, so this still being pollution.

Viscum album extract has been used as alternative complementary therapy to cancer treatment, because of the reducing of free radicals mutagenic effects during ratio or chemotherapy (2). Among the bioactive components in mistletoe,lectins, alkaloids, viscotoxins and polysaccharides, the most active compound in cytotoxity and immune-modulatory effect is lectins (3).

Knowledge of *Cladocolealoniceroides* chemical composition and pharmacological effect is limited. An aqueous extract (AE) of the fruit, shows antioxidant activity and cytotoxic effect on breast cancer cell line ZR75-1 (4).To the present, there is no knowledge about mistletoe AE components. Thus, the aim of the study was to investigate which proteins are present in the fruit and AE, to known the bioactive components of mistletoe.

Materials and Methods

-Protein extraction.

Sequential extraction of mistletoe protein groups was performed according to the method of Osborne (5). Each extraction step was performed twice al 4°C (during 8 and 6 h, respectively) using the corresponding extraction solution. In the first extraction a rate of 10 mL of extractant/g of defatted meal was used. In every step, extracts were isolated by centrifugation at 10000g during 20 min a 4°C. Albumins were extracted with solution A (0.1M NaCl, 0.01 M K_2HPO_4, pH 7.5, 0.001 Methylenediaminetetraacetic acid (EDTA), 0.02% sodium azide (NaN_3)). Globulins were extracted from the albumin-free pellet with solution B (1M NaCl, 0.01 M K_2HPO_4, pH 7.5, 0.001 M EDTA, 0.02% sodium azide (NaN_3). Prolamins were then extracted using solution C (70% ethanol, 0.01 M K_2HPO_4, pH 7.5) and glutelins with D (0.05 M acetic acid, 1% SDS).

Another sequential protein extraction was conducted according to the Padhye and Salunke method (6). Deffated mistletoe flour (50g) was mixed with 300 mL 0.04M Na_2SO_4 + 20mM β-mercaptoethanol and stirred for 30 min, centrifuged for 20 min at 13000g; the supernatant was mixed with 50%, 70% and 100% (sat.) $(NH_4)_2SO_4$ and dialysed for 24 h with four changes of distilled water; this method separated albumin 1 from globulin. Prolamins were obtained with 70% ethanol, stirred for 30 min and centrifuged for 20 min at 13000g. Finally, glutelins were separated with 0.05 M acetic acid, stirred and centrifuged at the same conditions.

Proteins were quantified using Kjeldahl method and Bradford reagent (7). Each sample was assayed in triplicate.

-Electrophoresis (sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)).

Albumin SDS-PAGE profiles were carried out according to the method proposed by Laemmli (8), using 12% (w/v polyacrylamide) separating gel and stacking gel (4% w/v polyacrylamide). All gels were run in mini-slabs (Bio-Rad, Mini Protean III) at 120 volts. Protein bands were visualized with Coomassie brilliant blue R-250. The molecular weight of proteins was calculated using the Sigma Marker Low Range (M. W. 6,500-66,000).

Results and discussion

Table 1 shows crude protein concentration (%N x 6.25) and protein assayed by Bradford method.

Table 1.Crude protein and Bradford protein content in proteinic fractions obteined by Osborne and Padhy and Salunke methods.

Extractionmethod	Extract	Crudeprotein (mg/g)	Bradford (mg/g)
Padhye y Salunke	Albumins	1.09±0.00	0.77±0.01[b,c]
	Globulins	0.00±0.00	0.00±0.00[d]
	Prolamins	0.56±0.00	0.25±0.02[c,d]
	Glutelins	1.68±0.00	0[d]
Osborne	Albumins	5.9±0.26	4.98±.06[a]
	Globulins	1.3±0.03	0.81±0.08[c,d]
	Prolamins	1.4±0.03	1.38±0.12[b]
	Glutelins	0	0[d]

Previous studies reported 12.65 ± 0.33 % of crude protein in whole mistletoe fruit (4). But sequential extraction of protein groups shows a very little content. Possibly, the nitrogen concentration is because alkaloids, nucleic acids and chlorophyll content.

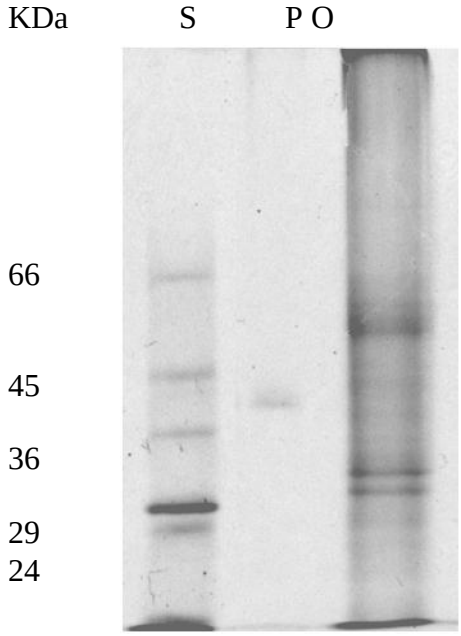

Figure 1.SDS-PAGE profile by using two different extraction methods for albumin from Cladocolealoniceroides fruit. (S)standart proteins; (P) Padhye and Salunke albumin; (O) Osborne albumin.

Osborne's method extracts a high content of crude protein (7.17 mg/g) compound mainly by albumins (4.98 ± 0.06 mg/g), so this method is better to extract proteins. ItsMr evaluated by electrophoresis profile was 29, 31, 44 and 55 kDa. Meanwhile, Padhye and Salunke's method extracts 1.02 mg/g of crude protein, albumin was the main fraction with Mr of 40 kDa.

Subsequently, AE proteins will be purified and biological activity will be evaluated on each fraction to know the cytotoxic effect active compounds in mistletoe.

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Protection Of Antioxidants Compounds With Multiple Emulsions In A Simulation Process Of Type Chihuahua Cheese

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Abstract
The objective of this work was the characterization of multiple emulsions for the protection of antioxidant compounds on in vitro tests simulating a type Chihuahua cheese process conditions. Several multiple emulsion (ME) formulations with different biopolymers were done: whey (W), Arabic gum (AG), maltodextrin (MD) and mesquite gum (MG) with binary and ternary blends. Stability of ME with antioxidant compounds was studied using particle size measurement for 45 days. Most stable formulations with antioxidant compounds were subjected to the Chihuahua cheese processing conditions (pH of 5 to 5.5 and temperature of 40°C). Stable multiple emulsions were compared with a control treatment without antioxidant compounds and total phenols, antioxidant capacity by DPPH and ABTS were measured. Results showed significant differences ($P < 0.05$) between formulations, binary blend (40% MG and 16% W) and ternary blends (66% MG, 17% AG and 17% MD) and (17% MG, 17% W and 66% MD). Greater concentration of MG showed largest external diameter and best stability for 45 days. ME with 66% MG, 17% AG and 17% MD was the best formulation in conditions simulated of cheese processing. Significant differences were observed in phenols and antioxidant capacity (DPPH and ABTS) ($P < 0.05$) between treatments, in all cases the best treatment was the antioxidant protected with ternary blend preserving 98.8% in concentration of phenols, antioxidant capacity 92% of DPPH and 87% of ABTS. The results showed that the implementation of multiple emulsions (66% MG, 17% AG and 17% MD) protect the antioxidant compounds in a simulation process of type Chihuahua cheese.

Keywords: antioxidant compounds, multiple emulsions, cheese.

Introduction
Polyphenolic compounds are secondary metabolites in plants. These bioactive compounds are considered an integral part of the human diet and animal found in almost all the plant materials (1). Nowadays the polyphenols researches are done about the applications in functional foods. The polyphenols give benefit effects than contribute to human health. However, its effectiveness depends on the preservation, stability, bioavailability and bioactivity of active ingredients. However, the unpalatable phenolic compounds most limit its uses. One option is decrease the unpalatable taste use encapsulation for replace use of free compounds (2). Encapsulation is a process whereby certain substances bioactive (vitamins, micro-organisms, etc.) are introduced into an array or system wall in order to prevent their loss, protecting them from the reaction withother compounds present in the food or to prevent serious reactions of oxidation due to the light or the oxygen (3). There are some techniques that exist for encapsulation of bioactive compounds, one of them being a multiple emulsions.Emulsions are dispersed systems of two or more liquids are insoluble, such as water and oil. A double emulsion or multiple emulsionsare an emulsion of emulsions. In the structure forthis emulsion coexistence of morphologies of water in oil (W/O) and oil in water (O/W).There are two main types of double emulsion: water in oil in water (oil in water in oil

(O/W/O) and W/O/W.) The size of an emulsion is a range of 0.1 to 100 µm, which is imperceptible sensorial to be added to food (4).On the other hand, the cheese has become an important ingredient in the traditional Mexican food and is used in various dishes so that in recent years, the available variety of cheeses in Mexico has grown considerably encountering present increasingly cheeses matured as the type Chihuahua cheese. The objective of this work was to get formulations of multiple emulsions for the protection of antioxidant compounds with in vitro tests, simulating the conditions of a cheese type Chihuahua process.

Methodology

Formulation of emulsions: emulsions were carried out by the methodology of the two phases, which consists of a primary emulsion (W_1/O) and through homogenization, emulsions part of this primary emulsion in another aqueous solution (W_2) with the purpose of obtaining a multiple emulsion (W_1/O/W_2) [5].Seven formulations of emulsions with binary and ternary mixtures of various materials proposed for this study were: rubber of mesquite (GM), gum Arabic (GA), serum (SL) milk and maltodextrin 10 (MD).In the internal aqueous phase W1, one antioxidant control was encapsulated (pH 4.5) for a selection process with canola oil as oily phase (O). Formulations to stabilize the external aqueous phase (W_2) were: $MD_{60\%}GA_{40\%}$; $MD_{60\%}GM_{40\%}$; $GA_{40\%}SL_{60\%}$; $GM_{40\%}SL_{60\%}$; $GA_{17\%}GM_{66\%}MD_{17\%}$; $GM_{17\%}SL_{17\%}MD_{66\%}$and $GM_{17\%}SL_{66\%}$ $MD_{17\%}$.The selection process was only a formulation and it was chosen to encapsulate with a solution of Nutriphy White Grape 100 Grape polyphenols (CHR. Hansen Mexico S.A. de C.V., Mexico, D.F.).

Particle size: Pictures of the emulsion was made using an optical microscope Olympus BX (Olympus Optical Co.) (Ltd, Tokyo, Japan), a software and scanner images Image-Pro Plus (version 7.0, Media Cybernetics, Inc., Silver Springs, MD, USA) were used [6].

Emulsion stability was determined up for 45 days by the quotient of the division of the internal with the external diameter obtaining a dimensionless measure that indicates the stability of the emulsion according to the scale: 0-0.34 stable, unstable 0.49-0.35, 0.5-1 unstable [7]. Due the inner diameter increases in size resembling the outer diameter of the emulsion, it gives an indication of instability of the emulsion, which means that you smaller internal diameter on the external, the emulsion will be more stable.

Conditions of simulation of process cheese type Chihuahua with emulsions: in the preparation of the cheese type Chihuahua, the maximum temperature reached is 40°C for one hour in the process of "chedarizacion". Another feature of this cheese is that according to established standards, its pH must be in a range of 5-5.5 with a minimum of seven days maturation.

Phenols: was performed using an adaptation of the methodology of Folin-Ciocalteau [9]. This test as well as the antioxidant needed in a dark environment to prevent loss of bioactive compounds by oxidation in the light. Folin-Ciocalteu reagent, distilled water and sodium carbonate 20% were added to the sample that was left to react a lapse of two h, the time elapsed, samples were read in a spectrum (UV-visible spectrophotometer CARY 100 Bio) to 760 nm and concentrations of phenols were calculated with a curve pattern of gallic acid.

Antioxidant activity: antioxidant activity, was determined by the chromogenic compounds; Acid 2, 2´-azino-bis (3-etilbenzotiazolin) - 6 - sulfonic (ABTS), 1, 1-diphenyl-2-picrilhidrazil (DPPH) [10]. For the antioxidant activity by DPPH added 0.3 mL of sample to 2.7 mL of DPPH (prepared in methanol 80%) and allowed to react for two hours, and then the samples were read in a spectrophotometer at 515 nm by calculating the percentage inhibition results. The ABTS was combined with potassium persulfate to generate the radical ABTS + which antioxidant activity was determined taking two readings per sample per minute one and six to 740 nm wavelength. Phenols and antioxidant activity: It was carried out through an adaptation to the methodology of Folin - Ciocalteau [7]. Antioxidant activity: was determined by the chromogenic compounds; Acid 2, 2´-azino-bis (3-etilbenzotiazolin) - 6 - sulfonic (ABTS), 1, 1-diphenyl-2-picrilhidrazil (DPPH) [8] [9].

Results and discussion

Due to the content of the antioxidant (grape polyphenols) encapsulated, the pH of seven formulations were acid with a range of 4.09±0.02 to 5.46±0.01, for this reason simulation of pH was not required (pH 5-5.5). For its stability, formulations $GM_{40\%}SL_{60\%}$, $GA_{17\%}GM_{66\%}MD_{17\%}$ and $MD_{66\%}SL_{17\%}GM_{17\%}$ were selected to be monitored for 30 days by determining the ratio of the inner and outer diameter (Figure 1) finding the three formulations in the range of "stable" with 0.110, 0.147 and GA 0.150 values respectively. Other formulations were discarded by instability in one period of less than 24 h. Considering the above, the encapsulation of a solution of grape polyphenols was in concentration of 0.051 g/mL, with the purpose of obtain 2.16 mg of grape polyphenols per g of cheese, taking as a reference the recommended daily intake (RDI) of the product (65 mg per day) per cheese portion (30 g, IDR).

This encapsulation was carry out in formulations $GM_{40\%}SL_{60\%}$, $MD_{66\%}SL_{17\%}GM_{17\%}$and $GA_{17\%}GM_{66\%}MD_{17\%}$, the first two formulations had instability in less than two hours, while the $GA_{17\%}GM_{66\%}MD_{17\%}$formulation was useful to encapsulate the solution of grape polyphenols, remaining stable for 45 days with a ratio of diameters of 0.139 after 45 days and a pH of 4.36±0.01, by such a situation continued to monitor antioxidant activity and total phenols at this formulation.

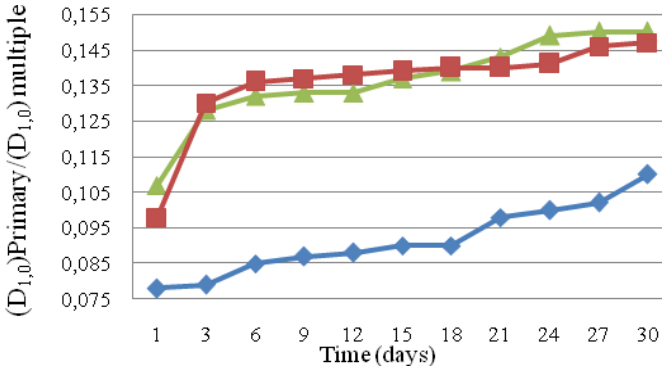

Figure 1. Ratio of diameters, internal and external: (♦) $GM_{40\%}SL_{60\%}$, (▲) $GM_{17\%}SL_{17\%}MD_{66\%}$ and (■) $GM_{66\%}MD_{17\%}GA_{17\%}$.

Figure 2 shows that there were significant differences ($P<0.05$) between the content of phenols between encapsulated and free grape polyphenols, exist a decrease in both, however, the encapsulated grape polyphenols solution demonstrates that the $GA_{17\%}GM_{66\%}MD_{17\%}$emulsion, preserved in a 98.8% the content of phenols which gives an indicative that the encapsulation is protecting the bioactive compounds of grape polyphenols to be subjected to a heat treatmentas a barrier against temperature. This phenomenon did not occurs in free grape polyphenols, which significantly decreased their total phenols content to find more exposed to external factors.

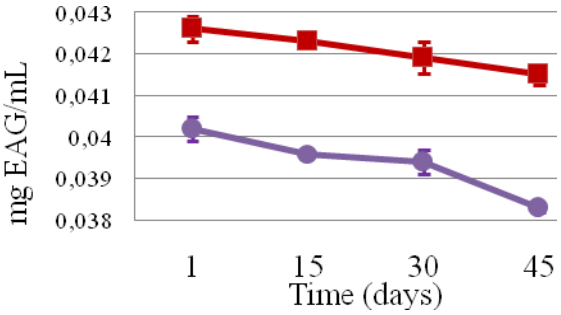

Figure 2. Content of phenols in (●) solution of grape polyphenols encapsulate (■) solution of grape polyphenols encapsulated with $W_1/O/W_2GM_{66\%}MD_{17\%}GA_{17\%}$.

The differences were also observed for the antioxidant activity in Figures 3 (DPPH) and 4

The differences were also observed for the antioxidant activity in Figures 3 (DPPH) and 4 (ABTS), obtaining for DPPH a percentage of 92% inhibition in grape polyphenols encapsulated versus 50.7% in free grape polyphenols after 45 days. The antioxidant activity by ABTS showed the same tendency to get is the highest percentage in grape polyphenols encapsulated (87%) with respect free grape polyphenols (84%). With the radical ABTS + can be measured the antioxidant compounds of hydrophilic and lipophilic nature, while the DPPH single can dissolve in organic compounds.The ABTS + radical has the advantage that its spectrum presents maximum absorbance at 414, 654, 754 and 815 nm in the middle an alcoholic, while the DPPH presents a peak absorbance at 515 nm [10]. However, on two ob both techniques, sample that presents greater antioxidant activity suggests be best to counteract the free radicals, which are involved in the cause of diseases cause oxidative damage in humans and for this reason the antioxidants those who neutralize the action of free radicals, play a critical role in disease prevention. That is why it is observed that solution of grape polyphenols encapsulated is a better option to preserve their bioactive compounds and functional in greater proportion and better conditions versus free grape polyphenols.Content of phenols and antioxidant activity could be seen positive in grape polyphenols encapsulated by formulating GM66%MD17%GA17% showing that this was useful for the preservation of the bioactive compounds of the encapsulated solution.

On the ternary mixture used in the formulation GM66%MD17%GA17% has been reported, that the mesquite gum is potentially a better barrier against the oxidation of lipids compared to maltodextrin and gum Arabic, however, a mixture of gum Arabic (17%), mesquite gum (66%) and maltodextrin (17%), provides excellent protection against oxidation of lipids compared to any other biopolymer using alone [5]. Other authors indicate that they have used a blend of maltodextrin (60%) and gum Arabic (40%) for the encapsulation of polyphenols from grape seeds by drying spray, obtaining an efficiency of the 88.84%, finding that the polyphenols had no changes during the drying process [2]. Gum Arabic is studied in greater detail, given its industrial importance since it has met the needs of industrial rubber for many years. However, has a chemical structure comparable to the mesquite gum, from which, in Mexico use in foods was approved by the Ministry of health in 1996. In addition, Mesquite gum, presents properties as a stabilizer, due to the possibility of forming concentrated solutions that (50% w/w), without increasing the viscosity of the solution.

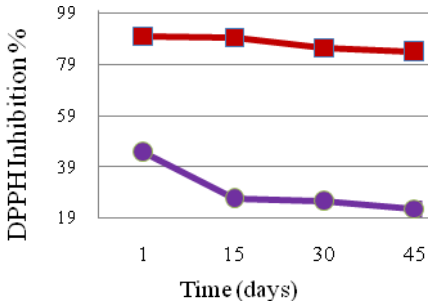

Figure 3. Antioxidant activity on DPPH of: (●) solution of grape polyphenols. (■) solution of grape polyphenols encapsulated with O/W1/W2% GM66% MD17% GA17.

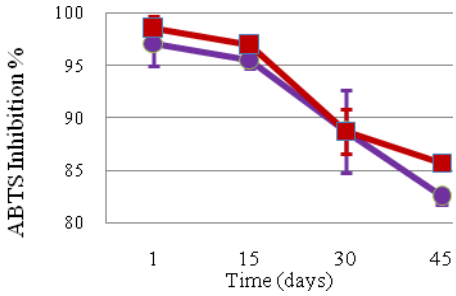

Figure 4. Antioxidant activity of ABTS: (●) solution of grape polyphenols. (■) solution of grape polyphenols encapsulated with O/W1/W2% GM66% MD17% GA17.

Conclusions

The formulation $GM_{66\%}MD_{17\%}GA_{17\%}$was the best option in order to protect bioactive compounds on simulated conditions during cheese processing. Significant differences were observed in phenolic content, antioxidant capacity (DPPH and ABTS) between encapsulated and free phenols in all cases the best treatment was the antioxidant protected with tertiary mixture preserving its 98.8% in concentration of phenols, antioxidant capacity 92% of DPPH and 87% of ABTS. The results showed that the implementation of multiple emulsions ($GM_{66\%}MD_{17\%}GA_{17\%}$) protected the antioxidant compounds in a simulation process of type Chihuahua cheese.

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Effect Of Extrusion Variables In Physical Characteristics And Antioxidant Activity Of Soybean-Corn Extrudate

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Introduction

Extrusion cooking is an adequate technology to obtain a series of products by changing the process variables, thus obtaining a wide variety of products such as pet foods, processed baby foods, snacks, breakfast cereals, etc. This extrusion process provides shorter cooking times and can be used to produce a variety of changes, the most significant are in starch and protein directly affecting the texture, appearance and degree of expansion of the final product (4). Moreover it has been reported that the extrusion process has the advantage of reducing non-nutritional compounds as inhibitors of trypsin (10), however it is desirable to retain some other compounds such as phenolic compounds which have antioxidant activity (8). In this study, we assessed the physical characterization and antioxidant activity of extruded soy.

Methods and materials

Extrusion

A central composite design was applied by using Design Expert 8.0.7.1 software. The independent variables were moisture content (18-26%), temperature (110-180°C) and proportions of soybean-corn mixtures (0-100%). Materials were processed in a single-screw extruder, 60 mm diameter and 950 mm length of screw. The compression ratio of the screw was 2.5:1. The barrels contained three controlled heating and cooling zones. The diameter of the hole in the die was 12.5 mm. Three thermocouples were used to monitor the barrel temperatures and one extra thermocouple was inserted in the die plate. The materials were fed to the extruder at 16 rpm, controlled with a Baldor Electric Co. Cat. GPP 7454 MDF (FT Smith, AR, USA).

Analysis of the Extrudates

Water Absorption Index (WAI) and Water Solubility Index (WSI)

They were determined as described by Anderson *et al.*, 1982. We used the following formulas:

WAI = *Gel weight / Sample weight,*

WSI = *(Weight of soluble/ Sample weight) x 100*

Expansion Index (EI)

It was determined according to the method reported by Jin *et al.*, (1994). We used the following formula:

EI = *Sample diameter / diameter of the extruder die.*

Color

The color was analyzed using a Sphere Spectrophotometer (Color Mate HDS Milton Roy) using the illuminant D65, the evaluation of color was made using the unextruded mixtures of soybean-corn (20.3-79.7, 50-50 y 79.73-20.27%) as standard samples. The CIELab values L, a* and b* were measured and the numerical total color difference (ΔE) was calculated by:

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$$

Free radical inhibitory capacity ($ABTS^{\cdot}+$)

The capacity for inhibition of radicals was measured by ABTS radical catión decolorization assay using the method of Scaatezzini *et al.*, 2006. The percentage was calculated by:

$$\textbf{\textit{\% de inhibition:}} \quad \frac{(Abs\ sample\ t_0 - Abs\ sample\ t_5)}{Abs\ sample\ t_0 \left(\frac{Abs\ solvent\ t_0 - Abs\ solvent\ t_5}{Abs\ solvent\ t_0} \right)} X\ 100$$

Statistical Analysis

The response variables were evaluated by response surface methodology using the Design Expert 8.0.7.1 software (Stat-Ease, Inc., Minneapolis, MN, USA).

Results and discussion

Characterization of extrudates

Expansion Index

In this analysis, a significant model with a determination coefficient $R^2 = 0.7371$ and $P = 0.0456$ was obtained: $\mathbf{EI} = -68.8676 + 6.3359\mathbf{H} + 0.3723\mathbf{T} + 0.3589\mathbf{C} - 0.032\mathbf{HT} - 0.0335\mathbf{H} - 0.149\mathbf{H}^2 - 1.1237 \times 10^{-4}\mathbf{T}^2 + 7.49 \times 10^{-4}\mathbf{HT} + 7.738 \times 10^{-4}\mathbf{HC}$, where **H** is moisture, **T** is temperature and **C** is the percentage of soybean in the mixture.

The temperature was the variable with the lowest effect on the EI. Figure 1(a) shows that when the soybean proportion was low of and moisture content was medium (around 22%), the values of EI were high. When the moisture content increased up to 26% and the proportion of soybean decreased, the result was a decrease in EI (Figure 1a). At 145°C the lowest EI values were shown at the same conditions (Figure 1b), with the highest proportion of soybean, the EI values increased. The same behavior was shown at 180°C, however, at this temperature but with a low soybean content and 22% of moisture, the values of EI decreased. This may be due to the fact that an increase in temperature and moisture can produce structural changes in starch and protein; this can result in less expansion of the material (7). On the other hand, when the moisture is high and temperature low, the friction of the material is reduced when in contact with the screw within the barrel, causing a lower gelatinization of the material and reducing the expansion of the extrudate (2).

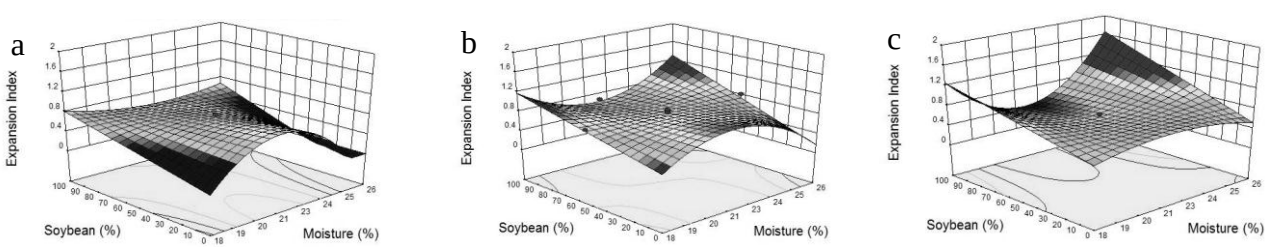

Figure 1. Influence of proportion of soybean and moisture content on the EI at temperatures of 110°C (a), 145°C (b) and 180°C (c).

Water Absorption Index (WAI)

Adjusted model: $\mathbf{WAI} = +47.1183 - 2.0554\mathbf{H} - 0.3046\mathbf{T} - 0.01175\mathbf{C} + 6.2882 \times 10^{-3}\mathbf{HT} + 2.139 \times 10^{-3}\mathbf{HC} - 3.5355 \times 10^{-4}\mathbf{TC} + 0.02517\mathbf{H}^2 + 6.675 \times 10^{-4}\mathbf{T}^2 + 1.4508 \times 10^{-4}\mathbf{C}^2$

$R^2 = 0.7588$ y $P = 0.0321$.

In the graphics of response surface (Figure 2a), it can be seen that regardless of the proportion of soybean, when the temperatura and moisture were high, the values of WAI increased. It was also observed that with low proportion of soybean, the WAI is higher even when the moisture decreased, while the low values of WAI were observed with lower temperatures and high moisture contents. This could be due to a reduced mass elasticity, thereby reducing the specific mechanic energy and therefore the gelatinization (9).

Moreover when the soybean proportion was 50% (Figure 2b) the lower values were observed around 145°C and 22% of moisture. When the proportion of soybean was 100%, with the highest temperatura and moisture content, the values of WAI were also the highest (Figure 2c). The WAI allows us to assess the degree of gelatinization of a sample (9). The high values of WAI at higher moisture contents and temperatures can be associated with a lower viscosity. In this case the starch gelatinization is favored by the temperature; we can say that the greater the gelatinization, the higher the water absorption capacity (13). On the other hand when the percentage of soybean was greater and the temperature was high, the values of WAI were low, and this may be due to a loss of protein solubility by denaturation (3).

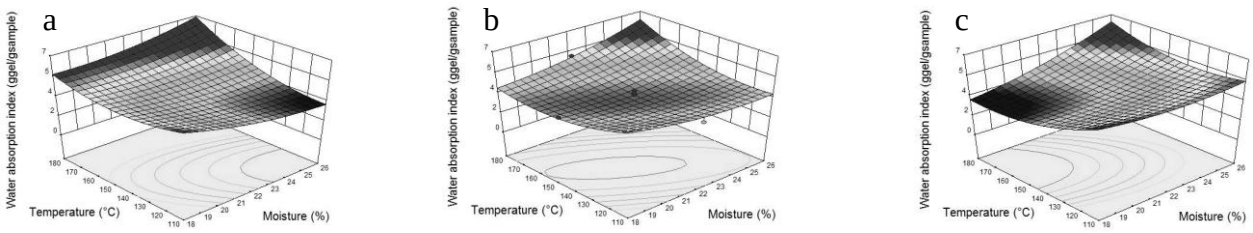

Figure 2. Influence of temperature and moisture of on the WAI at 0% (a), 50% (b) y 100% (c) of soybean content in the mixture.

Water Solubility Index

$$\mathbf{WSI} = -215.5714 + 12.1466\mathbf{H} + 1.0492\mathbf{T} + 0.7612\mathbf{C} - 0.03316\mathbf{HT} - 0.03955\mathbf{HC} + 1.4162X10^{-3}\mathbf{TC} - 0.1363\mathbf{H}^2 - 9.3542X10^{-4}\mathbf{T}^2 - 3.1236X10^{-4}\mathbf{C}^2$$

R^2= 0.7622 y P = 0.0302.

The major porcentaje of contribution in this evaluation corresponded the temperature with 32% and the interaction between soybean and temperaure are the factors with less contribution (2.38%). In this analysis the porcentaje of moisture was taken as a constant because it was not significant. It is observed that the higher values of WSI were obtained with moisture contents around 18% (Figure 3a), high temperature and high soybean proportion, and the lowest values of WSI were observed with low temperatures and soybean proportions; the same behavior was found when the moisture was 22% (Figure 3b). However, when the moisture increased until 26% (Figure 3c), the proportion of soybean influenced this response because with the lower proportion of soybeans, the higher values of WSI were observed and at the higher temperature and higher percentage of soybean, the lower values of WSI were obtained. One possible explanation for this behavior is that the protein tends to denature reducing its solubility (3). Stojceska *et al.,* (2010) found in extrudates made of rice, soybean, potato starch and corn starch that the WSI increased with high temperatures and decreased with low temperatures.

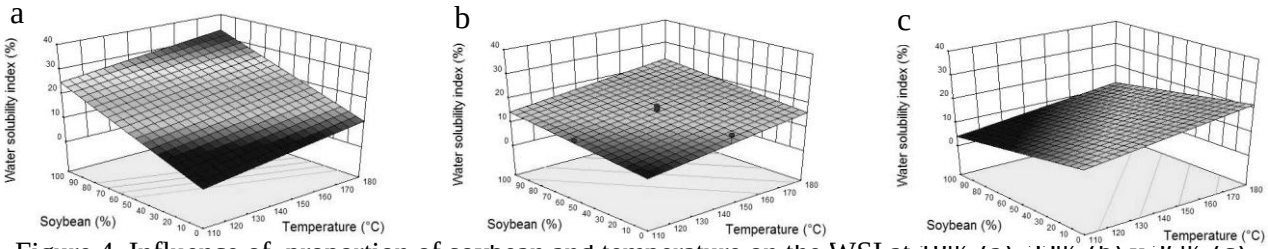

Figure 4. Influence of proportion of soybean and temperature on the WSI at 18% (a), 22% (b) y 26% (c) of moisture.

Color

$$\mathbf{C\ (\Delta E)} = -29.238 + 0.28446\mathbf{T} + 0.15026\mathbf{C}$$

R^2 =0.8150 y P = 0.0001

The data of the color evaluation showed that the difference in color increased at high temperatures of extrusion and high proportions of soybeans, having a high browning effect at the extreme conditions

(Figure 5), while at lower temperature and lower proportion of soybeans, the color tended to be lighter regardless of the moisture content. These changes can be due to Maillard reactions that occur between reducing sugars and free amino groups of soybean protein (4).

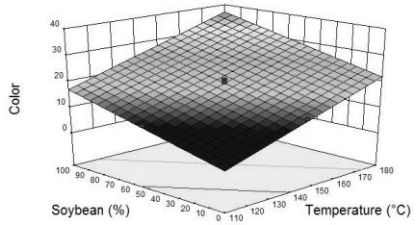

Figure 5. Influence of the proportion of soybean and temperature on the color at 22% moisture content.

Free radical inhibitory capacity (ABTS$^{\cdot}$+)

% of inhibition = + 371.1051 - 20.9396**H** - 1.9609 **T** - 1.0803**C** + 0.0463**HC** + 0.4589**H**^{2} + 7.1738X10^{-3}**T**^{2} + 5.3871X10^{-3}**C**^{2}

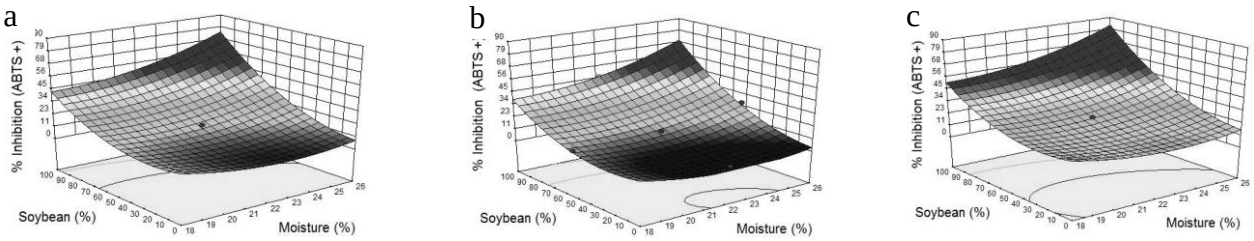

Figure 6. Influence of the proportion of soybeans and moisture on the ABTS activity at 110°C (a), 145°C (b) 180°C (c).

Free radical inhibitory capacity is heavily influenced by the moisture and proportion of soybeans. When the level of these variables were high, the ability for inhibition was higher, when the temperature reached 180°C, an increase in the inhibition values was also observed (Figure 6). This can also be due to Maillard reactions. Some research has reported that dark color is produced as a consequence of thermal treatment and this effect produced an increase in its antioxidant activity (6).

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Study of Alternative Conditions for Polyphenolics Extraction from Black Common Bean (*Phaseolus vulgaris* L.) Varieties.

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Abstract

Black common beans (*Phaseolus vulgaris* L.) contain phytochemicals, such as phenolic compounds, which can provide health benefits to consumers. In order to obtain extracts enriched in polyphenolics, different methodologies have been developed; most of them include toxic solvents as acetone and methanol. The objectives of this research were to study alternative conditions for the extraction of polyphenolic from the testa of "Jamapa" black bean using different ethanol concentrations at three different temperatures, and compare to the polyphenolic concentration in "Queretaro" variety extracts. Extractions using aqueous ethanol (100%, 80% and 50%) and different temperatures (5, 25 and 35 °C), during 8, 16 and 24 h were applied to lyophilized seed coat of "Jamapa" black beans. A traditional method for polyphenolics extraction (methanolic extraction at room temperature, during 24 h) was carried out for comparison. Conditions yielding the highest polyphenolics were applied to "Queretaro" common bean testa. Proanthocyanidins (vanillin-HCl assay), monomeric anthocyanins (pH differential method) and antiradical capacity (DPPH method) of extracts were evaluated. Methanolic extract from "Jamapa" beans (traditional method) presented 31.88 ± 1.56 mg eq. (+)-catechin/g seed coat, and 2.90 ± 0.23 mg monomeric anthocyanins/g seed coat. The highest amount of polyphenolics were extracted from "Jamapa" beans using 50% ethanol at 25 °C degrees during 16 h (103.86 ± 7.69 mg eq. (+)-catechin/g seed coat, and 5.62 ± 0.32 mg monomeric anthocyanins /g seed coat). Under the same conditions, only 78.86 ± 5.7 mg eq. (+)-catechin/g seed coat, but double (8.56 ± 0.3 mg/g seed coat) of monomeric anthocyanins were extracted and quantified from "Queretaro" beans testa. Antirradical capacities of extracts from both bean varieties were not statistically different. These findings suggest that it is possible to eliminate the use of highly toxic solvents such as methanol and acetone to obtain rich polyphenolic extracts from black common beans with antiradical capacity, which could be used as food additives.

Keywords: Polyphenolic extraction, *Phaseolus vulgaris*, black common beans, polyphenolic quantification.

Introduction

Common beans (*Phaseolus vulgaris* L.) are considered a staple food in Mexico and Latin-American countries. Besides being a good source of protein, carbohydrate, vitamins, minerals and fiber (1), beans contain phytochemicals, which have been demonstrated to have diverse biological activities (2). Phenolic compounds are one of the phytochemical classes present in common beans; they are mainly in the testa, and are partially responsible for the color. Different kinds of flavonoids had been identified in common beans, including flavonol glycosides and condensed tannins (proanthocyanidins); in black varieties, anthocyanins have been reported too (3-7); all those phytochemicals are of interest because of the potential health benefits they can provide to consumers. Presence of condensed tannins and anthocyanins in black bean varieties, make them a potential source for the extraction and use of those compounds as pigments and/or antioxidants in food (3). In order to obtain extracts enriched in polyphenolics, different methodologies have been developed; most of them include toxic solvents as acetone, ethyl acetate, acetic acid and methanol (4, 8-11). The objectives of this research were to study alternative conditions for the

extraction of polyphenolic compounds from the testa of “Jamapa” black bean using different ethanol concentrations at three different temperatures, compare to the polyphenolic concentration in “Queretaro” variety extracts, and determine the antiradical capacity of both extracts.

Materials and methods

Chemicals

Vanillin, (+)-catechin, 1,1-dipheny-l,2-picrylhydrazyl (DPPH), solvents and other general reactive were from Sigma Chemical Co (St. Louis, MO).

Biological material

Common bean (*Phaseolus vulgaris* L.) black varieties, “Jamapa” and “Queretaro” were obtained from local market at Lagos de Moreno, Jalisco, México, in 2008. In the laboratory, the samples were cleaned and stored at 5 °C, protected from light until manual separation of seed coats. The seed coats were lyophilized, powdered and stored in the dark at -20 °C until extraction.

Extraction of polyphenolics from bean seed coats

Independent samples of "Jamapa" bean seed coats were submitted to extractions using different proportions of ethanol:water: 100:0, 80:20 and 50:50; three different temperatures: 5, 25 and 35 °C; during three different extraction times: 8, 16 and 24 h. Briefly, 200mg of lyophilized testa were mixed with 10mL of the corresponding extracting solution. Extraction at 25°C were performed using magnetic stirring during all extraction time; extractions at 5 and 35°C were sonicated during 5 min at the beginning and the end of the extraction time. During all treatment, samples were protected from light. After extraction, the samples were centrifuged at 2500 rpm for 10 min, evaporated and redissolved in 10mL methanol in order to be analyzed. To compare the results with the most commonly reported method of proanthocyanidin extraction; samples of lyophilized seed coats from the same lot of beans were submitted, independently, to methanol (100%) extraction, following the protocol previously described by Cardador-Martínez et al. (8). Conditions yielding the highest polyphenolics were applied to “Queretaro” common bean testa.

Polyphenolics quantification

Condensed tannins (proanthocyanidins) were quantified using the vanillin-HCl assay, according to Deshpande and Cheryan (12), and results were expressed as mg of (+)-catechin equivalents per gram of sample.

Total monomeric anthocyanins were determined by the pH-differential spectrophotometric method, at pH 1.0 and pH 4.5, described by Giusti and Wrolstad (13).

Antiradical capacity evaluation

The scavenging activity of the extracts on DPPH radical was measured according to the method described by Brand-Williams et al. (14). The antiradical capacity was calculated according to the equation of Burda and Oleszek (15):

$$ARC = 100 \times (1 - \text{absorbance of sample} / \text{absorbance of control}) \quad (1)$$

Treatment of data

All tests were performed in triplicate. The statistical analysis was made for a completely random design with three repetitions per treatments; data obtained were subjected to variance analysis and means comparison by the Tukey test ($p \leq 0.05$).

Results and discussion

Table 1 shows the results of condensed tannins and anthocyanins quantified in extracts of “Jamapa” bean seed coats obtained at different conditions; and compared to data from an extract obtained with the traditional method reported elsewhere (8).

Methanolic extract from “Jamapa” bean seed coat (traditional method) presented 31.88 ± 1.56 mg eq. (+)-catechin/g seed coat, and 2.90 ± 0.23 mg monomeric anthocyanins/g seed coat.

Extraction with 100% ethanol yielded a very low concentration of both phenolic compounds analyzed at 24 h; extractions at 8 and 16 h were not developed.

For extractions with ethanol:water, 80:20, at all times (Table 1), it could be observed that room temperature (25°C) was the best temperature for extraction and conservation of both, anthocyanins and tannins. At all extraction times, refrigeration temperature (5°C) extracted the lowest quantity of polyphenolics. Conditions that extracted the highest amounts of polyphenolics (82.64 ± 7.38 mg eq. (+)-catechin/g seed coat, and 5.4 ± 0.37 mg monomeric anthocyanins/g seed coat) with ethanol:water 80:20, were 25°C during 24h; those concentrations corresponded to approximately 2.6 and 1.9 times the concentrations of condensed tannins and anthocyanins, respectively, extracted using the traditional method. Cardador-Martínez et al. (8) reported 36.3 mg eq. (+)-catechin/g seed coat, extracted from a pink bean variety (“Flor de Mayo FM-38”) using ethanol:water, 80:20 for extraction, during 24 h at 30 °C; that is a lower concentration compared to data obtained in this research. The difference between the results could be due to the natural concentration of phenolics in the different varieties of beans, since it is well known that common bean varieties with darker testa color contain higher concentration of polyphenolics (5-6, 16).

In general, extractions with 50% ethanol yielded higher proanthocyanidins (from 82.35 to 103.86 mg eq. (+)-cat/g testa, during 8 and 16h, respectively, at 25 °C) and monomeric anthocyanins (from 4.27 to 5.62 mg monomeric anthocyanins/g testa, with extraction times of 8h at 5 °C, and 16h at 25 °C, respectively). The highest amount of polyphenolics were extracted at 25 °C degrees during 16 hours; 103 mg eq. (+)-catechin/g seed coat, and 5.62 mg monomeric anthocyanins /g seed coat; that represents 3.26 and 1.95 times the concentrations of condensed tannins and anthocyanins, respectively, extracted using the traditional method. Similar quantities of proanthocyanidins were determined under other conditions (24 h at 35 °C; 8h at 35°C), but with statistically different content of anthocyanidins. The results published here are higher than data reported by Iniestra-González et al. (17) in other black bean varieties; they determined 47.01± 11.52 mg (+)-cat/g testa in “Negro Durango”, 72.6 ± 2.30 mg (+)-cat/g testa in “Negro Sahuatoba”, and 65.3 1.52 mg (+)-cat/g testa in “Negro Altiplano”

Table 1. Condensed tannins and monomeric anthocyanins quantified in extracts from "Jamapa" bean seed coats, obtained with different conditions and extraction times. [A]

Extracting solution	Time (h)	Temperature (ºC)	Condensed tannins (mg/g) [B]	Monomeric anthocyanins (mg/g) [C]
Methanol [D]	24	25	31.88 ± 1.56^{i}	2.90 ± 0.23^{g}
Ethanol:Water 100:0	24	35	0.96 ± 0.47^{k}	0.23 ± 0.04^{i,j}
		25	2.10 ± 0.55^{j}	0.37 ± 0.09^{i}
		5	1.69 ± 0.40^{k,j}	0.08 ± 0.07^{j}
Ethanol:Water 80:20	24	35	56.88 ± 10.05^{f,g}	3.94 ± 0.51^{e}
		25	82.64 ± 7.38^{c}	5.40 ± 0.37^{b}
		5	25.96 ± 4.89^{i}	2.14 ± 0.54^{h}
	16	35	54.41 ± 8.99^{g,h}	3.24 ± 0.53^{f}
		25	63.03 ± 6.22^{e,f}	4.90 ± 0.45^{c}
		5	48.78 ± 7.1^{h}	2.43 ± 0.61^{g,h}
	8	35	65.68 ± 5.23^{d,e}	4.01 ± 0.16^{e}
		25	70.80 ± 5.44^{d}	4.83 ± 0.50^{c}
		5	32.29 ± 6.78^{i}	2.54 ± 0.33^{g,h}
Ethanol:Water 50:50	24	35	103.21 ± 8.12^{a}	4.97 ± 0.40^{c}
		25	96.20 ± 7.13^{b}	4.96 ± 0.51^{c}
		5	100.10 ± 4.41^{a,b}	5.06 ± 0.36^{bc}
	16	35	86.13 ± 6.33^{c}	4.34 ± 0.27^{d}
		25	103.86 ± 7.69^{a}	5.62 ± 0.32^{a}
		5	88.77 ± 7.94^{c}	4.69 ± 0.53^{c,d}
	8	35	102. 23 ± 10.07^{a,b}	4.72 ± 0.55^{c,d}
		25	82.35 ± 9.23^{c}	5.50 ± 0.60^{a,b}
		5	85.18 ± 8.72^{c}	4.27 ± 0.42^{d}

[A] Results are the average of three independent experiments ± SD.
[B] mg/g = mg of (+)-catechin equivalents per gram of dry testa.
[C] mg/g = mg of cyanidin 3-glucoside equivalents per gram of dry testa.
[D] Extract obtained according to Cardador-Martínez et al. (8), using 100% methanol.
[a,b] Different letters on the same column express significant difference determined by Tukey´s test ($\alpha = 0.05$).

Conditions yielding the highest amount of polyphenolics in "Jamapa" beans (50% ethanol, 25 °C, 16h) were applied to "Queretaro" black beans testa. Condensed tannins extracted from "Queretaro" variety (78.86 ± 5.71 mg (+)-cat/g testa) represented only the 76% of the tannins extracted from "Jamapa" beans seed coats; while monomeric anthocyanins (8.56 ± 0.32 mg/g testa) represented 156% of the anthocyanins quantified in "Jamapa" bean seed coats (Figure 1); the results shows that concentration of polyphenolics in bean varieties is different, even in varieties of similar characteristics (5, 17).

The antiradical capacity of common beans has been directly related to condensed tannins concentration (18-19); the extract of "Queretaro" variety presented a lower content of total polyphenolics (condensed tannins + monomeric anthocyanins, Figure 1), however, its antiradical capacity (86.54 ± 5.89 %) was not statistically different from "Jamapa" extract (88.21 ± 2.53 %). It is important to highlight that "Queretaro" variety presented a higher amount of anthocyanins, which has been recognized as powerful antioxidants (20). Our results show that antiradical capacity does not rely on the total amount of polyphenolics, but on the kind of them.

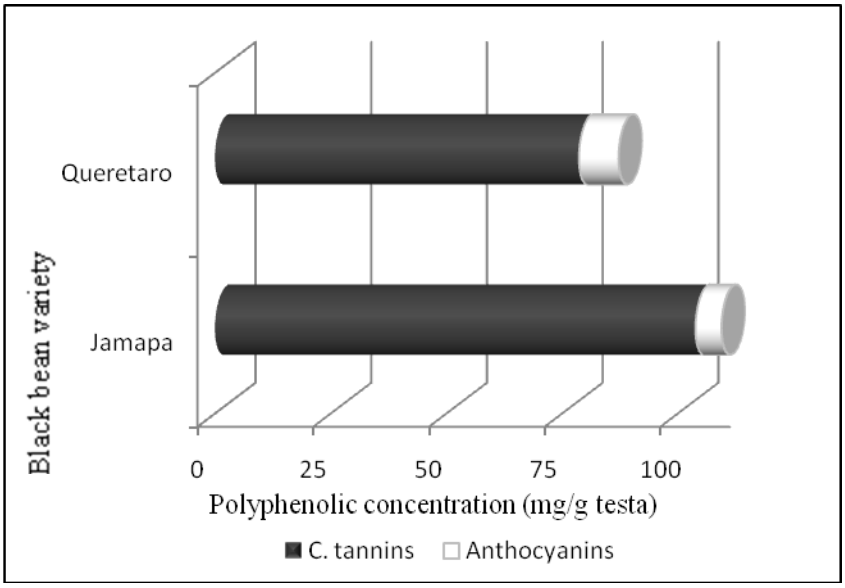

Figure 1. Condensed tannins and monomeric anthocyanins content in common bean "Jamapa" and "Queretaro" black varieties. Results are the average of three independent experiments.

Conclusions

These findings suggest that it is possible to eliminate the use of highly toxic solvents such as methanol and acetone to obtain rich polyphenolic extracts from black common bean varieties. The increase in the polarity of extraction solution (50% ethanol in water) resulted in a higher yielding of condensed tannins and monomeric anthocyanins. The concentration of each kind of polyphenolics relies on the bean variety. Extracts from black bean varieties presented high antiradical capacity; that is why the use of extraction conditions determined here can be suggested in order to obtain extracts which could be used as food additives.

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Study Of The Physicochemical Composition, Antioxidant Activity And Phenolic Content Of Edible Brown Macroalgae Macrocistys Piryfera

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Introduction

Seaweeds are frequently consumed in Asia countries, especially China, Japan and Korea; generally, in Europe and North America seaweeds are mostly used for animal nutrition or for extraction of hydrocolloids such as alginate principally (1, 2). From a nutritional point of view, marine algae are rich in non starch polysaccharides, dietary fiber (DF), protein and minerals. Moreover, seaweeds are an excellent source of bioactive substances like sulphated-polysaccharides and polyphenols with biological activities, such as antioxidant, anticoagulant, antiproliferative, antitumoral and anti- inflammatory (3, 4, 5). Its activity is due that the macroalgae are exposed to a combination of light and oxygen that leads to the formation of free radicals and other strong oxidizing agents, but the absence of oxidative damage in their structural components and their stability to oxidation during storage interfere they have good antioxidant defense system (6,7).

The compounds responsible of the antioxidant activity, in seaweed, include carotenes, vitamin E o α-tocopherol and chlorophylls and its derivatives within the lipidic fraction and polyphenols, vitamin C or and ascorbic acid (8). These properties increase the shelf-life of foods and are as well of interest to biologists and clinicians, due that they may help to protect human body against damage by reactive oxygen species (9). The reactive oxygen species include a wide variety of compounds as the superoxide anion-radical, hydroxyl radicals (O_2^- and OH^-) and non-radicals oxygen derivatives (H_2O_2 and $1O^2$). These molecules are unstable and highly reactive; circulate freely in the body with access to all tissues and organs. They can damage cells by chain reactions, such as lipid per oxidation or formation of DNA adducts that could cause cancer-promoting mutations or cell death (10). Chemical properties and biological behavior of seaweeds have been poorly investigated. Also, antioxidant activities of marine seaweeds have been studied mostly in non-polar extracts whereas those in aqueous ones have been rarely investigated [9]. Since the total antioxidant capacity of seaweeds could be due to both hydrophilic and hydrophobic principals, it is then interesting to determine antioxidant activities of them in hot-water extracts. The aim of the present investigation was to study the nutritional value of M. *Piryfera* specie, which represents natural resources, also of its aqueous extracts with potential economic value for use in human and animal nutrition.

Materials and methods

2.0. Raw material

The seaweed (*Macrocistys Piryfera*) used for this study was donated by Coyotefoods Biopolymer and Biotechnology Co., Saltillo, Coahuila. These dried seaweed samples were shredded to small pieces by a wiring blender to recuperate about 500 g of small dried seaweed (less than 1.0 mm particle size) and kept in airtight plastic bottles at room temperature until analysis.

2.1 Physicochemical properties

Water retention capacity in seaweed samples were assessed following the experimental protocol used in a European collaborative study. In addition oil retention capacity was measured.

2.1.1. Water and oil retention capacity (WRC)

Thirty millilitres of distilled water, containing 0.02% sodium azide was added to 500 mg of dry powdered samples in a 50 mL centrifuge tube. The sample was stirred and left at room temperature for overnight. After centrifugation at 3,000×g for 20 min, the supernatant was discarded, the residue was weighed and WRC calculated as g water/g of dry sample. The same protocol as above was followed, but using commercial virgin olive oil (0.4°) instead of water. ORC was expressed as g olive oil retained/g of dry sample.

2.2. Ashes and minerals content.

Seaweed samples were incinerated in a digitally controlled furnace. Temperature was gradually increased to 800°C and then maintained for 4 h. Ashes were quantified gravimetrically. The mineral content was determined by inductively coupled plasma atomicemission spectrometry (ICP-AES). Samples (200 mg) were digested with HNO_3 (5 ml) and H_2O_2 (3 ml) in closed vessels (XF100, Anton Paar) using a Multiwave 3000 microwave (Anton Paar). For the digestion, the microwave power was increased from 0 to 1150 W during 9 min, and was then maintained at 1150 W during 10 min. After cooled to room temperature, the final volume of the samples was adjusted to 100 ml, and they were analyzed by ICP-AES in a Thermo Scientific iCAP 6300 equipment.

2.3. Chemical analyses

The following analyses were carried out. Nitrogen content was determined using an Elemental analysis. A conversion factor of 6.25 was used to calculate protein content. The content of total fiber was enzymatically determined (a-amylase, protease, amyloglucosidase; BIOQUANT Total Dietary Fiber, Merck)

2.4 Amino acid analysis

Amino acids were analyzed by high-performance liquid chromatography, HPLC, (Waters Instruments) using a fluorescence detector (Waters model 470) and derivatization with carbamate in reversed-phase with an AccQ-Tag column (Nova Pak C18) at 37°C. In each sample, the amino acid content was measured triplicate, and values were expressed as mg/g of protein.

2.5 Extract preparation

Methanolic extracts were produced by mixing 1 g of ground seaweed (fine powder) with 20 mL of methanol solution (0, 70, 85 and 100 % v/v), under conditions of 70°C for 30 min.

2.6 Total phenolic content

The total phenolic content (TPC) in the seaweed extracts was determined. Briefly, an aliquot of 5 µl of each extract was mixed with 60 µl 15% sodium carbonate solution in 96-well microplate and allowed to react for 5 min at room temperature. Then 15 µl of Folin-Ciocalteu phenol reagent and 200 µl of distilled water and allowed to stand for 10 min at 60°C before the absorbance of the reaction mixture was read at 700 nm using a microplate reader. The TPC of the extracts was expressed as mg Gallic acid equivalent (GAE) per gram of each extract on dry basis and all determinations were performed in triplicate in two separate experiments.

2.7 Ferric reducing antioxidant power assay (FRAP)

Briefly, an aliquot of 10 µl properly diluted in methanol and filtered extract is mixed with 290 µl of FRAP reagent in a 96-well microplate. Then, the reaction mixture is incubated at 37 ºC for 15 min. After that, the absorbance is determined at 593 nm against a blank that is prepared using distilled water. FRAP values are expressed as millimoles of ferrous equivalent (mM Fe (II))/ g of dry weight of seaweed material.

2.8 Scavenging of diphenyl-picrylhydrazyl (DPPH) radicals

The scavenging activity of the extracts was determined based on DPPH-scavenging assay. Ten microliters of sample, duly diluted in methanol, are added to 290 µL of DPPH solution (6×10^{-5} M in methanol and diluted with methanol to an absorbance of 0.700 at 517 nm) in a 96-well microplate. The resulting solutions are vortexed in a spectrophotometric microplate reader, and allowed to stand for 30 min in darkness at room temperature. The absorbance is measured at 517 nm in a spectrophotometric microplate reader, using methanol as blank. The control solution consists in using methanol instead of the sample. The radical scavenging activity is expressed as the inhibition percentage using the following equation:

$$\% \text{ inhibition of DPPH} = (1 - A_S/A_C) \times 100$$

where A_C and A_S are absorbance of the control solution and the absorbance of the sample solutions, respectively.

Results and discussions

Physico-chemical characterization of M. *Piryfera.*

The nutritional physicochemical composition of the *M.Piryfera* specie under study, expressed as 100 g dry weight, is shown in Table 1. Regarding the main physicochemical properties, water retention capacity was high, while oil retention was low, related to the hydrophilic nature of fiber polysaccharides. The physiological properties are affects by influence of dietary fiber. The water associated with fiber is an important consideration when studying the effects of fiber in the diet. Such water will influence the metabolic activity of fiber along the gut. Ordóñez et al., (2010) reported that the physicochemical properties of powdered seaweed could be assumed to reflect those of the fiber present.

These properties could be related to the hydrophilic nature of the charged polysaccharides of soluble fiber: alginates and fucans in brown seaweeds. On other hand, oil retention capacity was low and similar a data reported by Ordóñez et al., (2010) on study of various species of seaweed (between 1.22–1.67 g/g dry weight).

3.1 Ashes and minerals content.

The ash content obtained from M. *Piryfera* (49.6 % ± 3.9) is among range that reported 10.8 to 50.9 g/100 g dry food. From point of view the high content of minerals such potassium, sodium, sulfur, calcium between others, some of which are essential for the proper functioning of the body, it is because these algae have great capacity to store various minerals from the marine environment where they live.

3.2. Amino acid analysis

Asp and Glu were the most abundantly occurring amino acids (AA) in the seaweed specie tested. *M. Piryfera* contained 6.5 g Asp/100 g protein and 8.2 g Glu/100 g N. In brown algae, the quantities of these AA were significantly higher ($P < 0.05$; Table 3).

Table 1. Characterization physicochemical of *M. Piryfera*

Properties	Average		Deviation standard
Protein (g/100 g sample)	8.07	±	0.1
Total dietary fiber (% dry matter)	30.4	±	2.3
Soluble fiber (% dry matter)	35.4	±	4.2
Insoluble fiber (% dry matter)	10.5	±	1.3
Water retention capacity	20.3	±	2.5
Oil retention capacity	2.8	±	0.8

Table 2. Mineral content in sample of *M. Piryfera* specie.

Composition (g/100 g alga)

Elements	Potassium	Sodium	Calcium	Magnesium	Sulfur	Phosphorous
	10.7 ± 1.30	1.3 ± 0.31	0.85 ± 0.11	0.55 ± 0.02	0.77 ± 0.01	0.28 ± 0.01

Composition (mg/100 kg alga)

Elements	Strontium	Iodine	ron	Boron	Aluminum	Zinc	Cobalt	Barium	Manganese	Chromium
	65.5 ± 2.3	21.2 ± 1.1	120.7 ± 3.7	65.4 ± 2.1	89.6 ± 9.2	I32 ± 5.3	17.5 ± 1.6	4.20 ± 0.8	23.3 ± 2.3	0.5 ± 0.01
	Vanadium	Cadmium	Copper	Molybdenum	Nickel	Tin	Lead	Selenium	Gallium	Mercury
	2.04 ± 0.02	0.36 ± 0.01	1.22 ± 0.04	0.31 ± 0.02	1.45 ± 0.01	<1.3 ± 0.1	<1.6 ± 0.02	<1.6 ± 0.06	<1.4 ± 0.04	<0.85 ± 0.01

Table 3. Amino acid composition given in means ± SD (g/100 g N)

AA	Ile	Leu	Lys	Met	Cys	Pha	Tyr	Thr	Val	Arg	His	Ala	Asp	Glu	Gly	Pro	Ser
g AA/ 100g protein	3.02 ± 0.14	4.82 ± 0.48	3.51 ± 0.28	1.36 ± 0.03	0.59 ± 0.09	2.28 ± 0.00	2.53 ± 0.01	2.42 ± 0.01	3.87 ± 0.02	4.74 ± 0.02	0.99 ± 0.01	3.90 ± 0.02	6.50 ± 0.03	8.20 ± 0.07	2.87 ± 0.01	2.47 ± 0.02	2.45 ± 0.01

The protein in algae contains all essential amino acids (EAA). The EAA contents of some species (e.g., *Porphyra sp*.) can be compared with those of soy and egg protein. In addition, high concentrations of Arg, Asp and Glu are found in many seaweed species (11).

Total phenolic content

The total phenolic content, is expressed as mg of gallic acid equivalent (GAE)/g of dry weight, is shown in Table 4. The TPC values showed a wide range (9.69 ± 0.58 mg GAE/ 100 g sw). The water and methanol extracts from the same seaweed showed different values. Methanolic extracts showed significantly higher phenolic content values ($P<0.05$) than their counterparts extracted with water alone.

Antioxidant activity as assessed by ferric reducing antioxidant power (FRAP) assay.

Antioxidant activity as measured by FRAP assay showed a wide range of variation among the extracts of seaweed used (Table 4). As with phenolic content, the water and ethanol extracts from the *M. Piryfera* showed different antioxidant activity.

Antioxidant activity as assessed by DPPH-radical scavenging assay.

DPPH assay detects scavenging of free radicals by the tested samples through the scavenging activity of the stable 2,2 diphenyl-1-picryhydrazyl (DPPH) free radicals. The scavenging activities of the seaweed extracts toward DPPH-radical are shown in Table 4. As in FRAP and phenol content assay, DPPH-scavenging activity was also the highest. The results of the current study have shown that most of the studied plants are potentially a good source of free radical scavenging compounds and support the traditional use of M. *Piryfera*. The phenolic compounds make a significant contribution to the antioxidant activity in these extracts as evidenced by the positive correlation between phenolic contents and antioxidant activities.

Table 4. Antioxidant activity and total phenolic content in the methanolic extracts produced from the edible seaweed.

Methanolic extract	Total phenol Mg GAE/ g seaweed	FRAP (µM Fe(II)/g seaweed)	DPPH (%)
70 % (v/v)	7.29 ± 0.92	2.18 ± 0.02	31.99 ± 1.51
85 % (v/v)	14.87 ± 1.82	3.01 ± 0.81	40.74 ± 2.63
100 % (v/v)	15.32 ± 2.91	3.91 ± 0.52	44.62 ± 3.04
water	1.28 ± 0.03	0.83 ± 0.01	3.72 ± 0.95

Conclusions

The antioxidant potential and total capacity antioxidant by the different methods revealed that reducing power of crude methanolic extract increased with increasing concentration. These results are of interest since antioxidant compounds are beneficial for the human health, and antioxidant compounds obtained from natural sources are preferred for consumption than synthetic antioxidants.

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Free Gluten Bakery Product for Celiacs

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Summary
Currently baked products based on wheat flour are consumed in masse. Including bread, looms worldwide. There is, however, a population group that has gluten intolerance, present not only in wheat, but also in oats, barley and rye. This syndrome is characterized by intestinal poor absorption, and it is called celiac disease and can lead to severe malnutrition. This disease occurs in genetically predisposed individuals, in which the ingestion of foods containing gluten damages the mucosal surface of the intestine thin leading to inability to absorb the nutrients. The treatment for this disease is exclusively dietary and involves the removal of cereals from diet: wheat, oats, barley, rye and products made from flour. In this paper we developed two kinds of bread (pound cake and shortbread), using gluten-free flours, added as quinoa, cassava, rice, amaranth and corn starch. Variables were defined as the amount of quinoa flour and corn starch, and were used as the selection index of visual appearance of the baked bread. Two tests were performed, discriminative sensory and preference orders. 70 semi-trained judges participated; both tests included shortbread wheat flour (control). To evaluate the experiment it was used a completely randomized design with factorial 32 with three replicates, where 3 is the number of levels of factor 1 (flour level) and 2 is the number of levels of factor 2 (type of flour). We generated a pancake and shortbread with gluten free flours with acceptable appearance. In the sensory evaluation was found that there is a difference in terms of taste, smell and appearance in general because the ingredients between the control and the test samples, but no difference were detected in the texture at $p = 0.05$.
Keywords: celiac disease, gluten, bread, sensory

Introduction
Bread is one of the most consumed food products from cereals in the world; however, because it is usually made from wheat flour, it’s not consumable by the whole population, as there are people who are intolerant to prolamins, major proteins of wheat (also oats, barley and rye. [4] Celiac disease is a permanent intolerance to a set of proteins called prolamins found in wheat, oats, barley and rye (TACC) and products derived from these four cereals. Prolamins receive different names according to the grain of origin: wheat gliadin, oats avenin, barley hordein and rye: secalin. Gluten cereals listed above are the most common form of presentation of celiac toxic prolamins. Gliadin is the most used at industrial level and is the biggest problem because wheat flour can be present as the main ingredient or as additive in many foods. Sometimes it can be present for cross contamination that can occur at industrial level or in the marketing of food [1].

The only treatment to reverse the symptoms of celiac disease is a strict diet and permanent TACC free gluten [1]. For this reason it is very important for patients with this disease to have gluten-free foods that are safe for their diet. In other words food that is not only cereal containing proteins TACC its composition but also did not undergo a cross-contamination grain-TACC in any stage, from process development to final marketing [2]. In this paper we developed two kinds of bread (pound cake and muffins) using gluten-free flours made from quinoa (*Chenopodium quinoa*), tapioca (*Manihotesculenta*),

rice (*Oryza sativa*), amaranth (*Amaranthuscaudatus*) and maize (*Zea mays*) in order to obtain products suitable for celiac disease patients.

Materials and methods

Acquisition of flour with no gluten

Flours (quinoa, amaranth, tapioca, rice and corn starch) were purchased atSaltillo Coahuila, Mexico supermarket,

Preparation and baking bread dough for gluten-free functional

The preparation of gluten-free breads (pound cake and shortbread) was held at the bakery 3 ESPIGAS S.A DE C.V, located in Saltillo Coahuila Mexico.

To prepare the pancake through the use of a mixture of gluten free flour (tapioca, quinoa, amaranth, rice, maize starch) and other ingredients as egg, oil, milk and yeast. Mixing was performed in a kitchen mixer brand, after having obtained the mass it is placed in a mold and allowed to ferment for 40 minutes, subsequently baked at a temperature of 392 °F for 20 minutes, elapsed the time the pan was removed from the mold, allowing to cool for 10 minutes and proceeded to taste by bakers who are working in the area.

To prepare the shortbread it was followed the methodology described above, with the difference that a viscous mixture was used, the mixture generated was placed in baking paper and the same in the corresponding molds, we proceeded to bake (oven Zucchelii) at a temperature of 392° F for 20 min. After baking the shortbread was allowed to cool for 10 minutes.

Sensory evaluation of breads formulated

The evaluation was conducted in the Department of Food Science and Technology in the sensory evaluation laboratory of the Universidad AutónomaAgraria Antonio Narro, campus Saltillo Coahuila.

Paired preference test

In order to know whether the judges preferred the gluten free shortbread compared withgluten shortbread on various factors (appearance, odor, flavor and texture). We worked with a semi-trained panel of 70 judges, the two samples made with gluten flour and glutenwas presented simultaneously; each panelist evaluated two samples only once.

Bromatological Evaluation of bread.

The evaluation was carried out in the Department of Nutrition and Food at the Universidad AutonomaAgraria Antonio Narro campus Saltillo Coahuila.

Compositional analysis was performed of the shortbread without wheat and the shortbread with wheat as a control, everything was worked with a replication, it was determined, fat, protein, carbohydrates, minerals, moisture, fiber and energy content.

Table 1 Results of the paired preference test applied to gluten free shortbread and gluten shortbread.

Sensory Characteristics	Shortbread with no wheat	Shortbread with Wheat
Appearance	17	53
Smell	35	35
Flavor	33	37
Texture	47	23

The results presented in Table 1 may be due to being replaced flour traditionally used in the baking industry, and to make this substitution generates a new product with unknown or different characteristics that provided free gluten meals, however the texture of shortbread formulated by mixing gluten free flour was mostly preferred by the evaluator judges.

Compositional analysis

In Table 2 it could be seen that the generated shortbread gluten free flours containing fat, minerals and moisture in greater amount (10.46, 20.74 and 3.31% respectively), and less protein and carbohydrates

(25.74 and 5.32% respectively), compared to traditionally muffins prepared with wheat flour. Our new formulation of muffins made with gluten free flour contains 2.48% of energy content.

Table 2 nutritional content

Nutritional Values		
Values per serving de 100 g		
	Shortbread with no wheat	Shortbread with wheat
Total Fat	39.39 g	36.20 g
Protein	4.99 g	6.72 g
Carbohydrates	38.77 g	40.95 g
Minerals	1.63 g	1.35 g
Humidity	15.27 g	14.78 g
Fiber	0.0 g	0.0 g
CALORIES	**529.35 Kcal**	**516.49 Kcal**

Gluten is considered a glycoprotein which makes up 80-85% of the protein content that wheat has [5], which is why our gluten-free formulation has a lower protein content, increased total fat, minerals and moisture, may be due to the fact that it was used twice amount of milk respected to the muffin made with wheat, and also to the use of 5 different types of flour (quinoa, tapioca, rice, amaranth and maize) containing different concentrations of the abovementioned.

Conclusions

It is possible to replace wheat flour for gluten-free flours (quinoa, tapioca, rice, amaranth and maize) in the baking industry, obtaining two different breads (pound cake and shortbread) which can be consumed by celiac patients perfectly, however, care should be taken in handling flour to prevent possible cross contamination.

The combination of Gluten free flours have allowed us to prepare pound cake and shortbread which were obtained sensory characteristics similar to those of a pound cake prepared with wheat flour and has similar nutritional content of the commercialpound cake wheat, which places the product within the range of the nutritional values of the recommended daily intake (RDI).

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Analysis Of Betalains Extracts In Water And Citrate-Phosphate Buffer Of Pitaya Stenocereus Pruinosus

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Introduction

Pitaya stands in our country by a wide variability of use and exploitation (1). In Oaxaca the pitaya of the species Stenocereus pruinosus is one of the most important fruits because of the amount produced and its nutritional value. A group of important compounds in these fruits are the betalains, water-soluble pigments that possess antioxidant properties and a range of vivid colors from yellow to red-violet therefore have potential industrial use of natural pigments (2). Betalains on fruits was identified by UV-Vis spectrophotometry, the procedure consisted in obtaining pitaya juice, the extraction of betalains using two solvents (water and citrate phosphate buffer pH 5.5). Juice of three varieties of pitaya (violet, red and orange) was analyzed. A spectrometer UV / VIS LAMBDA 25 PerkinElmer was used to obtain the spectra in the range of 200-700 nm. Stability of betalaines were determinates in acidic and alkaline conditions using HCl and NaOH solutions. In all samples were observed betalaines (wavelengths of 487 and 540 nm) in various proportions, besides were detected acylated betalaines a wavelength of 260 nm. The samples showed a stable behavior at acidic pH and unstable at alkaline pH. Alkaline conditions cause bond hydrolysis, while acidification induces recondensation of betalamic acid with the amine group of the addition residue. In the UV-Vis spectra to acidic conditions of maximum absorption peaks remain very close to the original wavelength, whereas the peaks alkaline conditions to change its position shifting to lower wavelengths, indicating instability or decomposition of betalains.
Keywords: betalains, pitaya S. Pruinosus, UV-Vis spectrophotometry

Methodology

MATERIAL. Pruinosus Stenocereus (Otto) Buxb. Pitayas from Santo Domingo Tianguistengo, municipality Chazumba, Huajuapan district, northwest of the state of Oaxaca. Pitayas stored frozen at -7°C.

EXTRACTION OF BETALAINS. The pitayas were divided according to the color of the pulp presenting: violet, red, and orange. Therefore were obtained from three different pitaya samples. The process consisted of the following steps: 1) Clean. Includes removing fruit thorns using forceps, washing with water and the shell manual peeling fruit to obtain pulp of pitaya. 2) Homogenisation. Pitaya pulp was shaken manually in a plastic container to form a homogenous mixture, 3) Filtration. The homogeneous pulp was passed through a sieve with apertures sized to remove seeds, husks and seeds adhering to waste, which was obtained pitaya juice.

The extraction of betalains of pitaya juice was performed using two solvents: a buffer or citrate-phosphate buffer (pH 5.5) and distilled water. The buffer solution comprises a citrate buffer and phosphate buffer. The betalains extraction process includes: 1) Magnetic stirring juice with one of the two solvents for 20 min in a dark room. 2) samples centrifuged for 15 min (centrifuge model brand solbat 2577), finally the supernatant was recovered and stored in amber containers frozen.

ANALYSIS UV-VIS SPECTROSCOPY. A spectrometer UV / VIS Lambda 25 PerkinElmer, with cells of 10 mm and a software version 2.85.04 WINLAB UV was used to obtain spectra in the range of 200-700 nm. The computer turns on and allowed to stabilize for half an hour, and then make measurements of the samples. The solvent to calibrate the equipment is the same found in the samples.

BETALAINS STABILITY OF CHANGES TO PH. The stability of betalains against pH changes was determined using two solutions: one solution of hydrochloric acid (HCl, 2N) and a solution of sodium hydroxide (NaOH, 3N).
Samples were dissolved in distilled water in a test tube, in a 1:5 ratio, each sample was divided into three: neutral (distilled water only), acid (HCl) and base (NaOH). Analysis was performed by UV-VIS spectroscopy to determine the behavior of each solution.

Results and discussion

The table 1 shows that the compounds of each extract have the same behavior regardless of the solvent used, water or buffer. In extracts violet shows two characteristic absorption bands at 534 and 489 nm. The extracts are red three absorption bands (535, 485 and 463 nm) and orange extracts have two at 483 and 463 nm. These values are consistent with those reported in the literature for betacyanins and betaxanthins. In all extracts a peak is observed at about 260 nm, which is attributed to the acylated betalaines, which are formed by the reaction of a carboxylic acid with an alcohol betalains a sugar (3).

Table 1. Wavelength of absorbance maximum of the extracts of betalains

SOLVENT	EXTRACT	λ MAX (nm)
CITRATE-FOSFATE BUFFER pH 5.5	VIOLET	1: 534
		2: 489
		3: 262
	RED	1: 535
		2: 485
		3: 463
		4: 261
	ORANGE	1:485
		2: 462
		3:260
WATER	VIOLET	1: 534
		2: 489
		3:262
	RED	1: 533
		2: 485
		3:462
		4:263
	ORANGE	1: 482
		2: 460
		3:260

BETALAINS STABILITY OF CHANGES TO PH. The results of the spectra of samples made acidic and basic solutions show stability at acidic pH and unstable at basic pH. Samples with sodium hydroxide show a shift of peak wavelength of maximum absorbance at shorter wavelengths (higher energy), because the energy differences between the bonding orbitals and antibonding where electronic transitions occur have changed due to an alteration in the structure of the compound.

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Ratio Of The Activation Energy Of The Drying Process Of Aqueous Solutions Of Biopolymers With The Efficiency Of Microencapsulation.

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Abstract

The microencapsulation has been used for protection against oxidation of lipid; the degree of protection depends on the encapsulation efficiency and oxygen diffusion to the oil. The activation energy was determined using the diffusion coefficient of isothermal drying of aqueous solutions of mesquite gum (GM), gum arabic (GA), chitosan (C) alone and in mixtures at temperatures of 50, 60, 70 and 80 °C. The effective diffusion coefficient (Def) was determined with the regular regime method, which evaluates the Def as a function of moisture content. Stabilized emulsions were prepared with 10% of biopolymers (GM, GA) and a mixture of 10% of GM with C 0.6%, 10% GA with 0.6% C. The emulsions were coded as GM-00C, GA-00C, GM-06C and GA-06C, respectively. The microcapsules were obtained by the spray drying method of the emulsion; the encapsulation efficiency was determined by the quantification of the surface oil and internal oil. The activation energy was evaluated of the drying process using the Arrhenius equation. The activation energy (kJ/mol) and encapsulation efficiency (%), respectively, in decreasing order was GM-06C (26.01kJ/mol, 86.81%), GA-06C (22.6 kJ / mol, 77.84%), GM (23.94 kJ/mol, 67.38%) and GA (19.2 kJ/mol, 63.89%). The microcapsules GM-06C showed the highest encapsulation capacity, because the interfacial membrane composed of a concentration of 0.6% and 10% C and GM formed a thin and dense structure which reduced the spread of linseed oil during drying spray.

Key words: mesquite gum, activation energy, efficiency of microencapsulation.

Introduction

Microencapsulation is a technique widely used in the industry of pharmaceutical, cosmetic, health, food and secondary petrochemicals. In the case of the food industry, microencapsulation of food ingredients is used to reduce the degradation due to environmental factors such as oxygen, light, temperature and pro-oxidant agents; to improve the stability during processing or use, to achieve a controlled release, or simply to have free flowing powder and used as a food ingredients. Generally, the microencapsulation has the ability to provide some functional properties, modifying and improving the physical form and properties of a substance (1).

Several researchers have attempted to measure the retention capacity of the wall materials studying the isothermal drying curves, but only they have done the qualitative form (2, 3, 4). The tendency of the materials to form a dense skin during drying is difficult to evaluate. The isothermal drying of a solution of wall material is characterized by the dependence of the drying speed on the isothermal drying over the moisture content.

Matsuno y Adachi (1993) shows the characterized curves of the wall materials during the isothermal drying and its relation with the protection degree against lipid oxidation and grouped into 4 types. The shape of the drying curves is a qualitative method that does not allow a proper choice of the best barrier materials, when several of them form isothermal drying curves type 1. The isothermal drying rate is governed by the diffusion rate of water through the wall material therefore is necessary to develop

methods that allow a better selection of quantitative manner and this is achieved by determining the coefficient of diffusion during the process of isothermal drying aqueous solutions of the wall materials.
There are different methods to determine the diffusion coefficient from drying curves such as the simple method, the regular regime method and numerical method (5). The moisture diffusivity in liquid foods and polymer solutions show a strong dependence on the moisture content (6,7); the regular regime method allows calculating the diffusivity as a function of moisture content from drying isothermal curves, where the drying rate is governed by mass transfer within the material, (8), in addition to considering the shrinkage suffered the sample during drying.
Several studies have shown that the determination of the activation energy of the drying processes is a method that allows to predict which material can increase the encapsulation efficiency and decrease the oxidation degree of the encapsulated lipids (9, 10). The objective of this study was to correlate the activation energy of the drying process with the encapsulation efficiency.

Experimental

Biopolymers Aqueous Solutions: The aqueous solutions of each biopolymer were prepared at 30% (p/p), considering the ratio ($g_{(GM\ o\ GA)}/g_C$ = 16.667), biopolymers were dispersed in a 1% aqueous solution of acetic acid, since chitosan is soluble in acidic solutions.

Isothermal drying of biopolymers and mixtures: The isothermal drying experimental data were obtained with thermogravimetric analyzer TGA 2950 de TA-Instrument (New Castle, DE, EUA), and allowed to reach the experimental conditions in the drying chamber. A sample 15 to 20 mg of each solution of biopolymer was placed in the oven equipment and solutions were dried at 50, 60, 70 and 80 ° C for 90 min nitrogen was used as purge gas flow 100 cm3/min. The data obtained were the weight loss over time and temperature as well as the derivative weight loss versus time.

Regular regime theory: Isothermal drying curves contain two periods, the first is called the induction period, in this zone the drying rate depends on the initial distribution of the moisture, and the second term is called the normal regime, where the drying rate is independent of the initial velocity and thus of the initial distribution of moisture. If this period there, all curves of the same material and geometry at a specific temperature and different initial moisture, converge to a single curve called regular regime curve (6, 8, 10, 11, 12).

Preparation of emulsions: the emulsions were formulated to have a dispersed phase fraction ($\phi = g_{oil}/g_{emulsion}$), ϕ=0.10, and ratio of emulsifier material and linseed oil 1:1 (w/w). All emulsions were prepared with acetic acid solution 1% (w/w) because chitosan is soluble in acidic solutions. Four emulsion were formulated and were dissolve 10% GM o GA and the mixed with 0.6%C , that give a ratio of ($g_{(GM\ o\ GA)}/g_C$ = 16.67), emulsions were coded as: GM-00C, GA-00C, GM-06C and GA-06C.

Microencapsulation: The emulsions were dried in a mini spray drying Büchi model 190 (Büchi Laboratorio Technik AG, Flawil, Suiza) at a temperature of inlet air at 170 ± 5 ° C and an outlet air temperature of 95 ± 5 ° C and an atomization pressure of 4.5 bar.

Encapsulation efficiency: Content was determined by performing a surface oil extraction with isooctane and magnetic stirring for 5 minutes; internal oil content was determined by rehydrating the microcapsules and breaking the emulsion formed to quantify internal oil using a mixture of the isooctano/2-propanol (3:1, v / v) and centrifuging for 10 minutes at 6000 rpm.

Results and Discussion

Effective diffusivity and activation energy of drying process

A plot of moisture content against effective diffusivity for blend GA-06C a different drying temperature, show in figure 1. It is observed that the diffusion coefficient depends on the content of moisture, so that it is necessary to determine the average effective diffusivity which is obtained by integrating the area under the curve in the range of moisture. Similar results were obtained for the GA, GA, and GM-06C

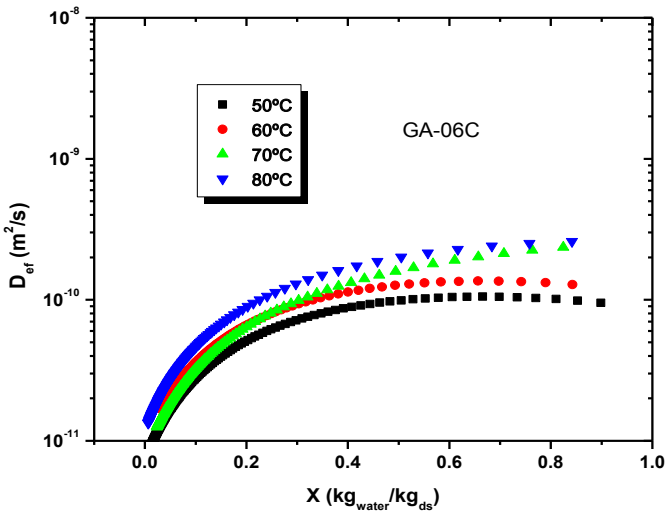

Figure 1. Diffusion coefficient for systems based on GA-06C

To find the activation energy was linearized Arrhenius equation and the equation is obtained from a straight line, as shown by the following equation.

$$\ln \overline{D_{ef}} = \ln D_o - \frac{E_a}{R}\left(\frac{1}{T}\right)$$

Linear regression was performed with the data in Table 1 and the value of the slope is calculated the activation energy of the drying process.

Table1. Effective diffusion coefficient average a different temperatures and the activation energy of drying process

Temperatura de secado isotérmico (ºC)	$\overline{D_{ef}}_{GM}$ (m^2/s)	$\overline{D_{ef}}_{GA}$ (m^2/s)	$\overline{D_{ef}}_{GA\text{-}06Q}$ (m^2/s)	$\overline{D_{ef}}_{GM\text{-}06Q}$ (m^2/s)
50	7.51×10^{-11}	9.04×10^{-11}	7.84×10^{-11}	6.10×10^{-11}
60	9.61×10^{-11}	11.27×10^{-11}	10.22×10^{-11}	8.26×10^{-11}
70	12.29×10^{-11}	13.62×10^{-11}	12.72×10^{-11}	10.43×10^{-11}
80	16.06×10^{-11}	16.69×10^{-11}	15.81×10^{-11}	14.09×10^{-11}
E_a (kJ/mol)	23.94	19.2	22.6	26.01

These results show that it is possible to form fine and dense skin at the beginning and during drying which controls the diffusion of water molecules, and the larger the Ea the skin formed is thinner and denser (3, 9, 10).

Encapsulation efficiency

The results of the encapsulation efficiency are shown in Table 2. These results can be correlated with those obtained for the activation energy of the drying process. The materials that show the shape of the curve type 1 during isothermal drying and higher activation energy showed the higher encapsulation efficiency. These results support the hypothesis of the formation of a fine and dense skin which limits the diffusion of water and therefore any substance having a size equal to or greater than water; therefore, a higher activation energy greater is the encapsulation efficiency, as long as the biopolymer has surface activity, capable of forming and stabilizing emulsions.

Table 2. Encapsulation efficiency of linseed oil in the microcapsules

Formulation	Encapsulation efficiency (%)	Formulation	Encapsulation efficiency (%)
GA-00Q	63.89	GM-00Q	67.38
GA-06Q-MT	77.84	GM-06Q-MT	86.81

The GM and GA are good emulsifiers (13); by the results of this study, is the material that can guarantee good retention and protection of the encapsulated material during the formation and storage of the microcapsules. Electrostatic interactions between chitosan and GM or GA allow to form a fine and dense skin compared to these biopolymers alone. In this work it was decided to use GA, GM and Q, as emulsifying and microencapsulating materials, because they exhibit high values of the activation energy of the drying process indicating that the diffusion coefficient is lower in them because the polymeric matrix formed at the beginning and during the drying is more compact making it difficult for water molecules to diffuse from the interior of the drop to the surface by the drying effect.

Conclusions

The use of TGA guarantees isothermal conditions during the drying process. The biopolymers effective moisture diffusivity was dependent on moisture content and temperature. It is recommended that drying be performed at temperatures lower than 80 °C for an adequate analysis of the moisture diffusion mechanism using regular regime methodology. Activation energy provides a quantitative measurement for selecting potential good wall materials against lipid oxidation. Existe una buena correlación entre la energía de activación y la eficiencia de encapsulación, a mayor energía de activación mayor es la eficiencia de encapsulación.

Acknowledgements

The authors are grateful for the financial support of this research from the Consejo Nacional de Ciencia y Tecnología (CONACyT) through grant Apoy-Compl-2009 119047.

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Shelf Life Of Cookies With Flour Lentil And Microcapsules Of Flaxseed Oil As A Source Of Polyunsaturated Fatty Acids

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Abstract

Five formulations were prepared for the production of cookies: The original recipe with wheat flour (F1) was the control; F2 and F4 formulations containing flaxseed oil *per se* and formulations F3 and F5 were added 150g of flaxseed oil microcapsules; F4 and F5 have a 40% replacement lentil flour instead of wheat flour. Proximate analysis was determined according to AOAC techniques. The microcapsules were prepared by the spray drying method of an emulsion with a disperse phase (ϕ=0.1) and a ratio of wall materials: flaxseed oil of 2:1; the value peroxides was determined with the Shanta and Decker method from the extracted oil in 10 g of cookies. The sensory evaluation was determined under three affective type methods (acceptance test, preference test and hedonic test). With the addition of lentil flour and linseed oil microcapsules were found positive trends in the welcome and acceptance of cookies, itself as a nutritional improvement in the amount of protein observed. The cookies were stored at 4, 25 and 35°C. ASLT method was used to evaluate the shelf life of cookies. The proximate analysis was very similar for all formulations, the protein content of the formulations increase the F4 and F5 by replacement of wheat flour by lentil flour (10.70% and 12.70% protein, respectively). The activation energy (kJ/mol) for the formation of lipid peroxides in decreasing order was: F5 (30.26), F4 (26.49), F3 (19.61), F1 (17.12) and F2 (15.82). The cookies made with formulation F5 at 35 ° C storage, can have a shelf life of up to 70 days while the F2 formulation only has a shelf life of 20 days. These results show that microencapsulation is an effective method to retard the formation of hydroperoxides because the polymeric membrane formed by mesquite gum reduces the diffusion of pro-oxidant agents to the interior of the microcapsule; lentil flour also contains polyphenols which act as antioxidants.

Keywords: Microcapsule, flaxseed oil, Shelf life.

Introduction

Cookies are popular products made of wheat, that contain sugar and fats in their formulation, they have varieties of flavors, long shelf life and allow the incorporation of ingredients to increase their nutritional content (1,2,3). Bakery products can be added with oils rich in polyunsatured fatty acids as flaxseed oil, which is an oil seed that contains 38 – 45% oil, which alpha linolenic acid represent 52%. Microencapsulation is used to facilitate the usage and protection of the flaxseed oil from possible factors that propitiate oxidation, like the presence of oxygen, temperature, metallic ions and light, it changes the oil's liquid phase into solid microcapsules (4). Recent studies have found that lentil flour has a high protein content, compared to other legumes, and contain polyphenols with high antioxidant activity, these phytochemicals, that can be categorized as phenolic acids, flavonoids, saponins, fitic acid and condensed tannins; even though in the past were considered as antinutritional compounds, now days have been associated with the prevention of chronic diseases (5,6,7).

Bakery products that contain lipids high in polyunsaturade fatty acids present oxidation reactions, which can be quantified through the quantification of hydroperoxides. The products from the lipid oxidation are considered the main causes of deterioration; the oxidation process can be slow at room temperature and shelf life is difficult to predict for industrial needs, due to the time necessary for this evaluation, it might last a couple of months. The most used method to decrease time is using accelerated shelf life tests (ASLT), during storage; oxidation can accelerate when changing temperature, light and humidity, mainly. The most useful parameter is temperature because it is a critical factor in lipid oxidation (8, 9). ASLT method consists in measuring the oxidation speed of the different samples stored at at least 3 different temperatures; the reaction speed at a desired temperature can be extrapolated using the Arrhenius model. The goal of this study was to determine the shelf life of oatmeal cookies replacing wheat flour with lentil flour and polyunsaturated fatty acids by including flaxseed oil *per se* and microencapsulated, stored at different temperatures.

Experiment

Microcapsule preparation: The emulsion was prepared with a disperse phase (ϕ) = 0.1 flaxseed oil and a relation 2:1 mesquite gum: flaxseed oil, using distilled water to dissolve the mesquite gum and was added drop to drop using an homogenizer Ultra Turrax (Ika Works, Inc., Wilmington, VA. EUA) at 7600 rpm for 5 min. Emulsions were dried in mini spray drying Büchi 190 (Büchi Laboratorio Technik AG, Flawil, Suiza) at an entrance air temperature of 170 ± 5°C and an air exit temperature of 95 ± 5°C and an atomization pressure of 4.5 bar.

Encapsulation efficiency*:* The oil content in the surface of the capsule was determined by an extraction with isooctane and magnetic agitation during 5 minutes, the content of oil inside was determined rehydrating the microcapsules and breaking the emulsions using a mixture of isooctane/ 2- propanol (3:1, v/v) and centrifuging 10 minutes at 6000 rpm.

Cookies preparation: The cookie preparation materials and quantities are described in Table 1.

Table 1. Cookie formulation

Ingredient	F1	F2	F3	F4	F5
Wheat flour (g) Bleached : whole, (1:1)	1000	1000	1000	600	600
Lentil flour (g)	0	0	0	400	400
Sugar (g)	300	300	300	300	300
Margarine (g)	400	400	400	400	400
Vanilla (teaspoon)	1	1	1	1	1
Oatmeal (g)	500	500	500	500	500
Egg (unit)	1	1	1	1	1
Water (g)	200	200	200	200	200
Baking powder (g)	30	30	30	30	30 g
Shortening (g)	200	200	200	200	200
Cinnamon (g)	25	25	25	25	25
Flaxseed oil (g)	0	50	50 *	50	50 *

*Microencapsulated flaxseed oil

Cookie Proximal Analysis: The proximal composition (water, ash, protein, fat, crude fiber and NFE) was determined by the AOAC procedures (AOAC, 1990)

Cookies Sensorial Evaluation*:* Three sensorial affective type tests were used to evaluate the five formulations of cookies and determine their level of pleasantness. For their identification, the different formulations were assigned a key three digit number: F1 (320), F2 (542), F3 (103), F4 (834) and F5 (435), so that the judges would not make speculations and alter results. The affective test were applied to 45 non

trained judges (potential consumers), determining the level of acceptation or rejection, preference of the formulations and pleasantness level (0 to 10).

Shelf Life Determination: The shelf life of cookies was determined by the ASLT method that consists in measuring the speed of oxidation by the quantification of hydroperoxides thru the spectrophotometer method UV- vis described by Shanta y Decker 1994 (10) to the samples stored at 4, 25 and 35 °C. Starting with the equation 1, the quantity of hydroperoxides is measured according to the time and the experimental results adjust to a reaction order = 0, 1 and 2.

$$-\frac{d[C]}{dt} = k[C]^m \qquad \text{Eq.1}$$

Where:

$C = peroxide\ concentration\ \left(\frac{meqO_2}{kg_{oil}}\right)$; $k = reaction\ constant$

$t = time$ (days); $m = reaction\ order$ (adimensional)

Arrhenius model (eq. 2) allows determining the dependence of temperature

$$k = k_0 e^{\left(-\frac{E_a}{RT}\right)} \qquad \text{Eq.2}$$

Where:

k = reaction constant; k_o = pre-exponential frequency factor (adimensional); E_a = activation energy of the oxidation process (kJ mol^{-1}); R = Gas Constant (8.314 J K^{-1} mol^{-1}); T = absolute temperature (K)

Results and discussion

Encapsulation efficiency: The encapsulation efficiency was 67.38% ± 1.32, this results probably are because the polymeric matrix formed during the spray drying process permitted the escape of the flaxseed oil leaving a minimum part in the microcapsule surface.

Proximal Analysis of the Cookies: On table 2 an increase in the protein content is shown for F4 and F5 because of the lentil flour content. ANOVA shows that there is a significant difference between the formulation and a mean comparison (Tukey) shows no significant difference between F1 and F2 with a significant level of 5%. Fat content showed significant differences at a 5% level between F2 and F3, F4 and F1, F4 and F2, F4 and F3 and F4 and F5.

Table 2. Proximal composition of the different cookies formulation.*

	F1	F2	F3	F4	F5
WATER	6.86 ± 0.24	5.59 ± 0.45	6.60 ± 0.15	5.51 ± 0.42	6.06 ± 0.15
ASHES	1.75 ± 0.01	1.79 ± 0.01	2.00 ± 0.34	2.10 ± 0.02	1.95 ± 0.03
PROTEIN	7.29 ± 0.07	7.39 ± 0.07	8.54 ± 0.03	10.04± 0.57	10.26± 0.02
FAT	21.08 ± 0.20	21.36 ± 0.21	20.67± 0.06	21.84± 0.31	20.79± 0.16
FIBER	0.48 ± 0.001	0.49 ± 0.09	0.61± 0.04	1.11 ± 0.08	0.84 ± 0.04
N.F.E.	62.54 ± 0.26	63.38 ± 0.26	61.58± 0.29	59.40± 0.37	60.10± 0.13

*Results are expressed as the mean of three repetitions.

Sensorial Evaluation

Acceptance Test: The results for the acceptance test in from best to worst were F1 (69%), F2 (67%), F5 (73%), F4 (84%) and F3 (91%). When comparing the number of judges that accept the samples vs. the corresponding critical ordinal sum difference, this is smaller, then all formulations are accepted significantly by the judges at a significance level of 5%.

Preference Test: the test shows significant difference between formulations F1 and F4, F2 and F4, F3 and F4; and F4 and F5, at $p<0.05$. F4 was sensory different to the other formulation, the order of preference observed was F1, F2, F5, F3 and F4, where F1 is the worst and F4 the best.

Hedonic Test: The results obtained with the Fisher DMS test indicate that there is significant difference between the five formulations, as well as in the judges opinions, with a significance level of 5%.

Hydroperoxide formation kinetic

The lipid oxidation in the cookies was measured by the concentration of hydroperoxides formed during the storage at different temperatures. Figure 1 shows the results of the oxidation in the cookies stored at 25ºC. F1 does not contain flaxseed oil or lentil flour, it is a formulation that contain mostly saturated fatty acids (shortening and margarine), F2 to F5 contain a higher level of polyunsatured fatty acids because of the addition of flaxseed oil. F2 presents a higher level of oxidation due to its content of flaxseed oil *per se* (50 grams). F3 was additioned with microcapsules of flaxseed oil using mesquite gum as wall material, using this technique; the lipid oxidation was lowered compared to F2. The samples stored at 4 and 35º C showed a similar tendency.

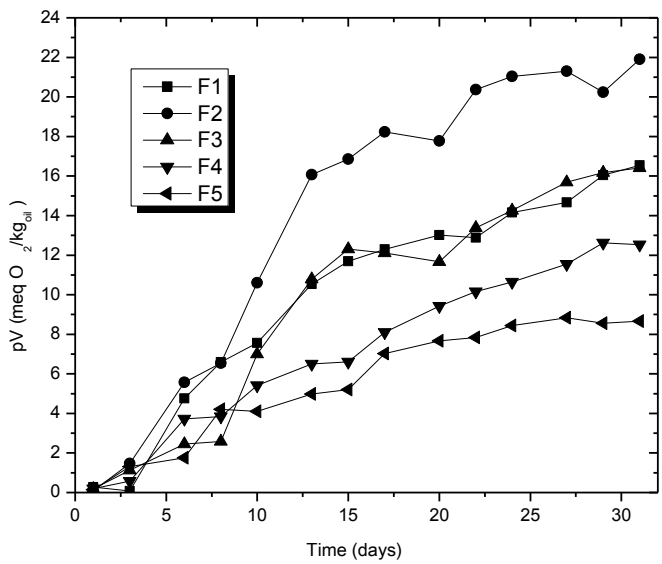

Figure 12. Hydroperoxides formation thru time in the different formulations.

To determine oxidation kinetic order 0, 1 and 2 kinetic adjustments were proved. The best adjustment was achieved with the order 0 with values of R^2 greater than 0.9. This results match with the ones obtained by Calligaris and col., 2007 (11). In Table 3 the order 0 kinetic values are shown, this means that the hydroperoxide formation does not depend on the hydroperoxide concentration.

Table 3. Kinetic order cero parameters in the different cookie.

Temperature (°C)	Kinetic Parameters	F1	F2	F3	F4	F5
4	*k*	0.262	0.461	0.288	0.168	0.108
	R^2	0.95	0.96	0.95	0.91	0.90
25	*k*	0.533	0.745	0.577	0.420	0.295
	R^2	0.92	0.88	0.92	0.98	0.93
35	*k*	0.523	0.922	0.657	0.518	0.396
	R^2	0.95	0.98	0.97	0.97	0.98

Converting into a line the Arrhenius equation and graphing (ln*k*) vs (1/T) parameters as activation energy (E_a) and k_0 are obtained; Table 4 shows the results of the kinetic parameters.

Table 4. Arrhenius model and energy activation parameters

Formulation	Ln k_0	m	R^2	Ea (kJ/mol)
F1	6.133	-2059.05	0.90	17.12
F2	6.091	-1902.74	0.99	15.82
F3	7.288	-2359.06	0.98	19.61
F4	9.739	-3186.31	0.99	26.49
F5	10.928	-3639.86	0.99	30.26

Activation energy can be interpreted as the energy necessary for the hydroperoxides to form during lipid oxidation; at less activation energy, hydroperoxides formation is higher because less energy is required for the lipids to oxide. F5 presents the highest activation energy; therefore it is the most stable formulation and the one that presents the least oxidation.

The model represented by equation 3 allows determining the hydroperoxide concentration with respect to time as a function of temperature. To estimate shelf life the reference is 20 $\frac{meqO_2}{kg_{oil}}$ of hydroperoxides (11), considering that at this level cookies are no longer suitable for consumption.

$$C = k_0 e^{\left(-\frac{E_a}{RT}\right)} t \qquad \text{Eq. 3}$$

The most stable formulations are F4 and F5, F2 has a 20 days shelf life and F4 and F5 up to 50 – 70 days, respectively. These results show that microencapsulation is an effective method to retard the formation of hydroperoxides because the polymeric membrane formed by mesquite gum reduces the diffusion of pro-oxidant agents to the interior of the microcapsule; lentil flour also contains polyphenols which act as antioxidants.

Conclusions

The best formulation was F5 because besides containing more protein levels, it contains alpha linolenic acid in very optimum levels, its acceptance is 73%, and even though is not the best (preferred) its statistically accepted according to non trained judges (consumers), besides it is preferred over the original formulation (F1). F5 has a longer shelf life, 70 days stored a 35º C. F5 is the best formulation nutritionally and from the food technology point of view. A functional food was elaborated that is very palatable and practice for most part of the population, from babies to adults.

Acknowledgements

The authors are grateful for the financial support of this research from the Consejo Nacional de Ciencia y Tecnología (CONACyT) through grant CB-2010 157511.

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New Germplasm Of Pink Pulp Guava: Physicochemical And Nutritional Characterization

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Introduction

Mexico ranks third worldwide in the production of guava (1). Producing areas in the country are: Michoacán (104 000 548 ton), Aguascalientes (69 thousand 428 tons), and Zacatecas (32 000 746 tonnes), which represent 88% of the cultivated area in the country, representing an important economic activity (2). For many decades the material was available generically known as Type "China" and "Chinese middle" cultivated by 90% of the national area (3, 4, 5). However, the international market demand rose fruit pulp, features not present national rates (4) result, export to international destinations such as the United States is reduced.
There are currently studied genotypes that will diversify the crop by selection, characterization, evaluation and utilization. The diversity of these resources will also deal with the emergence of new pests and diseases, or changing exotic microorganisms (6).

The fruits possess secondary metabolites such as carotenoids, which are responsible for the color, taste, smell and defense system (7) that contribute to the prevention of chronic degenerative diseases (8). These compounds have antioxidant capacity (9), ie they have a chemical structure conducive to capture, delay or eliminate free radicals (10, 11), responsible for oxidative stress. Ascorbic acid is found in high concentrations in the guava has specific functions in collagen metabolism, steroid hormones and aromatic amino acids, in addition to its antioxidant (7, 9). Inside the nutritional composition of guava grown in Mexico provides for Vitamin C 160.0 mg per 100 g edible portion for Type "half Chinese" (5).

Materials and Methods

Genotype was investigated L10A1 pink pulp and the new variety called S-XXI Calvillo having characteristics similar to commercial "Media China". The material was obtained through the Germplasm Bank belonging to the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). The fruits were analyzed in a mature state.
Was used for pH Pinnacle CORNING potentiometer. Total soluble solids (° Brix), were taken from a manual refractometer ATAGO with range 0-35 ° Bx. The instrumental color of the pulps were analyzed through the Hunter Lab colorimeter reflectance COLOR FLEX values for L *, ° ° Hue and Chroma. The weight was recorded for each fruit using a scale LEVAD (220 g). For the water retention rate and oil was performed according to methodology proposed by Sosulski, (1962), as amended, which was to get three particle sizes, 1mm, 500 micron and 250 microns, the samples were mixed with substances and then centrifuged for calculating weights differences. The AA content according to AOAC method 43 056 (12), using as dye solution dichlorophenolindophenol 2-6 where 5g sample was homogenized with extraction solution and centrifuged. The end point of the titration is considered when the solution turned pink persistent for 15 s. Lycopene was quantified by spectrophotometry proposed by Fish, (2002) (13), the

sample was added 5 ml of ethanol and 10 ml of n-hexane, stirred and then added 3 ml of deionized water for later reading absorbance. An analysis of variance with a Tukey-Kramer test was performed at a significance level of 0.05, with three replicates.

Results and Discussion

For pH, genotype recorded L10A1 3.88 (± 0.11), while for S-XXI Calvillo was 4.17 (± 0.14), showing significant difference (P <0.05). They correspond to the characteristic acidity of guava (5).

Total soluble solids were 13.53 (± 1.44) in L10A1 and 11.83 (± 0.44) for S-XXI Calvillo, showing significant difference (P <0.05), the maturing coincides with the increase in TSS content attributed to the hydrolytic degradation of carbohydrate polymers such as cellulose, starch and pectin, and thus the appearance of glucose units and the one provided galacturonic acid sweet taste and soft consistency (14).

For the flesh color of the parameters obtained for genotype L10A1 were * L 46.36 (± 1.93), Hue 29.91 ° (± 1.18), Chroma 29.92 ° (± 1.18) . Calvillo S-XXI results were L * 71.9 (± 2.13), Hue 91.38 ° (± 2.59), Chroma 26.25 ° (± 0.68). White pulp Calvillo introduced S-XXI higher brightness value with respect to the pulp pink due to red pigments such as lycopene present. The genotype Hue L10A1 ° corresponds to red shades found in tomatoes.

Calvillo S-XXI presented a weight of 57.00 g (± 9.47) placing in the second category (<60g) while weight was higher in L10A1 weighing 91.91 g (± 0.12) Extra corresponds to category (> 90g) (15). Weight is an important parameter for the performance offered to producers.

The water capacity and oil provides information about the functionality of the fibers in the human body as well as in foodstuffs behavior (16). The water absorption capacity was provided by the best Calvillo S-XXI genotype with a particle size of 250 μ, where the particle size directly affects the water retention. However, L10A1, in three particle sizes showed greater water absorption capacity that can be attributed to the constituents of the fiber (17), independent of particle size.

As for the oil absorption, S-XXI Calvillo showed no clear trend in the L10A1 particle size, showed an increase in oil absorption of the particle size dependent, so a larger particle size has greater oil absorption (Matos-Chamorro and Chambilla-Mamani, 2010).

AA concentration of Calvillo S-XXI matches reported for commercial type "Media China" (5) with a concentration of 162.01 (± 9.99) mg / 100 g. The L10A1 AA was 384.83 (± 57.01) and mg/100 g, more than twice the concentration.

Within sensory quality attributes related to consumer products fresh, the color, is closely linked to consumer perception (18). The color is determined by the appearance and / or disappearance of pigments in plant tissues (Melendez, et al., 2004) and lycopene. Lycopene concentrations were 1.36 ± 0.54 and 14.69 ± 3.67 mg / 100 g dry sample, respectively. The genotype L10A1 coloration meets international market demands while providing high levels of AA.

The genetic material belonging to INIFAP germplasm field has great potential for the exploitation of new varieties with competitive advantages.

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Viability Of *Lactobacillus paracasei* Encapsulated By Spray Gun

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Summary

Probiotics are live microorganism that confer various health benefits to consumers, provide there are consumed in adequate amounts (10^6-10^7 cfu/g). However, are affected by different factors, during processing and storage of food in which they are added, viable preventing them from reaching its site of action in the gastrointestinal tract. That is why is necessary to develop and apply new technologies to ensure the protection of these microorganisms against adverse conditions. So the aim of this work was the encapsulation and feasibility study of *Lactobacillus paracasei* by the method of spray gun and freeze-drying. Was evaluated the formulation of the emulsion to spray, as well as its particle size and viability in the emulsion. Assessing the physicochemical characteristics of the capsules obtained, such as humidity, color scale according to the CIE L*a*b*, flow properties and the effect of drying on viability of microorganisms encapsulated within 6, 8 and 10 hours. Was selected an emulsion formulation with a weight of 2 % sodium alginate, 2 % of native potato starch and 86% water, obtaining a particle size of 0.817 microns and a viability of $7.8x10^{11}$cfu/g of sample. The wet dry showed a humidity of 79%, a viability of $2.3x10^{10}$cfu/g of dry sample, while the capsules at the end of 10 hours of drying showed 77% moisture, a viability of $1.76x10^{10}$cfu/g of dry sample and good flow properties. While the color values for the dry capsules were L=83.35, C=11.06 and °Hue=-85.62.Concluding that at the end of the 10 hours of drying is more viable and less humid ensuring higher presence of viable organisms in the sample. The present study demonstrates that spray gun is an appropriate process to generate dried capsules of defined dimensions containing probiotic bacteria *Lactobacillus paracasei*, which retain viability during the encapsulation with alginate.

(Keywords: Encapsulation, Viability, Coacervation, Probiotics, Spray Gun)

Introduction

Today,the consumption offunctional foodshas increased significantlydue to itsimpact onthe preventionof gastrointestinal diseasescaused byvariousfactorssuch as stress andpoor eating habits (1). So thesearch for new products, to maintainthe health of thebody, has servedfor the development ofnew technologies forfunctional foodswithout affectingtheir sensory characteristics (2). The mainfood groupsusedas a basisfor the development offoodsthat benefithealth throughconsumptionarefermented dairy products.The dairy industryis theleader inthe creation, development, application and commercializationof products containingprobiotic (2). According toFAO andWHO(3), probiotics viablemicroorganismsare thosethat offerhealth benefits, provided they areconsumed inadequate concentrations(10^6-10^7CFU/g) (3).Among themicroorganismsreferredtoas probioticsfoundlactic acid bacteria, more particularly the*Lactobacillusparacasei*[2, 4].This microorganism is classifiedas anon-spore-forming lactic acid bacteria, lacking flagellum, whichcan growunder anaerobic conditions, but toleratesoxygen. Its dimensions are0.8 to1.0µmwideand 2.0 to4.0µmin length.Itsgrowth temperatureranging from 10°Cto 40°C, with the optimal37°C.Isisolatedfromchemical sources, sewage andfermented milk

products,whichiswhyonespeciesofprobioticmicroorganismsmost usedinthe food industry (5, 6).Probioticsmust meetcertain characteristicsthat improvethe resistanceof microorganismsduring processing andstorage of food, and, once it reachesconsumers, probioticsmust resistthe digestive process, tolerategastric juicesandbile saltsin order toreachaviableplaceof action andits effectssoensurehealth benefits (5).The technologies usedto protectthe microorganismsisencapsulation,for this,there are various typesofencapsulation methods, either by physical meanssuch asspray dryingitandchemicals such asliposomesentrapmentand thecoacervation (5, 7). However, somemethods such asspray dryingorliposomalentrapment, not been effectiveintheencapsulation,as they requirehigh temperaturesortheuseoforganic solventsthatdirectly affectthe viabilityofthe microorganisms.The coacervation is the separation of the solution into agglomerated particles in a coacervate phase called, in this phenomenon can be observed dissolving a gelling forming an emulsion with the core material. The simplecoacervationcontemplates the additionofstronglyhydrophilicagentto only one typeofpolymer.The complex coacervation, two or more polymers areusedfor different purposes (8).Two ofthe polymersmost used inencapsulationbycoacervationaresodiumalginateandstarchpotatoes.The alginateis a polysaccharideobtained fromseaweedconsistingofβ-D-guluronicacidand α-L-guluronic acid. Themost usefulalginatecontaining higher concentrations ofα-L-guluronic acid, improving the mechanical strength, high porosity andtolerates somesalt. Also it is highly used in the dairy industry, improving the texture of various products as yogurt, not to mention, it does not present any toxicity (9).Furthermore,thestarchisusedintheencapsulationpotentialretainencapsulated volatilecompoundsby other methods.Andinconjunction withthe alginateincreases theviabilityof the probiotic bacteriaencapsulated,thus demonstratingtheirpotentialprebiotic (8).The coacervation has been complemented by a new method for the preparation of capsules, the spraying by gun (10). This new method uses an air gun, not used in high temperature, gives a better structure to the capsules and their cost is low.So theaim of this studyis to evaluatethe viability of*Lactobacillusparacasei*encapsulatedbycomplex coacervationusingtwo polymers, sodiumalginateandpotato starchaswall materials, sprayedbyair gun. However,studies show thatthe digestive processaffects the viability ofprobioticmicroorganisms, thereby preventingthe bodyhealthyeffect. Therefore is necessary to developnew technologies that offerbetter protectionof probiotic microorganismsensuringits effectson the siterightaction.

Materials and Methods

Materials

The *Lactobacillusparacasei*strainwas provided bythe Instituteof Basic Sciencesat the Universidad Veracruzana, in its pure form, frozeninhalfwater/glycerol50:50 (v/v), kept at -40°C.The starch was obtained from local market potato, in the city of Xalapa, Veracruz.

Reactivationof strain and preparation of the emulsion

Reactivating a gram of strain according to the methodology proposed byHamayouni*etal.*(11) andPimentel-Gonzales(12).After reactivation,strain was separated from the culture medium by centrifugation at 1800 rpm for 10 minutes using a Universal Hettich centrifuge D-78532.Once separatedreactivatedstrain, twoformulationswere preparedemulsion accordingto themethod proposed byHamayouni*etal.*,(11). The first formulation (F1)consisted of anemulsionof 2%concentrationofnative potatostarch, 2% ofsodiumalginate, 10% purecanolaoiland 86% sterile distilledwater. The secondformulation (F2) waspreparedwith 1%ofstarch, 1% alginate, 5% oil and 93% water; preparing100 gofemulsion per formulation. The potatostarch, canolaoiland water werehomogenizedat 10,000rpmfor5 minuteswitha homogenizerWigger-HausserD-500. Subsequently sodium alginate is added and the mixture homogenized at 4,500 rpm for 5 minutes using a homogenizer IKA Eurostar 600.

Sprayingtheemulsion

Theemulsionwassprayedusingan air gunSURTEK108003, with a 0.5mmnozzleexit diameterand aspraypressureof 35 psiusingfor this,an air compressorDeWaltD2002M-WK. The emulsionwas

sprayedonto acalciumchloride solutionat 2%forthe crosslinkingofthe capsules formedinthe emulsion.Since it has beensprayedtheemulsion, the capsules are collectedby centrifugationat 2000rpmfor5 minutes. Freshly harvestedcapsuleswere driedby a freeze-dryerIlshinTFD5505,at a temperatureof-55°Canda vacuum pressureof5mmTorr.

Analytical Methods

Was identified the size of the capsules prepared in two formulations, through the quantification of percentage of more uniform size of the capsules, using a Coulter-Counter LS32. Additionally, the total count of viable cells encapsulated in the emulsion according to the methodology described by Semyonov*et al.*,(13).Physicochemical analysis was performed on the dry capsules of the selected formulation. Humidity according to AOAC (14), the study of color based on the CIEL*a*b* scale, and the chromaticity and color hue angle using a colorimeter V1-72 Flex. Was evaluated the impact of freeze-drying the viability of encapsulated organisms to 6, 8 and 10 hours of drying. Finally were evaluated the flow properties of the dried capsules (13).

Results and discussion

The capsules obtained with F1 showed a larger particle size (0.817 µm), while the capsules obtained from F2 had a smaller particle size (0.696 µm), yet no significant difference in the particle size or morphology of the capsules obtained in both emulsion formulations. As reported bySandoval*etal*.,(7),witha higherparticle size,the more likelyencapsulation, so thatthe viabilitywill depend onhow largethecapsuleprepared andencapsulationcapacityof thecapsule[7].Forthis, we conductedan analysis ofthe viabilityin bothemulsion formulations, resulting in improved viabilityin the formulationF1(7.8x10^{11}CFU/gof wetbase), while forthe formulationF2showed alower growthof4.5x10^{10}CFU/g(wet base).However,nosignificant differenceobservedin the microbiological countsofthe twoemulsionformulations (Table 1).

Table 1.Viability of *Lactobacillusparacasei*encapsulatedin differentemulsionformulations.

Sample	F1 Formulation (CFU/g)	F2 Fromulation (CFU/g)
Emulsion	7.8x10^{11}±9.0738	4.5x10^{10}±12.124
Fresh Capsules	1.82x10^{11}±7.0238	5.04x10^{10}±12.22
Dry Capsules	1.16x10^{8}±2.5166	7x10^{4}±3.4641

Jimenez (2011), reports a better viability (2.4x10^{8} CFU/g), with concentrations of 2% native potato starch and 2% sodium alginate (2). So that F1 was selected to perform this work. We obtained a value of 79.13% (wet basis) and a water activity of 0.987 ± 0.0023 for fresh capsules.After obtaining the fresh capsules was evaluated the effect of drying optimizing the time of drying of the capsules to 10 hours, in order to ensure better viability and less stress for the microorganism (Table 2).

Table 2.Effect ofdrying timeon viabilityof themicroorganism*Lactobacillusparacasei*.

Time (hoursdrying)	CFU/g (wb)	%Solid/g (wb)	a_w	CFU/g (db)
0	4.95x10^{9}±0.7071	20.8704±0.2039	0.987±0.0023	2.3x10^{10}±1.0677
6	9.9x10^{8}±14.364	22.7761±0.5185	0.388±0.0095	4.3x10^{9}4.773
8	4.1x10^{8}±3	21.803±11.363	0.182±0.0157	1.8x10^{9}±90.884
10	4.04x10^{9}±4.2624	22.7106±0.0745	0.127±0.0538	1.76x10^{10}±12.162

After dryingthecapsuleswasnecessary to evaluatetheir flow propertiesto evaluate the effectof drying onthe wall of thecapsulesand offeran ideaof howholloware thecapsules made. The values foundinthe capsules areshown in Table3.As reported byKagami*etal*.,(2),the percentageofcompressibilitydepends directly on thesize and number ofthegroovesorrecessespresent inthecapsule wall. Inthiscase the valueofthe particle densitygives an ideaofthe gapsinourcapsule.So, looking atthese valuesin the above table, we can deduce thatthedry capsuleshavemanyporesin its wall. The flow of powder is
determined by the static angle of repose.

The angle of repose (without considering as measured) can be used as an approximate indicator of fluidity. According to Carr (14) the angles less than 35° indicates free-flowing, some cohesiveness 35-45°, 45-55° cohesiveness (free fluidity loss) and 55° or larger and high cohesiveness free flowability very limited (14).ln the next table (Table 3) shows a value of 31.8859 ± 1.617 for the angle of repose, indicating that the capsules have a free flow, this value can be affected by several factors such as the size of the capsule, the cohesion or the morphology of the particle. A borderless particle has a better flow than those with irregular or granular.

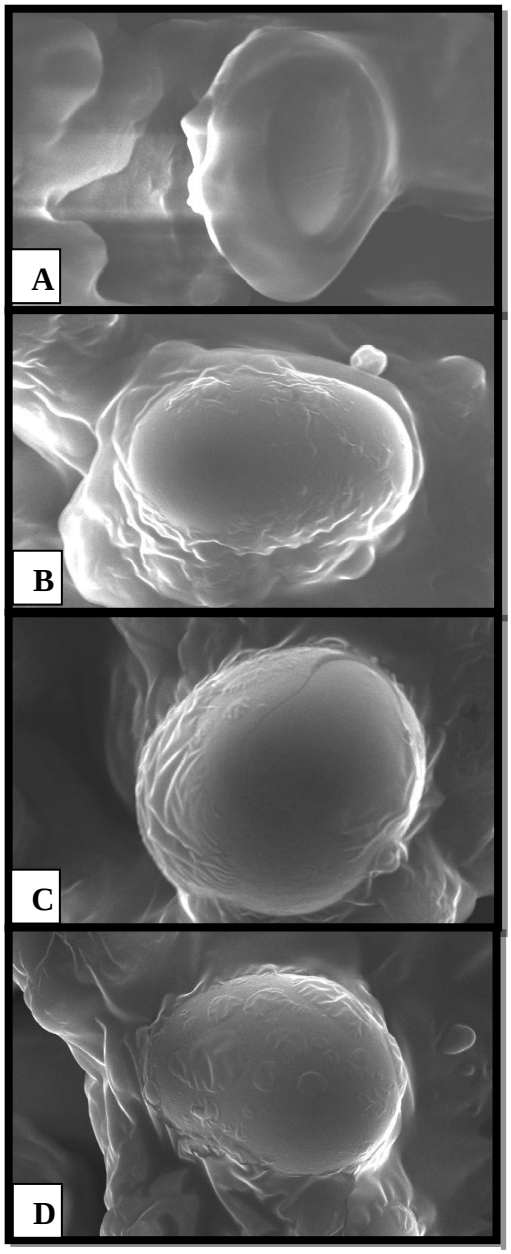

Figure 1. Dry capsules under different water activities 0.0 (2.A), 0.536 (2.B), 0.765 (2.C) and 0.903 (2.D) storage at 25°C for 30 days.

Table 3.Flow Propertiesofthedry capsulesprepared

FlowProperties	Value
BulkDensity (g/mL)	0.1954±0.0146
TappedDensity (g/mL)	0.244±0.0089
Particle Density (g/mL)	0.634±0.0615
Angle of Repose	31.8859±1.617
Hausner Index	1.24±0.096
Compressibility	79.78±6.32

To better observe the morphological characteristics of the capsules produced, it was necessary to use electron micrographs (Figure 1), which allowed us to observe the behavior of these capsules when subjected to environments with different water activities, (0.0, 0.536, 0.765 and 0.903),storaged at 25°C for 30 days.It can be seenthatahigherwateractivity, thecapsule wallundergoes a modificationinitsstructure,because it absorbs water by the membrane wall and begins to form a aqueous layer around the capsule.
For analysis of color, the L*a*b* parameters were evaluated, which are shown in Table 4. From these values is obtained and the hue angle chromaticity of dry capsules.

Table 4.Colorparametersof dry capsulesby freeze-drying.

Parameter Color	Value
L*	83.0933±0.74
a*	-1.06±1307
b*	10.8166±0.3189
Chroma	11.0618±4157
°Hue	-85.6257±1.6974

Conclusions

Greater protection and viability ($7.8x10^{11}$CFU/g) was obtained in an emulsion with greater amount solids. Is possible to encapsulate the microorganism *Lactobacillus paracasei*bycomplexcoacervationmethodand sprayed by air gun at a flow pressure of 35psi, a nozzle of0.5mm and a height of 10cm. Obtaining capsules with a moisture content of 79.13%, and activity water of 0.987 and aviability of $1.82x10^{11}$CFU/gw hich were dried by freeze-drying. Was standardized drying time of capsules to 10 hours by freeze-drying, capsules obtaining a solids content of 22.7106% and a viabilityof$4.1x10^{9}$CFU/g. The dry capsules exhibit high fluidity, according to the value of its angle of repose, however, has an irregular shape and a high compressibility. Dry capsules were observed under different water activities at a temperature of 25°C, highly hygroscopic behavior reported by the capsule wallat water activities medium-high. While for water activities close to 0.0, the wall of the capsuleremained intact. The dry capsules showed a color with a high luminance (L*=83.0933), a low concentration of color (Chroma=11.0618), and a very wide angle(°Hue= -81.6257). Describing bright white capsules, but they are very sensitive to light and high temperatures, changing its color parameters.

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Antioxidant Activity Of Blackberry (*Rubussp*) Juice In Powder

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Abstract

Blackberry is an important source of colored substances, mainly anthocyanin, which are known for their high antioxidant activity. These pigments are principally flavonoids which give blackberries their characteristic red to blue color. Also, the total pigment extract which is produced from blackberry fruits has been widely used as a natural colorant in beverages, baked products, chewing gums, jellies and fruit wine making. Many studies have demonstrated the antioxidant activities and health benefits of the anthocyanins occurring in various fruits and vegetables, however, one of the disadvantages of anthocyanin is its low stability, which limits its use as a food colorant. So that, the objective of this study was to evaluate the antioxidant activity, flow properties and color on a blackberry juice powder, obtained from blackberry juice with gelled starch. Blackberry juice was clarified, containing 6% of soluble solids; which was mixed with an equal volume of water and 5% of native starch, the mixture was heated to 90°C. Then it was dried until to water activity was 0.311. The loss in the anthocyanin content in blackberry juice in powder was 67.86%. The polymeric color content was 73.17%,the scavenging activity in powder was of 83.98%, and total phenols content of 5.7 mg GAE/100g SS. The content of moisture in powder was 5.13%. The color values for the blackberry juice in powder were L=31.34, C=27.65 and H°=14.84. The powder has a good flow and efficiency according to their flow properties.Therefore, the powder produced in this study represents an interesting food additive for incorporation into functional foods, both as an antioxidant and as a colorant.

(Keywords: anthocyanin, food colorant, antioxidant activity, blackberry, powder).

Introduction

Today the food industry uses synthetic additives, between which we can find the dyes, but the use of these additives causes adverse effects on the health of consumers. Recent research has tried to find stable and less toxic dyes, like natural colorants presents in the fruits (1,2,3). Blackberry (*Rubus sp.*) is a small fruit belonging to the *Rosaceae*family,these fruits have a high content of water and fiber, and also is a major source of anthocyanins, which are responsible for the attractive red to purple to blue colors (4), these pigments making to the blackberry an important fruit to obtaining natural dyes (5,6,7). Anthocyanins are plant pigments,with antioxidant activity because the anthocyanins have a chemical structure suitable to act as antioxidant (8), this factcauses that anthocyanins are associated with disease prevention such as cardiovascular diseases, neurological diseases and cancer (9). In this case, theantioxidant potential of the anthocyanins depends on the number and position of hydroxyl groups and conjugation, as well as the presence of electron donors in the structural ring.These plant pigments also have a high potential as natural colorant. Anthocyanins are relatively unstable and often undergo degrative reactions during processing and storage, that is why its application is limited. The production of the anthocyanins powder is the answer to its limitation, because this technique helps to extending shelf life, bioavailability and processing stability. There are many ways to combat with the problemthat presented the anthocyanins in terms of stability. Microencapsulation is one of the forms of protection. This suggests several methodologies as physical processes, physicochemical processes, and chemical processes. After taking

into consideration the substance to be encapsulated must be considered encapsulating process, as well as the encapsulating material. There are many types of hedges used for microencapsulation as gums, celluloses; lipids, proteins, and carbohydrates. Starch is the one of the carbohydrates most used in the food field. The gelled starch enclosing the anthocyanins within the matrix protects from many factors that affect their stability, during processing and their shelf life, like temperature, pH, and oxygen mainly.The aim of this work was to evaluate the antioxidant activity, flow properties and color on a powder, obtained from blackberry juice with gelled starch.

Material

The blackberries used to carry out this work were obtained from an organic garden located in the city of Coatepec in the state of Veracruz, Mexico. The fruits were collected in optimum ripe state.

Storage conditions

Fruits were stored at -40°C while being used.

Methods

Juice extraction

Fruits were crushed and then were passed through a sieve to avoid the presence of seeds. This juice obtained from the crushed was sonicated, with a digital sonifier model 250 Branson (Eagle Rd., Danbury, U.S.A), to promote the break of the cell and count with a greater presence of anthocyanins. Then the juice was clarified by centrifuging in a centrifuge HettichZentrifugen Universal 32 R (D-78532 Tuttlingen, Germany) at 4000 rpm for 5 minutes at 5°C, finally was filtered with vacuum.

Preparation of powder of blackberry juice

The preparation of powder was to develop a mixture of 47.5% water, 47.5% of clarified juice and 5% of native starch. The mixture was heated with stirring to 90°C. Later of gelled starch present in the mixture, this was served in plastic boxes, which were entered into an air oven model 132Felisa (Alfonso GarzónSantibañez Nº 7, Zapopan, Jalisco, México) at 40°C until the mixture in the plastic boxes get to awater activity of 0.300. The films formed in the plastic boxes were separated and pulverized using a blender model 6640 Osterizer (México).The resulting powder was sieved with a laboratory sieve number 60 to give a uniform size.

The physicochemical properties analyzed were moisture, °Bx according to the AOAC (16), and the water activity was measured with water activity meter AquaLab series 3 (Labcell Ltd., Basingstoke, Hants, UK).

The antioxidant activity was measured in juice and powder. The determination of DPPH radical scavenging activity was analyzed, the reducing power was determined according to the method of Oyaizu (17); the content of anthocyanins and percent polymeric color also were evaluated by differential pH method, total polyphenol content was estimated by the Folin-Cicalteu assay according to the method of Singleton (18).

The flow properties of powder angle of repose, bulk density and tapped density were evaluated by method of Shah (19);compressibility index and Hausner ratio were calculated from compressibility data by method of Aulton (20). All determinations were carried out in triplicated to average data and get the final results.

Results and discussion

The table 1 shows physicochemical properties as antioxidant activity found from juice and powder. The soluble solids in clarified juice was of 6%, this represents half of that reported in a study to blackberry variety Brazos (21), because in this case the juice was clarified; and water activity of 0.980, whiles the powder of 0.311. The figure 1 shows the powder made with the blackberry juice, the moisture in powder was 5.13% which is than that reported for blueberry microcapsules (22). Regarding to the antioxidant activity the powder presents a decrease of 67.86% of anthocyanins; another works reported that the anthocyanins content between a blackberry puree and a powder made from puree is the same, which is because the powder was elaborated by freeze dried, rather than by thermal treatment (23). It is widely known that phenolic compounds present in fruits are responsible for their antioxidant activity due to the

number of hydroxyl groups (24);in this work the powder had 5.7mg GAE/100g ss.regarding to the scavenging activity express by DPPH, in powder was 83.98% which is above that reported following the spray-drying methodthat was 56% (25). The absorbance reported for the powder in the reducing power is low with respect to the juice but higher than the reported for powder made with spray drying (25), indicating a good antioxidant activity for powder. Finally the polymeric color was increased in the powder.

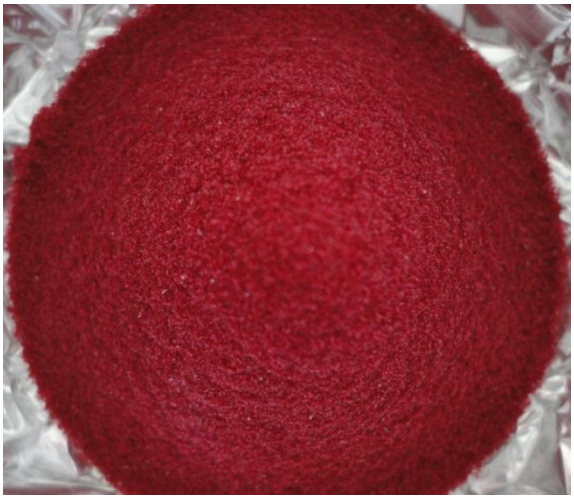

Figure 1. Powder made with blackberry juice.

Color parameters of the powder are reported too in Table 1. Previous research have been interpreted the increase in L* value in model system colored with anthocyanins from different vegetables sources as indication of discoloration due to anthocyanin degradation (26, 27, 28). The intensity of color (Chroma) shows higher values for the powder due to addition of the starch. While the °Hue increased by one unit. The total color difference between the juice and powder was of 27.57 units, this maybe because of low retention of anthocyanins that is achieved during the process and the degradation of anthocyanins for a thermal treatment.

Table 1. Values of phisycochemical properties and antioxidant activity of juice and powder.

Analysis	Juice	Powder
°Brix	6 ± 0.200	-
Moisture %	-	5.13±0.140
a_w	0.980±0.005	0.311±0.008
Anthocyanin (mg cya-3-glu/100g ss)	784±0.207	252±0.162
Total phenolics (mg GAE/100g ss)	11.9±0.019	5.7±0.007
Polymeric color %	32.35±14.88	73.17±14.95
DPPH scavenging activity (%)	87.60±4.110	83.98±1.820
Reducing power (Abs)	1.149±0.100	0.989±0.113
Color parameters		
L*	6.68±0.281	31.34±0.945
a*	14.96±0.346	26.73±0.804
b*	3.51±0.389	7.08±0.210
°Hue	13.18±1.245	14.83±0.372
Chroma	15.37±0.399	27.65±0.811
ΔE	27.57±0.831	

The table 2 presents the results with respect to the flow properties of powder. The angle of repose was of 18.76 which is a low value, according to Carr (1976), this angle indicates free flowing.This factor is favored with a smaller particle size, lower humidity and greater homogeneity, principally (30). The Carr's

index representing the percent of compressibility was of 10.94 and the Hausner ratio 1.12 according with these results the flow and efficiency in the powder is very acceptable (29, 30).

Table 2. Flow properties of powder made with blackberry juice.

Properties	Powder
Angle of repose	18.76±0.52
Bulk density (g/cm^3)	0.65±0.04
Tapped density (g/cm^3)	0.73±0.04
Carr's index (%)	10.94±0.94
Hausner ratio	1.12±0.01
Particle density (g/cm^3)	0.53±0.06

Conclusions

The results indicates that the blackberry *Rubus sp.* is rich source of anthocyanins, and antioxidants, since in this work shows a content of 784 mg cya-3-glu/100 dw in juice, although the total content in powder was of 252 mg cya-3-glu/100 dw. Total phenolics were reduced by almost 50% with respect to the juice, while the polymeric color was increased. The scavenging activity and the reducing power were slightly decreased.

The total color difference was of 27.57 units between juice and powder, the L* parameter shows increased because of the anthocyanins degradation.

At last and in accordance with the results obtained in this work, the powder shows a very acceptable flow, movement and efficiency.

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Antioxidant Activity Of The Capsules Of Juice Blackberry (*Rubus fructicosus)* Using Spray Drying

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Abstract

Blackberries have an excellent source of vitamins, especially vitamins A and C, who makes this fruit a good antioxidant, and the abundance of anthocyanins and carotenoids. This antioxidant activity they possess can help combat degenerative diseases such as cancer. A practical way to obtain the antioxidants of this fruit is through the encapsulation, and to protect them, for easy handling, spray drying is widely used, because it is very simple and provides a high encapsulation efficiency, this process need of a wall material, which helps to create a cover to protect the bioactive substances, one of the most used are maltodextrins, however it is believed that the wall material interferes with the antioxidant activity once encapsulated juice, is why the aim of this study was to evaluate the effect of wall material on the antioxidant properties of the juice of blackberry (*Rubus fructicosus*) encapsulated by spray drying. The methodologies used to evaluate this activity were determination of anthocyanins, total polyphenols, reducing power and DPPH colored polymer. The results obtained in the juice were anthocyanins: 603.99 mg / L + SD 103,776, in polyphenols were obtained: 1100.86 + SD 186,327 in reducing power: 1,670 + 0,201 SD, polymeric color: 64.8 + 8,980 SD and DPPH: 91. 611 + 2,668 SD, while the capsules obtained are as follows blackberry; of anthocyanins: 381.20 mg / L, Polyphenols: 1271.13 + 122.14 SD in reducing power: 1,283 + SD 0.0602, polymeric color: 74.2 + SD 16,585. It is observed that the anthocyanins in the capsules decreased in comparison to the juice, but the amount of anthocyanin is still acceptable since the coloration in the capsules is good, in Polyphenols there is considerable variation in the results of the juice in terms of capsules, indicating that these compounds are not lost and that the antioxidant activity is high, in terms of reducing power shows no significant decrease in the capsules so that the redox capacity is not lost, and color polymer is observed which is greater in the capsules

(Key words: Antioxidant activity, Rubus fructicosus, capsules, spray drying)

Introduction

Currently there are a number of serious diseases affecting man, as cancer, which occurs when the balance between radical free and antioxidants is lost for the first, then is when trigger processes harmful, generating in turn called oxidative stress that is caused by various factors. However there is a way around it and one way is obtaining natural antioxidants. BlackBerry (Rubus fructicosus) is a fruit rich in vitamins A and C, which makes it a good antioxidant, in addition to the abundance of anthocyanins, phenolic acids and carotenoids that owns. This type of fruit is the most important sources in the diet, thanks to the anthocyanins and phenolic compounds, BlackBerry has its characteristic color of natural pigments (2). However, to maintain the stability of these compounds, there are methods of encapsulation and spray drying is one of the best today. This in turn requires wall materials that help to protect to the active metabolite, encapsulated, one of the most used are the maltodextrins, however such material interferes with the stability of the compounds. That is why the objective of this study was to evaluate the antioxidant properties of the juice of BlackBerry (Rubus fructicosus) as well as also to evaluate the antioxidant stability of capsules, using drying spray.

Materials and Methods

Material

Juice Processing. The fruit of BlackBerry defrosted at room temperature of 25 C, after this they macerated with pistil in a colander and Bowl where the juice (protected from light) was collected. The

pulp of the fruit pass through an ultrasound, model and brand Digital Sonifer C / probe watts 250 time 10 min at intervals of 10 seconds, with a range of 65% and 800.

Encapsulation process. Was used as material of wall maltodextrin (MD), once the juice was prepared a proportion to 100 ml of juice to encapsulate, 62.5 ml juice of fruit, 22.5 g MD and 15 ml of water. Everything is mix and process in the hair spray.

Method anthocyanins monomer. According to the technique of Wrolstad, (3), (with some modifications) prepared the necessary reagents: KCI 0.025 M buffer pH 1 and 0.4 sodium acetate buffer pH 4.5 M. Found the specimen dilution factor making a sweep of 200 to 700 nm wavelength in a spectrophotometer UV-Vis Varian, model Cary 100 Conc. The anthocyanin content expressed as cianidin-3-glucoside using your 510 nm maximum absorbance. The dilution factor used in this study was 54.5 ml juice and capsules was 26 ml. After you determine the dilution factor samples were prepared in triplicate using buffer of pH 4.5 and pH 1, remained idle for 15 min and read the absorbance at 510-700 nm.

Total polyphenols. A container in amber placed 0.1 mL of the sample and 0.5 mL of Folin-ciocalteu reagent, after 5 minutes added 0.4 NaC02 mL to 7.5% and conveyances to 5 mL. It was left undisturbed sample for 60 min and read at 740 nm. For the calculations was a calibration curve using as reference the Gallic acid from the equation of the straight line calculated the amount of acid present Gallic/L according to the absorbance of the sample (5).

Power reducer. 0.25 ML of sample were added 2.5 mL pH 6.6 sodium phosphate buffer, 2.5 mL of 1% potassium ferrocyanide mixed and incubated for 20 minutes in a water bath at 50 ° C, added 2.5 mL of 10% trichloroacetic acid. They were 5 mL of the mixture and 5 mL of distilled water and 1 mL of ferric chloride were added. This solution was immediately read and up within an hour of having been prepared at 700 nm using as white (6) sodium phosphate buffer.

DPPH* (2*, 2-difenil-1-picrilhidrazilo). A DPPH 0.1 methanolic solution used mM, to prepare samples placed 0.1 mL of this in 2.9 mL of DPPH and rested for 30 minutes covered with the light to read the absorbance at 517 nm in a spectrophotometer JENWAY model 6305. At the same time prepared a target with 0.1 mL of the sample and 2.9 mL of methanol and a control with 0.1 of methanol and 2.9 mL of DPPH, which they stored and read at the same time the samples. The percentage inhibition of the radical DPPH was calculated by an equation. (1). % inhibition = Abs control - shows Abs / Abs control.

Determination of color. The color was measured using a spectrophotometer for color Color brand flex, Hunter Lab model 45/0, calibrating the equipment previously (as indicated by the provider) before eading the samples, using as a parameter more important a* It was triplicated.

Polymeric color. For polymeric color and density of color analysis, samples were prepared in triplicate in accordance with the retrieved dilution factor to know the ml of sample, this added 0.2 ml of 0.2 ml of distilled water and potassium Metabisulphite.Samples were read at 420 nm, 510 nm and 700 nm and color density is calculated as follows: color density = [(A420_nm_-_A700nm) (A510 - A700 nm)] x DF (dilution factor), while the color and polymeric color % polimericose calculated using the following formulas away: polymeric color = [(A420nm-A700_nm) (to vis-max - A700 nm)] × DF and polymeric color percent = (polymer color / color density) × 100. (1)

Results and Discussion

Once evaluated the antioxidant activity of juice and capsules from juice of BlackBerry (Rubus fructicosus) results were as follows in the juice anthocyanin content was 603.99 mg / L 103,77,en polyphenols: 1100.86 186,327 mg, 1,670 reducing power 0,201, polymeric color 64.8 8,980% and DPPH: 91.611 2.668%, while the capsules was obtained in anthocyanins 381.20 mg / L, polyphenols: 1271.13 122.14 mg, reducing power: 1,283 0.0602 and polymeric color 74.2 16,585% (table 1). The contents of anthocyanins is greater in the juice in comparison to the capsules you, however the coloring on them is high as shown in Fig. 1

Fig 1. The juice of BlackBerry (Rubus Fructicosus) capsules.

As regards the polyphenols content is slightly larger in capsules by what its antioxidant capacity is lost, reducing power is more high in the juice for what the wall material interferes with this oxide reduction capability, the percentage of polymer color is also more high in the capsules, this means that there is greater resistance to discoloration after encapsulation. Without a doubt wall material used (MALTODEXTRIN) has an important effect in each test already in some helps and protects to antioxidants but others not. These anthocyanins values reported in previous research: 440.34 9.21 mg, total polyphenols: 1.134 0.73 mg and antioxidant capacity of 2.49 0.57 µmol ET/100 g (3). This work also do a physico-chemical analysis where measured the color and properties of flow of the capsules, the results can be seen in table 2.

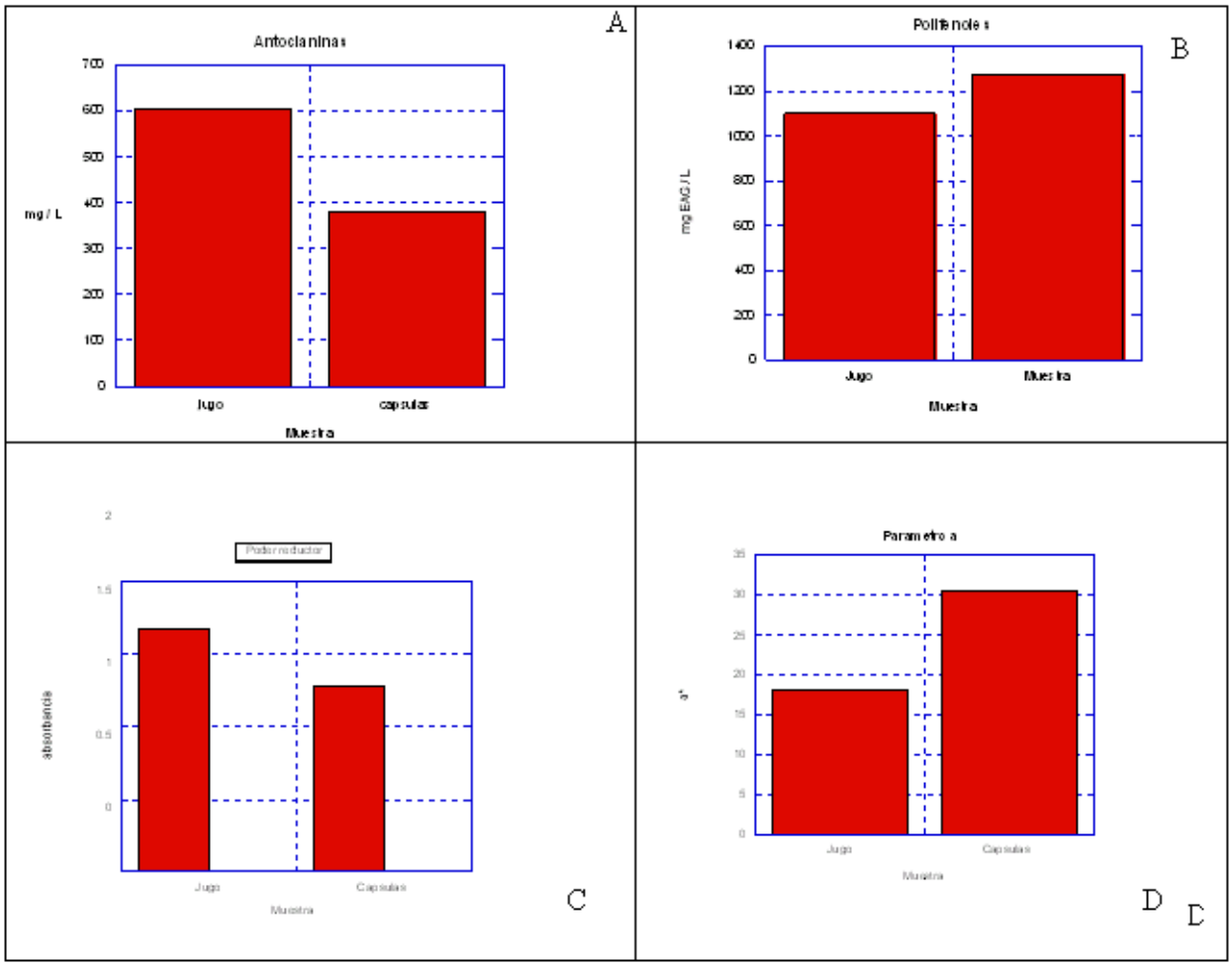

Fig. 2. Graphs showing the behavior of the juice and capsules during the tests carried out. Anthocyanins (A), (B) polyphenols, reducing to (C), (D) parameter a*

Table 1. Correlation between anthocyanins, color polymer, power reducer, DPPH and polyphenols of capsules with juice

Property	Juice (Average)	Capsules (Average)
Anthocyanins (mg/L)	603.99 + 103.776	381.20
Polymeric color %	64.8 ±8.980	74.21 ± 16.585
Power reducer	1.670±0.201	1.283± 0.0602
Polyphenols (mg)	1100.86±186.327	1271.13 + 0.0602
DPPH %	91.611±2.668	

Table 2. Flow properties in capsules

Property	Capsules
Bulk density (g/ cm^2)	0.36096 ± 0.00644
Particle density (g/ cm^2)	0.7392 ± 0.1684
Compact density	0.4926 ± 0.0137
Compressibility	90.7933 ± 0.6572
Index of Hausner	10.8966 ± 0.7492

Conclusion:

In conclusion, the wall material used in the encapsulation is certainly an important factor, since it intervenes in the analysis that is applied to the sample, helping to protect antioxidants or not, since then each wall material will speak differently according to their characteristics.

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Development And Formulation Of Symbiotic Petit Suisse Cheese

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Introduction

The development of functional foods is one of purpose of the investigations in the industry of food. In the last few years, great attention was dedicated to probiotics and prebiotics or their combined use (synbiotics) to improve human health in a natural way. Some lactic acid bacteria (LAB), particularly *Bifidobacterium* make an extremely important group of probiotic bacteria. As members of the autochthonous microflora of the gastrointestinal tract of humans and animals, they offer considerable potential as probiotics because of their history of safe use and the general body of evidence that supports their positive roles (*1–4*). Namely, probiotics are microorganisms which had been included in food for many years without any adverse effects or which were present in the gastrointestinal tract of the healthy humans and animals (*5*).

A strategy for increasing their number is to supply those already present in the intestine with selective carbon and energy source that provides them with competitive advantage over other bacteria in this ecosystem, thus selectively modifying the composition of the microflora using dietary supplements. These selective dietary components were named »prebiotics« by Gibson and Roberfroid in 1995 (*6*). They are specific, naturally occurring carbohydrates, mainly of plant origin, and fructo-oligosaccharides (FOS) are mostly known representatives. The concept of »synbiotics« (a mixture of probiotic and prebiotic) has recently been proposed to characterize health-enhancing food and supplements used as functional food ingredients in humans (*6–8*).

During the last decade, many studies have been conducted on the viability of probiotic bacteria in fresh fermented dairy products (9-10). However, due to the acidic nature of these products, probiotics show low viability in yogurt and fermented milk (11). On the other hand, cheese may offer certain advantages over fermented milk products in terms of delivery of viable probiotics. This results from the fact that cheese tends to have a higher pH, a higher fat content and a more solid consistency, all of which should afford added protection to the probiotics in the gastrointestinal tract (12).

The Petit Suisse cheese has a great acceptance in the public child; this could likewise be consumed by adults because of its unique flavor, texture and nutrients by the organism. However, studies show that the addition of probiotic cultures directly in food presents undesirable organoleptic changes and it has not shown high viability of the bacteria from preparation to consumption of food, because these are subject to conditions of the gastrointestinal tract, resulting in the reduction of such bacteria, lowering the beneficial effect that might make, which is why, that microencapsulation presented as a solution that allows the bacteria to introduce a capsule, protecting them from the environmental conditions and release at the required time, so that the encapsulation is a viable alternative to prevent these phenomena (13).

A crucial point in the encapsulation process is to maintain the concentration of probiotic cultures higher to 10^6UFC/g of product, where the latter is required to add it to a food, allowing their proliferation and provide the beneficial effect on the host. One of the methods used in the encapsulated bacteria is spray drying, due to the short times employed and the particle size obtained (~ micrometers) make it suitable for the formulation of food (14). That is why in this paper we used different formulations of Petit Suisse cheese with added probiotic cultures encapsulated by spray drying, which were subjected to sensory

analysis, where the accepted formulation submitted gastrointestinal simulations to evaluate the viability of probiotic cultures as well as the presence of the fibers (prebiotic).

Development of Petit Suisse cheese symbiotic. Petit Suisse cheese was obtained in a conventional manner, and various formulations were prepared from cheese, cream, strawberry, sucrose, maltitol, xanthan gum, soluble fiber (Nutriose) encapsulated probiotic cultures and carmine dye.

Determining shelf life of Petit Suisse cheese symbiotic. The shelf life of the product was determined from the acidity (lactic acid production), viscosity and viability of probiotic cultures. Where the acidity analysis was performed to 9 grams of sample was titrated with 0.1 N NaOH using phenolphthalein as indicator, thereby calculating the percentage of lactic acid is considered as one acid present in the product, using the following equation:

$$\%Ac.\,lá\,ctico = \frac{(N_{NaOH})(V_{NaOH})(meq\ Ac.\,lá\,ctico)}{g\ de\ muestra} \times 100$$

The viscosity was evaluated using a Brookfield viscometer with a No. 7 needle temperature, noting the percentage of torque. And finally the viability of the probiotic cultures was carried out using the technique described above.

Simulation gastric Petit Suisse cheese symbiotic. Two grams of the product were subjected to simulated gastric, which was conducted using a simulated gastric juice solution (HCl and pepsin at pH 1.9 for 30 min) and continuously, to a solution of simulated intestinal juice (bile salts and pancreatin at pH 7.5 for 5.5 h) at a temperature of 37°C verifications changes in the total content of the live bacteria in the digestive mixture.

Sensory analysis. The product was presented to 100 judges trained in age from 6-12 years (children), using a five-point hedonic scale (very good, good, fair, poor, bad) by faces, analyzing the attributes of color, taste, smell, texture and overall assessment.

Results and discussion

The table 1 shows the viability study of probiotics cultures, where *Bifidobacterium lactis* and *Bifidobacterium infantis* present a high resist to encapsulation process using skim milk like encapsulante agent (15 w/v) and a temperature of 170°C, with the loss of one logarithmic cycle. Also, the spray powder of *B. lactis* was added to a food presented a $3.1x10^{12}$ CFU/g of product. FAO indicates that like a product could be named "probiotic", these have to present a $1x10^{6}$ CFU/g of product; our concentration is higher in the finished product. The gastro-intestinal simulations show the presents of $8.1x10^{7}$ CFU/g of product of probiotic culture (*Bifidobacterium lactis*), this allows ensure that the benefic effect of probiotics culture will be to exercise in the gastro-intestinal gut human.

Tabla 1. Viability study of probiotics cultures of *Bifidobacterium* genes in the encapsulation process and finished product

Exp.	*Probiotic culture Bifidobacterium genes*	UFC/g of product before of encapsulation process	UFC/g of product after of encapsulation process	CFU/g in finished product	CFU/g in finished product front gastro-intestinal simulation
1	*B. lactis*	$3,2x10^{12}$	$1x10^{11}$	$3,1 \times 10^{10}$	$8,1 \times 10^{7}$
2	*B. infantis*	$4,5x10^{10}$	$2,0x10^{9}$	-	-
3	*B. lactis*	$5,0x10^{11}$	$3,5x10^{10}$	-	-
4	*B. lactis*	$4,4x10^{11}$	$3,3x10^{10}$	-	-

The table 2 shows the different formulations by the development of Symbiotic Petit Suisse Cheese, where the final formulation presents to be the most optimal for a symbiotic food with a 3,2% soluble fiber content and 2,0% of probiotic cultures. In the table 3 presents the composition of final product, it shows a 3,2% dietetic fiber content. Investigations show that concentrations higher than 1,2% of fiber in a food, it can be to consider like prebiotic (Cardarelli *et al*., 2008). Moreover, if a food presents adequate concentrations of probiotic cultures and prebiotics, these allow defining to Petit suisse cheese like a symbiotic food.

Petit Suisse cheese like a symbiotic food was evaluated by microbiological analysis, using the different technical of the Norma Oficial Mexicana (NOM-121-SSA1-1994) specific to QUESOS: FRESCOS, MADURADOS Y PROCESADOS. The present of total colifoms, *Staphyloccus aureus*, mushrooms and yeast and *Salmonella*, do not exceed the permissible limits, this indicates that the product enjoy of a high sanity quality (table 4).
In the determination of shelf life of product, in the table 5, we observed that 23 day, the production of lactic acid is of 2,3%, also the viscosity was of 7200 cps; in this day the product presents flavor, smell and consistency good by the consumers; moreover for 27 day, the product was reject becose the acid flavor and unpleasant consistency; we can conclude that the Symbiotic Petit Suisse Cheese presents 23 days of shelf life.

Table 2.Formulation of Symbiotic Petit suisse cheese

Ingredients (%)	Formulations				
	1	2	3	4	Final
Cheese	63,9	63,9	63.9	60,0	59,0
Cream	14,0	14,0	14,0	16,0	12,5
Natural Fruit	11,0	11,0	11,0	10,0	14,0
Sacarose	10,0	5,6	10,0	13,0	4,5
Maltitol	-	4,4	-	0,4	4,5
Gum xantan	0,5	0,3	-	0,5	0,3
Grenetin	0,5	-	0,5	0.4	-
Flavoring	0,04	-	0,1	0.1	-
Dye	0,06	1gota	-	-	1gota
Soluble fiber					3,2
Probiótics					2,0

Table 3. Percentage composition of Symbiotic Petit Suisse Cheese

Composition	Energetic Information (kcal)	
	Percentage (%)	Portion de 45 g
Carbohidrate	18,58	8,36
-Dietetic fiber	3,2	1,44
Protein	4,51	2,03
Fat	2,98	1,34
	73,3	0
Ashes	0,63	0
Total	**100**	**46,88**

Table 4. Results microbiological realized to final product (Symbiotic Petit Suisse Cheese)

Microorganisms	Permisible limit	Results
Fecal coliforms (UFC/g)	100 UFC/g	<10 UFC/g

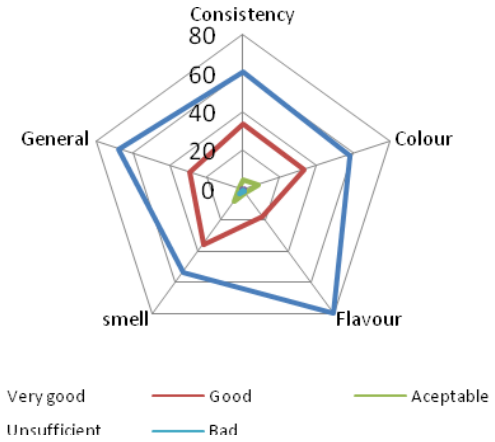
***Staphylococcus aureus* (UFC/g)**	1000 UFC/g	< 100 UFC/g (coagulasa -)
mushrooms and yeast (UFC/g)	500 UFC/g	<10 UFC/g
Salmonella en 25 g	AUSENTE	AUSENTE

Table 5. Analyzing of production of lactic acid and viscosity of final product

Day	Lactic Acid (%)	Viscosity (cps)
1	1,1	55000
3	1,2	58500
6	1,5	63200
15	1,7	65100
19	1,9	68700
23	2,3	71200
27	-	73000

Finally, the sensorial analysis, it shows that the attributes of consistency, colour, flavor and general evaluation. It shows that Symbiotic Petit Suisse Cheese presents that the judges (child of 6-12 years old) considered the product like very good (60-85%), good (20-40%). Finally the product presents to be competent.

Graphic 1. Sensorial Analysis of Symbiotic Petit Suisse Cheese



Conclusions

In the present study, we obtained the formulation of Symbiotic Petit Suisse strawberry flavor, with the presents of fiber (3,2 g) and probiotics cultures (2g), where these latest were encapsulated and they maintained higher concentrations (> 10^6 CFU/g). *Bifidobacterium lactis* presented to be the culture more resistant to encapsulation process. The Symbiotic Petit Suisse Cheeese shows 23 days of shelf life with good organoleptic characteristic and high quality.

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Effect of Crosslinking of Malanga Starch (*Xanthosoma sagittifolium*) With Sodium Trimetaphosphateon Their Functional and Textural Properties

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Introduction

Starch is an excellent raw material to modify the texture and consistency of a large number of foodstuffs(French, 1984; Biliaderis, 1991).. Native starches are used because they regulate and stabilize the texture and its thickening and gelling properties(Cousidine, 1982). . In order to diversify the use of starches, it has studied the chemical modification that modify the starch structure and function.In this respect, the aim of this work was to evaluate the effect of sodium trimetaphosphate on the functional and textural properties of malanga starch (MS).

Materials and Methods

Raw Material
Malanga tuber was acquired in a cultivar of Alamo, Veracruz, México.
Malanga Starch Extraction
Malanga Starch was extracted following the Ganga and Corke method (1999). This method provides a high yield of starch with little loss. Tubers were washed, peeled and cut into 2 – 3 cm cubes. This was mixed with distilled water in a ratio 1:3 (malanga:wather) for 1 min. in a food processor Hobart. The mixture was left to stand for 40min. The liquid was decanted and the remaining solids were mixed for another 1 min. The mixture was added back to the sediment and held at room temperature for 40min. The liquid was decanted and discarded, and a further of water was added tothe sediment before sieving with a 250 µm sieve. Theremaining sediment in the sieve was washed off with distilled water and resuspended; afterwhich it was mixed for 1 min. in the food processor Hobart and sieved (250 µm sieve). The starch in the filtrate and the rinse water were allowed to settle for 30 to 45min and the liquid decanted and discarded. The starch was resuspendedin a distilledwater and passed through a 250 µm sieve. The remaining solids on the sieve were rinsed with distilled water before they were discarded. The starch was dried in a convection oven at 40°C for 24 hours and then ground with a mortar and pestle and passed through a 250-mm sieve. Samples were stored in air-tight containers at room temperature until use.

Starch Modification WithSodium Trimetaphosphate (STMP)
In a 100 mL solution of NaCl 5% were dispersed 100 g of native starch and 2 g de STMP; the pH of the suspension was adjusted to 10.5 by adding 1 N sodium hydroxide. The mixture was stirred for 20 h at temperature of 40 ° C; the alkaline conditions was constant in all time of reaction. The reaction was stopped with HCl 1 M until a pH of 6.5. The suspension was filtrated in a Büchner funnel using a Whatman paper No. 4. Finally, the cross-linked starch was washed three times with distilled water and dried in a convection oven at 40 °C. for 24 hours(Lim &Seib, 1993; Seib& Woo, 1999).
For the purpose of comparing native (NMS) and crosslinked (CMS) malanga starch were evaluated chemical composition, water holding capacity (WHC), solubility (% Sol), Swell Factor (SF), clarity, thawin stability, retrogradation, viscosity and TPAusingthe methodologiesdescribedbelow. In addition, the morphology of starch granules was determinatedby optical microscopy.
Chemical Composition

The chemical composition of native and modified malanga starch was performed according to the standard methods described in the AOAC (1990), i.e. moisture, ash and fat. Protein content was estimated from nitrogen content by Kjeldahl method multiplied by 6.25. Carbohydrate content was calculated by difference.

FUNCTIONAL PROPERTIES

Water Holding Capacity (WHC)

In preweighedcentrifugue tubes were added 10 mL of 1% (w/v) NMS and CMS suspension and then heated at 50 and 80 °C for 15 min, with shaking at 5and 10 min. The tubes were then centrifuged for 15 min at 2300 rpm 10 min. The supernatant was decanted and the tubes were allowed to drain for 10 min at a 45° angle. The tubes were then weighed and the gain in weight was used to calculate percent gain as the WHC. (Bryant and Hamaker, 1997; Niba et al., 2001).

Swelling Power (SP) and Water Absorption Capacity (WAC)

Briefly, 10 ml of1% (w/v) NMS and CMS suspension was prepared in a previously tared centrifuge tube and it was kept at a constant temperature (50, 70 and 90 °C) in a water bath for 30 min. The suspension was then centrifuged at 4500 rpm for 15 min, the supernatant decanted and the swollen granules weighed. From the supernatant, 10 ml were dried in an air convection oven at 120 °C for 4 h in a crucible to constant weight.(Crosbie, 1991)

Clarity

10 ml of 1% (w/v) NMS and CMS suspension were added in tubes with threaded caps and were placed in a water bath at 100 °C for 30 min, agitated by vortexing every 5 min, and left to cool to room temperature. Percentage transmittance (%T) was determined in these suspensions at 650 nm (Crosbie, 1991).

Retrogradation

10 ml of 2% (w/v) NMS and CMS suspensions were prepared by stirring in a water bath at 90 °C for 10 min and then, cooledinan ice bath. Samples were storedfor 24, 48 and 72 hoursinrefrigeration(4 °C). Finally syneresis rate was calculated as the weight of leaked out water at 4000 rpm 15 min divided by the weight of the original gel. (Lovedeep*et al*., 2002; Ishiguro *et al*., 2003).

Flow behavior

flowbehavior of NMS and CMS dispersions (1 and 4 % w/v) was performed in aPolivisc viscometer at 1-100 rpm.

Morphologyofthe Starch Granules

The morphology of NMS and CMS granules was performed in an optical microscope using a 40x and 100x lenses.

Statistical analysis

Means of all results were subjected to statistical analysis using the SPSS statistical software (V. 17.0). Analysis of variance and Tukey tests were used to interpret data and establish significance at $P<0.05$

Results and discussion

Water Holding Capacity (WHC)

The values forWHC increased when temperatureincreases (1.24 and 0.85 at 50 °C to 6.37 and 5.40 at 80 °C for NMS and CMS respectively). WHCwas significantly higher inNMS than in CMS. This behaviorindicates adesirablefunctionalpropertyofthe starchesusedintheformulationofpastes.(Crosbie, 1991).

Swelling Power (SP) and Water Absorption Capacity (WAC)

SPshowedvalues below10 g water/g starch at 50°Cfor both typesofstarch (NMS and CMS). However,at90 ° Cshowed an increaseup to 1060 and 366 %(53.7 and 18.1 g water/g starch for NMS and CMS respectively). As shown in figure 1. Theswelling powerofthestarchis a propertyofthe contentofamylopectin,amylosebeinga diluentandswellinginhibitor(Cheng etal., 1996).

Clarity

Claritywas significantly lowerin NMS than inCMS (0.69 and 1.68 of absorbance respectively). This behaviorindicates adesirablefunctional propertyof starchesto be used inthe manufacture of beveragesand other foodssuch asatole, a Mexican drink.(Lovedeep et al., 2002).

Retrogradation

CMS showed highervalues of syneresisduring storage than NMS at 24, 48and72 hours(489.16, 548.34and626.62 % and 61,84.2, 104.34%, for CMS and NMS respectively). These values areextremelyhigh, whichindicates thatthe modified starchpossesses a highertendencyto retrogradation.Accordingtotheseresults,CMS can be usedinfoods which requirebeingstored in refrigeration and requiresomemoistureexudationto providea freshappearance,likecustardsorsauces(Betancur et al., 2001).

Flow behavior

The viscosity ofNMS and CMS decreasedwhenincreasedshear rate. Characteristic behavior of shear thinning fluids. However, NMS showed values of viscosity higher than CMS (2000 and 100 mPa s for NMS and CMS dispersions at 4 % and at 50 rpm). Crosslinkslimitingswellingofthe starch granules, whichindicatesthatthecrosslinkedstarch granuleshave a lowervolume thanthe native starchgranules;incontrast,crosslinkedstarchs showed viscosityvalues lower thannative starchs, thisbecause theswellingwas limitedby thecross. (Jyothi& col., 2006; Singh & col., 2007; Hirsch&kokini., 2002).

Morphologyofthe Starch Granules

Figure 1 showed the spherical shape of Malangastarchgranules, similar tothepotatostarch granules thatare ovaltospherical (Swinkels, 1985). NMS granules showed an average size of 39.85 μmin diameter while CMS granules showed an average size 34.53μm, similar values to those reported by Moorthy(2002).

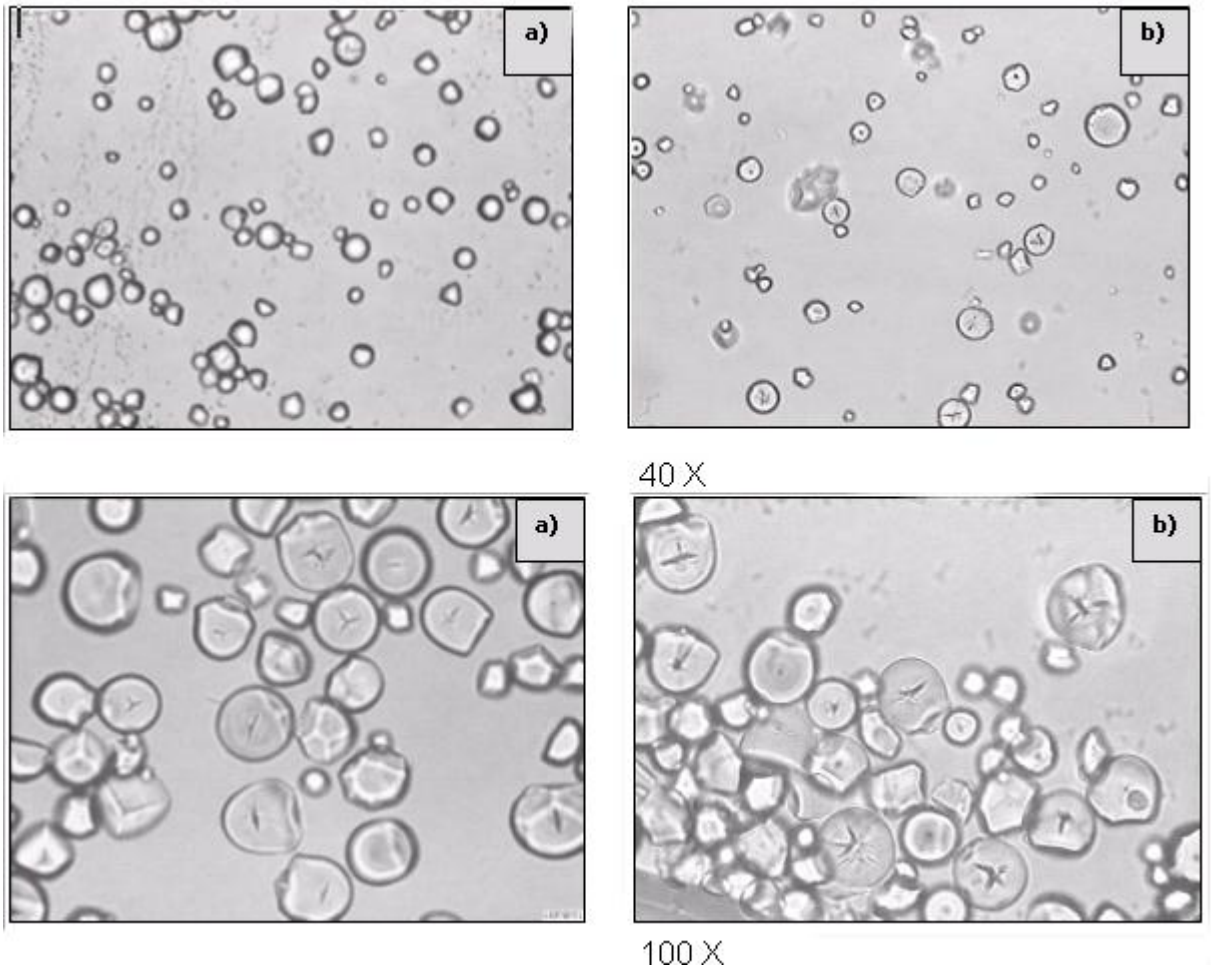

Figure1.-Micrographs of NMS (a) and CMS (b). at 40 and 100X

Conclusions

The present study demonstratesthat the malanga tubers (*Xanthosomasagittifolium*) represent apotential new source ofstarch withhigh yield.
CMS showedvariousphysicochemicaland functionalpropertiesso itscanbesusceptibletouse in variousfood systemsor other industrial applications.
The results inthis study showed thatthecrosslinkedstarchwithsodiumtrimetaphosphatenot affectthe morphologyand size of granule.

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The Wild Mexican Blackberries, Plants For Antioxidant And Human Food

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Introduction

Blackberries are bushy plants with ornamental and protective components that respond to environmental changes, showing diversity of morphological structures that vary with environmental conditions and this fact make it difficult to identify, reporting from 250 to 700 species worldwide (1). In Mexico, it's been reported the presence of numerous species in several states where highlights Michoacan; locals of the localities where they develop leverage their fruits, which are temporarily collected for sale to local or regional markets, ans this activity represents an alternative source of family income for low-income people. Some species with commercial potential reported for the state of Michoacan are *Rubus adenotrichos, R. pringlei, R. coriifolius, R. sapidus and R. pumilus* (1).

Globally strawberries have become important because of the high content of bioactive compounds such as polyphenols. In Mexico, studies of these compounds in wild blackberry are scarce and those reported highlight the higher antioxidant properties than those of cultivated species. They also highlight the content of anthocyanin type compounds in concentrations up to 10 times higher compared to a commercial variety (2). Similarly, evaluations of the antioxidant potential of Mexican wild species, specifically those collected in the state of Michoacan, have been reported and compared with species developed in the U.S., highlighting the high values exhibited by these wild materials in antioxidant capacity analyzes, such as ORAC and FRAP, and have also been directly correlated with the high content of total phenols and anthocyanins. Thus, these results provide strong evidence on the functional properties of wild species developed in our country and the importance of continuing their study (3).

Compounds such as ascorbic acid, chlorogenic acid, malic acid and ferulic acid and arbutin, hydroquinone and tannins are present in leaves and young stems of blackberry. Similarly, the astringent, antidiabetic and diuretic properties exhibited by blackberry leaves infusions have been used in traditional medicine to treat stomach disorders, sore mouth, among others (4). Nutritionally, blackberries possess important features, including the presence of vitamin B, E and C, the latter enhances antioxidant capacity along with phytochemicals (5); these berries present 7-16 % sugar, about 80 % water and 3 % of nitrogen compounds. It also contains 4% soluble fiber, which helps in better digestion of food (6).

As already mentioned, the species of *Rubus* genus are elements with important nutraceuticals and nutritional values, some of which are wild distributed in Mexico, fact that has attracted the interest of researchers to study and evaluate the content and action of polyphenols present in different genotypes of blackberries and thus produce new evidence for defining their utility or application, and to suggest them as an alternative food that provides health benefits. Thus, in this paper we proposed the evaluation of the antioxidant potential of blackberry fruits developed in the wild in the region of Uruapan and commercial varieties coupled with quantification of polyphenolic compounds both total and major groups individually (flavonoids, anthocyanins). To complement this, we determined the most important physicochemical parameters as they are a reflection of the quality of the fruits.

Methods and Materials

Among the physicochemical parameters determined is humidity content, pH and titratable acidity expressed as percentage of citric acid according to the official methods (7). Total soluble solids expressed as ° Brix were determined using a hand refractometer Atago model 300003 at 20 ° C. Additionally, total

reducing sugars were determined by the method of the 3,5-dinitrosalicylic acid, DNS (8), which is based on reducing the yellow DNS by glucose or other reducing sugar to brick red colored 3-amino-5-nitrosalicylic, whose presence is detected by reading absorbance at 570 nm in UV-visible spectrophotometer; for determining the concentration of reducing sugars a standard curve was performed at different concentrations of fructose.

For the quantification of total polyphenols we followed the methodology described by Singleton and Rossi (1965). First, plant extracts were obtained from a gram of sample and 80% ethanol as the extractant, after which the extract was added to a mixture of acids, because they undergo redox reaction with the polyphenols producing blue oxides . Finally, the absorbance reading tof the samples was performed at a wavelength of 760 nm in UV-Vis spectrophotometer and results were expressed as mg of gallic acid per gram of fruit. Furthermore, the total flavonoid content was determined by the method described by Chang and his colleagues (2002), performing an extract from one gram of sample and 9 mL of 80% ethanol for a period of 48 hours. Subsequently, a mixture was prepared containing fruit extract, 1 M potassium acetate, 10% aluminum nitrate and 80% ethanol, then absorbance was read with an UV-Vis spectrophotometer at 415 nm and the results were expressed as mg equivalent of quercetin per 100 g of sample based on a standard curve. The total anthocyanins were determined by the pH differential method according to Wrolstad (2000), based on the reaction of anthocyanins to pH changes in the medium, and at pH 1.0, they exhibit their maximum coloration, while at pH near 4.5 they lose it. This method allows to accurate measurement of total anthocyanins. Extracts were prepared with one gram of sample and acidified ethanol; also, buffer solution was prepared at pH 1.0 with potassium chloride at a concentration of 0.025 M and another at pH 4.5 with sodium acetate 0.4 M. Absorbance was recorded in a spectrophotometer at 520 and 700 nm.

Determination of antioxidant capacity was performed by the method of free radical DPPH (1,1-diphenyl-2-picrilhidracilo) developed by Brand-Williams and colleagues (1995). For this, extracts dilutions were made and an aliquot of 100 µL of each sample extract was taken to subsequently add 900 µL of DPPH solution prepared with 80% methanol. The reaction was allowed to be effected for 30 minutes and the absorbance reading was performed at 515 nm in a spectrophotometer.

Results and Discussion

Table 1. *Total polyphenols, flavonoids, anthocyanins content and antioxidant capacity of wild and domesticated materials*

MORPHOSPECIES	TOTAL POLYPHENOLS (mg/100 g of sample)	TOTAL FLAVONOIDS (mg/100 g of sample)	TOTAL ANTHOCYANINS (mg/100 g of sample)	ANTIOXIDANT CAPACITY (µM/g trolox equivalents)
Wild Blackberry "Vellosa"	3014.85 b	580.96 b	18.45 a	29.90 b
Wild Blackberry "Cerosa"	2285. 25 d	435.40 c	17.85 a	15.35 d
Wild Raspberry	3154.80 a	364.10 d	8.75 a	25.15 c
Tupy Blackberry	2851.35 c	933.65 a	4.30 c	82.85 a

Little letters indicate significative differences. Tukey ($p < 0.05$)

At established sites three wild morphologically distinct materials were collected, arbitrarily called by their outstanding morphological characteristics as: "Vellosa", which is distinguished by the presence of reddish hairs, fruit from 1.5 to 2 cm black colored at mature stage and inflorescences may be range from 30 to 70 berries. The material called "Cerosa" is a plant that is characterized by the presence of a whitish layer that covers the entire stalk, giving the appearance of a waxy texture. The fruit has a black colored at mature

stage, it may vary in size from 2 to 3.5 cm and the plant shows generally 5 to 6 fruits per inflorescence. Finally, the material known as "Raspberry", unlike the other materials, is characterized as a fruit that at its mature stage is dark red and at the time of collection, it releases from receptacle, a typical feature of a raspberry.

In total polyphenol content, the Wild Raspberry highlighted as the material with the highest concentration of these compounds (3154.80 mg/100 g). These data are comparable with those obtained in a similar research (13) in which the reported concentrations are 1347 and 1703 mg/100 g in blackberry; another study reported concentrations of 2349 mg/100 g (14). Furthermore, in the quantification of total flavonoids with methanolic extracts of the fruits, it was noted that blackberry Tupy had the highest content of these compounds (933.65 mg/100 g), compared to wild individuals studied, and the Wild Raspberry showed the lowest level of flavonoids (364.10 mg/100 g), as shown in Table 1. On the other hand, the analysis for the determination of total anthocyanins showed that materials with higher content of these compounds, expressed as concentration of cyanidin-3-glucoside, were the blackberry species called "Vellosa" (18.45 mg/100 g) and "Cerosa" (17.85mg / 100 g) and the variety "Tupy" presented the lowest content (4.30 mg/100 g). The results are comparable to the values reported for species of the *Rubus* genus (15) and are within the ranges of anthocyanins reported for blueberries, strawberries and raspberries (14, 16). Finally, the analysis to determine the antioxidant capacity of the materials was performed for the purpose of a comparison between the wild materials and the cultivated variety, Tupy. Thus, the higher antioxidant potential was found on the commercial blackberry Tupy (82.85 uM / g trolox equivalents), with a much higher value compared to wild species studied. The results are comparable to those obtained in previous evaluations (17, 18) carried out with blackberry fruits and the found values were 13 to 146 and 37.6 to 75.5 µM trolox equivalent, respectively. Importantly, several authors have found that polyphenols and anthocyanins content directly influences the antioxidant capacity (3, 13, 15, 17), however, caution should be exercised in interpreting results. In this context, it is noted that the differences in the test results reported above indicate the possibility that each analyzed species has a different polyphenolic profile. Furthermore, it can be inferred that, based on the values obtained in each test, the total polyphenol concentration of berries has no direct relationship with their antioxidant capacity.

Table 2. *Physicochemical analysis of wild and cultivated materials*

MORPHOSPECIES	**Wild Blackberry "Vellosa"**	**Wild Blackberry "Cerosa"**	**Wild Raspberry**	**Blackberry Tupy**
Weight (g)	1.5 c	2.2 b	1.1 d	7.4 a
TS (°Brix)	12.4 a	7.6 c	10.7 b	9.8 b
Total titratable acidity (% ascorbic acid)	0.84 a	0.91 a	0.99 a	1.10 a
pH	3.1 b	3.2 ab	3.3 a	3.3 a
Reducing sugars (g/100 g)	1.34 a	1.55 a	1.48 a	1.55 a
% of Humidity	75.1 c	87.7 a	82.6 b	86.9 a

Little letters indicate significative differences. Tukey ($p < 0.05$)

The values in Table 2 show the results of the physicochemical analysis performed, where the species "Vellosa" highlighted in the total soluble solids content indicating that it has a sweetest taste compared with the other wild berries analyzed. This is a remarkable characteristic as this

would reflect an appealing taste for the consumer. The reducing sugar content and acidity did not show any difference between the analyzed materials.

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Use Of The By-product Of Soy Milk (Okara) For The Development Of Pasta As A Functional Product

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Summary
Nowadays, Mexico reports high rates of obesity; thereby the prevalence of chronic degenerative diseases has increased. Functional food emerges in order to attempt this problematic helping to offset the alimentary unbalance and improving the intake of nutrients as fiber and protein. Food industry produces big quantities of by-products with high nutritional content that are not used for human feeding, one of them, the okara, by-product of soy milk which contains considerable quantity of protein (10-20%) and fiber (9-20%). Pasta, one of the most consumed alimentary products between the populations, has been worked recently, modifying it with the addition of new ingredients in order to enhance its nutritional properties. The objective of this research was the use of the okara to elaborate pasta from wheat semolina and okara. To obtain the okara flour two dryers were tested: Tunnel dryer and dryer trays. Physicochemical properties were evaluated: absolute density, moisture content and particle size. Obtained flour, three formulations were -tested: 70/30, 75/25 and 80/20 of wheat semolina and okara respectively, the tests performed were: cooking time, water absorption and percentage of solids, in addition, the proximal composition was made for the best formulation chosen and for two commercial pastas: Santiveri ® and Pasti Bambino ®. The results obtained from the drying curves show that the dryer tunnel (0.23 g H_2O/SS in 60 min) compared to the dryer trays (0.23 g H_2O in 230 min) decrease the drying time. The physicochemical properties of the flour obtained were evaluated: density (0.47 g/ml), moisture (10.44%) and particle size (427.3 microns). The formulation 70/30, wheat semolina / flour okara showed the largest increase in properties: cooking time (14.3 min ±0.45) water absorption (142.39% ± 0.45) and percentage of solids (0.104% ± 0.09). Dietary fiber content was acceptable (23.79% ± 0.59) although there was less protein content (3.67% ± 0.61) compared with both commercial pastas.
Keywords: okara, dietary fiber, pastas, residue, functional foods.

Introduction
According to the latest information released by the National Nutrition Survey in 2006[1], overweight and obesity are problems that affect about 70% of the Mexican population (women, 71.9% men, 66.7%). Obesity is the result of an imbalance between energy intake and expenditure, following ingestion of diets with high energy density and low fiber, combined with low physical activity[1].

The primary role of diet is to provide the necessary nutrients to meet the nutritional needs of people; however, with the current lifestyle it becomes increasingly difficult to consume an adequate diet. Functional foods, considered as foods that are consumed as part of a normal diet containing biologically active components, provide health benefits, reducing the risk of disease[2]. The pasta,today has a high degree of acceptability among the people because of its easy preparation and economic access in addition to its long shelf life. Unfortunately, in terms of their nutritional value, pasta is not an especially rich source of minerals, is low in fat and dietary fiber, plus it is a food rich in digestible and indigestible carbohydrates[3].

In recent years we have worked with the dough formulation to enhance their nutritional properties[4, 5, 6 and 7]. Among the various ingredients used in meal replacement or durum wheat semolina are: fish protein concentrate, soy flour, pea, lentil, chickpea, soy isolates, egg albumin, non-starch polysaccharides, cassava starch and banana, among others.

The okara by-product of soy milk, with high nutritional content, is little used in the food industry because of its high moisture content making this a highly perishable product. The addition of okara flour in pasta making, offers an alternative use of this product to improve the nutritional content of traditional pasta.
This research proposes the development of apasta,this formulation aims to incorporate new sources of fiber and protein from the use of okara flour as a functional ingredient thus affecting the presentation of alternatives for promoting the consumption of healthier foods.

Methods and materials

Obtained okara
The insoluble residue from soymilk elaboration was obtained the okara. It tested two types of dryers: tunnel dryer and the drying trays. The samples were milled to a particle size of less than 1.0 mm before analysis. Moisture content was determined by drying at 105 °C in an oven for 16 h. Obtained flour, three formulations were -tested: 70/30, 75/25 and 80/20 of wheat semolina and okara respectively, the tests performed were: cooking time, water absorption and percentage of solids, in addition, the proximal composition was made for the best formulation chosen and for two commercial pastas: Santiveri ® and Pasti Bambino ®.

Rheological testing

Water Absorption and Cooking Time
These tests were done together, weighing 5 g of raw pasta and adding100 ml of water,subsequently the pasta with water was heated untilcooking (10-15 min). The optimum cooking time was obtained by taking a sample of the pulp at intervals of one minute and pressing it among two microscope slides; the optimum time was calculated when observing the disappearance of the nucleus in the sample, indicating that the starch is gelatinized. Thereafter, the volume and weigh of the cooked pasta was measured based on the next formula:

Water Absorption= $$\frac{\textbf{Final weight of the pasta} - \textbf{initial weight of the pasta}}{\textbf{initial weight}} \times 100$$

% Solids
Was obtained, taking an aliquot of 50 ml of cooking water and weighed in a taredglass beaker, put in the furnace at 80-90 ° C and evaporated to dryness and was measured based on the next formula:

$$X = \left(\frac{Weight\,residue}{Weightsample} \times Volcooking\ water\right) \times \frac{100}{5}$$

Proximal chemical composition

Protein
Total nitrogen in defatted soybean seeds and okara was determined according to the method described in official AOAC 991.21, the protein content was determined indirectly by quantification of total nitrogen using the Kjeldahl method. Protein was calculated as nitrogen x 6.25.

Ashes
Were analyzed with the method described by AOAC No. 942.05 was weighed 1 g sample, defatted soybean seeds and okara were incinerated in a furnace at 550 °C for 16 h and weighed.

Lipids
Fat determination was performed in triplicate in accordance with the method described in the official AOAC (963.15). Fat was removed from dried okara using Soxhlet extraction with petroleum ether (100 ml for a 3 g sample at 60 °C for 8 h).

Total dietary fiber
The analysis of total dietary fiber (TDF), soluble fiber composite (SF)and insoluble fiber (IF) was quantified by enzymatic-gravimetric method of AOAC (985.29)[9] using an enzymatic hydrolysis enzyme kit that includes the thermostable alpha-amylase, protease and amyloglucosidase enzymes.

Statistical analysis

Results were calculated as the mean values of at least three replicates and expressed as mean ± SD. The data obtained were analyzed statistically using the Minitab statistical software (version 15.1.30.0). We used analysis of variance (ANOVA) one-way with a significance level α = 0.05, when statistically significant differences between the samples we used a multiple comparison test of Tukey at 5%.

Results and discussion

After obtaining okara according to Codex and Food (Codex Stan 152-1985) the flour then meets the standards did not exceed 15.5% moisture content. The flour obtained has an approximate size of 427 microns, and a moisture content of 10.41%, these data are consistent with those reported by Constanza et al. (2010), it is possible to produce a pasta with flour 355-500 microns in preparing a pasta with sorghum flour and semolina.

From the flour obtained was the drying process in which were obtained for the tray dryer, air velocity is 1.7 m / s, the inlet temperature is 59 ° C and outlet 57 ° C while that the tunnel dryer, air velocity is 1.9 m / s inlet air temperature is 69 ° C and the outlet is 64 ° C. Due to the results obtained are elected tunnel dryer to carry out drying okara general, since the drying time should be minimized to prevent decomposition reactions.

Rheological tests in each of the formulations shown in Table 1. According to the statistical analysis we can see that there is no significant difference for each test performed between formulations except the cooking time where the formulation 80/20 proved to be statistically different from the others.

Table 1 Results for rheological testing: Water absorption, cooking time, % solids for different formulations.

Formulation	Water absorption	cooking time (min)	% Solids
70/30	142.39 % ± 0.45[a]	14.3 ± 0.57[a]	0.10% ± 0.09[a]
75/25	151.17% ± 0.75[a]	16 .0±1.00[a]	0.08% ± 0.02[a]
80/20	186.34% ± 0.85[a]	10.3 ± 0.57[b]	0.11% ± 0.02[a]

Mean values of triplicate determinations ±SD

The proximate analysis results (Table 2) showed that the protein content in each sample was significantly different, the formulation 70/30 found to have a protein content of 3.67% low compared with pasta and SantiveriPasti ® 8.68% Bambino ® 6.03%, this was attributed to the ingredients for preparing the pastas according to other label include egg and other legumes, in addition to the proteins provided by the wheat. Note that the nature of soy protein is more nutritious because of its high content of sulfur amino acids as reported by Ridner[10].

The percentage of lipids present in the formulation 70/30 was 10.08% and was considerably greater than theSantiveri ® (3.88%) andPasti Bambino ® (3.47%)pastas, this was expected because soya is considered a oleaginous seeds with a high content of fatty acids. The fatty acids contained in soy are mostly unsaturated type (Ridner et al, 2006), which provides nutritional quality. The ash percentage of 1.05% of theSantiveri ® pastaand content of 1.25% of the formulation proposed no significant difference which occured with the Pasti Bambino ® pastawhich contains 0.68% ash. However, the proposed formulation is the one with higher ash content, which indicates a higher content of vitamins and minerals.

Table 2 Proximal chemical composition of the formulation 70/30 and commercial pasta: Santiveri ® and Pasti Bambino ®.

	Protein	Lipids	Moisture	Ash
Santiveri®	8.68% ±0.78[a]	3.88% ± 0.24 [a]	13.11% ±0.91[a]	1.05% ±0.10[a]
Pasti Bambino®	6.033% ±0.73[b]	3.47% ±0.36[a]	12.87% ± 0.33[a]	0.68% ±0.06[b]
70/30	3.67% ±0.61[c]	10.08% ±0.12[b]	11.31% ± 0.34[b]	1.25% ±0.13[a]

Mean values of triplicate determinations ±SD

In the case of moisture content, making 70/30 showed a percentage of 11.31%, the lowest this as reported by Vasiliu[11]. This behavior is attributed to the decrease of gluten protein network base protein responsible for water retention.

Data obtained in total dietary fiber (Table 3) show that the dough obtained a Santiveri ® pulp 31.12% and 20.68% Pasti Bambino ® compared to the percentage of the formulation 23.79% 70/30 we can see that the formulation 70/30 has a fiber content intermediate between the commercial pasta.

Table 3 Composition of total dietary fiber

	Total dietary fiber	Fiber Insoluble	Fiber Soluble
Santiveri®	31.12% ±0.69[c]	28.03% ±1.14[c]	3.09% ±0.45[a]
Pasti Bambino®	20.68% ±0.82[a]	17.10% ± 0.69[b]	3.58% ±0.12[a]
70/30	23.79% ±0.59[b]	20.41% ±0.31[a]	3.37% ±0.72[a]

Mean values of triplicate determinations ±SD.

Conclusions

The worldwide production and intake of soybean seeds and derived products (mainly soy milk and tofu) are on the increase, with the consequent rise and accumulation of okara, their mainby-product. The generation of new products such as pastas and more fiber content of proteins from the flour using okara (by their physicochemical properties: high swelling and water retention capacity), as a functional ingredient and generate alternatives for promoting consumption of new functional foods with prebiotic effect.

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Protein Hydrolysates Obtained from Azufrado (Sulphur yellow) Beans (*Phaseolus vulgaris*): ACE-Inhibitory and Antioxidative Characterization

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The goals of the present work were to evaluate the nutraceutical and functional properties of protein hydrolysates obtained from three cultivars of Azufrado (sulphur yellow) beans. Bean protein concentrateswere obtained and digested with Alcalase™, Thermolysin™ and Pancreatin™. The Azufrado Higuera cultivar showed the best protein yield/concentration ratio, having a higher amount of hydrolysatescompared to the rest of the varieties. All treatments presented ACE-Inhibitory activity, these values rangedfrom 0.109 to 319.96 mg/ml. Regarding to antioxidant activity, the Azufrado Higuera/alcalase treatment showed the highest DPPH scavenging activity (40%), however with the ABTS method the Azufrado Regional ’87-alcalase was the best treatment (99.89% of scavenging). Overall, the results of our work showed that Azufrado beans proteins hydrolysates are suitable for development of a nutraceutical product for prevention andcontrol of degenerative diseases such as hypertension and those derived from cellular oxidation.
Keywords: nutraceutical, biopeptide, hydrolysates, ACE-Inhibitory

Introduction

Functional foods have components that possess certain biological activity, also known as nutraceutic compounds. There are many ways to make functional foods such as the modification of native compounds that have certain biological activity, making them bioavailables when they are consumed (1). Bioactive peptides are nutraceutic compounds, normally released during gastric hydrolysis of many proteins. Many of these peptides have the ability to reduce blood pressure through inhibition of the modular blood pressure control enzyme called Angiotensin Converting Enzyme (ACE). Thus, these peptides could be an alternative for hypertension (HT) treatment (2). Several reports have demonstrated that bioactive peptides showed antioxidative activity, with possibility to delay cellular oxidation process in vivo lowering the risk of development of chronic diseases (3,4,5). Nowadays there is an increasing interest in finding nutraceutic compounds from natural sources that until now have been unexploited; like common bean (*Phaseolus vulgaris*), a legume whose grain is an important source of protein for Mexican population. Common bean contains high digestibility protein (up to 22 g/100 g seed) making it a good candidate to explore as a source of bioactive peptides. Mexican population is severely affected by HT, the goal of this work was to obtain protein hydrolysates from three types of Azufrado beans grown in Northwestern of Mexico, and to analyze ACE-Inhibitory (ACE-I) and antioxidative potential with in vitro assays.

Materials and methods

Common bean cultivars grains: Azufrado Higuera(Reg.No.FRI-045- 170205), Azufrado Noroeste (Reg. No. FRI-044-170205) and Azufrado Regional ’87 (Reg. No. FRI-008-251104) were donated by the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Valle del Fuerte, México. ACE (from rabbit lung), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,20-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), hippuryl-histidyl-leucine (HHL), alcalase, thermolysin and pancreatin were purchased from Sigma (Sigma Chemicals, St. Louis, Mo., U.S.A.).

2.1. Protein isolations and hydrolysis

Protein concentrates were by alkaline extraction and isoelectric precipitation as described by Morales-de León et al. (6)with modifications. Protein hydrolysis was carried out according to Humiski and Aluko (7) and Adler-Nissen (8) with modifications, with the use of three different proteolytic enzymes: alcalase, thermolysin and pancreatin, using appropriated conditions for enzymes optimum activity. Degree of hydrolysis (DH) was determined, with the o-phthaldialdehyde(OPA) method (9), using serine as standard.

2.2. ACE-inhibitory activity

Antihypertensive potential of each protein hydrolysate wasdetermined by their ACE-inhibitory (ACE-I) activity according toMiguel et al. (10) with modifications.ACE hydrolyses HHL to generate hippuric acid and the peptide His-Leu. The method is based on the quantification on hippuric acid after the reaction of ACE, HHL and the potential ACE-inhibitor. The concentration of hippuric acid was determined at 228 nm using water as a blank. Inorder to determinate the IC_{50} of each protein hydrolysate, data wereadjusted to a non-linear regression model using Hill's equation (11).Significant differences between treatments were found usingFisher's test (LSD, $\alpha = 0.05$).

2.3. Radical-scavenging activity (DPPH and ABTS)

Scavenging activity of the protein hydrolysates against DPPHradical was performed according to Hou et al. (12). ABTS radical cation assay was also carried out following the methodology of Re et al. (13). Scavenging activity of the samples wasexpressed as the percentage of free radical-scavenging effectfollowing the equation:

Scavenging effect = [(Abs Blank - Abs sample)/Abs Blank] x 100

Additional, the antioxidant activity obtained calculated wasplotted as a function of antioxidant Trolox concentrations in the standard reference data. The Trolox equivalent antioxidant coefficient (TEAC) was calculated by dividing the absorbance percentageinhibition absorbance versus antioxidant concentration slope by the Trolox plot slope.

2.4. Statistical analysis

For the statistical analysis the Graph-Pad Prism 5 and Statgraphics Centurion XV were used. Data were analyzed by a twowayANOVA. The results were expressed as mean values. Differencesbetween treatments were assessed with Fisher's test,considering significant differences when $P \leq 0.05$.

Results and discussion

3.1. Protein isolations and hydrolysis

Azufrado Higuera and Azufrado Noroeste were the cultivars with better protein/flour yield (13 g of protein/100 g offlour). These yields were similar to those reported for other grains,but significantly lower than those obtained from other *Phaseolus* seeds by Torruco et al. (14), theauthors reported a higher flour/protein yield (up to 28 g of protein/100 g of flour). The protein content of the concentrates obtained for Azufrado Higuera, Azufrado Noroeste and Azufrado Regional '87were 61, 58, 66 g/100 g of concentrate, respectively. These results indicate that Azufrado Higuera is the cultivar that provides betterprotein yield/concentration protein ratio.OPA method results showed that alcalase and thermolysin were the enzymes with higher hydrolytic activity, followed by pancreatin over common bean proteins (Data not shown). No significant differences were detected between alcalase and thermolysin regarding DH, whichranged between 69 and 73% for the Azufrado Higuera and Azufrado Noroeste cultivars, but there was significant difference among the DH of these protein hydrolysates and those from Azufrado Regional'87, that had between 33 and 38% when alcalase and Thermolysin were used as proteases, respectively. These differences amongenzyme behaviors could be explained due to differential proteinprofile found in seed storage proteins of Azufrado Regional '87, and those present in Azufrado Noroeste and Azufrado Higuera, where through a proteomic approach up to thirty proteins were alreadydetected as differentials in seed (15).The protein hydrolysates from pancreatin presented the lowest DH with values around 12 and 34%. This could be due to the fact that pancreatin is a mixture of several serine-proteases andamylases. In this case, the concentrates did not have any thermaltreatment and some remaining serine-proteases inhibitory activitymight have caused the low hydrolytic activity of the enzyme.

3.2. ACE-I activity

To determine the potential antihypertensive activity in a compound, IC_{50} value has to be calculated, which is defined as the concentration needed to inhibit 50% the activity of Angiotensin Converting Enzyme (ACE). The IC_{50} values are shown in Table 1. The treatments with the lowest values of IC_{50} were the protein hydrolysates of alcalase and thermolysin for all three cultivars (0.11-2.78 mg/ml). The lowest were those resulting from the treatments with Azufrado Higuera for both enzymes (0.12 and 0.11 mg/ml, respectively); on the other hand, the treatments that showed higher IC_{50} values were the protein hydrolysates of pancreatin for all three cultivars (60.36-319.97 mg/ ml). The lowest was that resulting from Azufrado Regional '87 (319.97 mg/ml). According to the statistical analysis (Two-way ANOVA), the enzyme factor had a significant effect ($P = 0.0001$) on the IC_{50} values, but the cultivar factor did not have any effect ($P = 0.0927$). Thus, the different antihypertensive potential among protein hydrolysates relied mainly on the enzyme used for hydrolysis rather than on the origin of the protein concentrate. The results obtained in this work are encouraging, because IC_{50} values were lower than those reported for fermented products of soybean (80-360 mg/ml) (16), storage protein from chickpea (17), and other protein sources like that reported by Hatanaka et al. (18) who purified bioactive peptides from *Euphausia superb* fish, with an IC_{50} of 2.75-4.26 mg/ml, which was higher than those found in the alcalase and thermolysin hydrolysates in this study. Torruco et al. (14) reported that IC_{50} values for two cultivars of beans hydrolyzed with alcalase and flavourzyme were higher than those here reported. On the other hand, since pancreatin is a mixture of several digestive enzymes, its hydrolysates could provide information of the nutraceutical benefits of bean protein consumption. These hydrolysates showed lower IC_{50} values (Table 1) than many otherhydrolysates from vegetables sources.

3.3. Antioxidant capacity

The DPPH radicalscavengingactivity values of the protein hydrolysates rangedfrom 24 to 44% (0.0146-0.027 mmol/L TEAC/mg protein) (Table 2). The best treatment was Azufrado Higuera/alcalase (44%); this was significantlydifferent from the rest of the treatments (proteinconcentration = 1 mg/ml). The results obtained in this study werehigher than those reported in canola (19) and pea (7) hydrolysatesthat showed up to 8 and 11% of DPPH radical-scavenging activity,respectively. Zhang et al. (20) reported similar DPPHradical-scavenging activities (up to 29%) of soy protein hydrolysates obtained with alcalase, but evaluating a highest protein concentrate(100 mg/ml). Regarding antioxidative activity of the protein hydrolysates by the ABTS method, all protein hydrolysates exhibited a good antioxidative capacity ranging from 50.2 to 99.9% (1.903-3.788 mmol/L TEAC/mg protein) (Table 3). The treatment Regional '87-alcalase was the more effective being significantly different from the rest (99.9%). The values obtained by the ABTS method were higher than to those found by the DPPH method; even when the protein concentration assayed was lower (0.25 mg/ml protein). This could mean that the ABTS method is a more sensitive to determine antioxidative capacity of protein hydrolysates samples, because it can determine its capacity at lower inhibitor concentrations.The radical-scavenging activity detected was similar to those obtained by Raghavan et al. (21) in tilapia protein hydrolysates, but lower than those reported by Torruco et al. (14) for other beans types (8.42-11.55 mmol/L TEAC/mg protein).

Conclusions

The bean hydrolysates evaluated showed nutraceutical properties that make them suitable to be used in novel product development. Regarding ACE-I activity we found IC_{50} values that ranged from 0.1089 to 319.99 mg/ml, these values are lower than those found in legumes and other protein hydrolysates. The hydrolysates showed good antioxidative capacity with values up to 99% radical scavenging capacity that complement ACE-I, highlighting alcalase and thermolysin high hydrolytic power. Overall, we can conclude that the Azufrado bean protein hydrolysates could be used as an ingredient for development of functional foods. The following steps are the development of value-added foods that include bean protein hydrolysates as functional ingredient.

Table 1.Free radical (DPPH) scavenging activity of protein hydrolysates.

	Treatment	IC_{50} (μg/ml)
Alcalase	Azufrado Higuera	0.19**g
	Azufrado Noroeste	0.45f
	Azufrado Regional '87	1.30e
Thermolysin	Azufrado Higuera	0.11g
	Azufrado Noroeste	0.35f
	Azufrado Regional '87	2.78d
Pancreatin	Azufrado Higuera	60.36c
	Azufrado Noroeste	203.65b
	Azufrado Regional '87	319.97a

**Different letters indicate significant difference (LSD = 0.137 mg/ml, α = 0.05).

Table 2. Free radical (DPPH*) scavenging activity of protein hydrolysates*.

Enzyme	Az. Higuera		Az. Noroeste		Az. Regional '87	
	% of inhibition	mmol/L TEAC mg protein	% of inhibition	mmol/L TEAC/ mg protein	% of inhibition	mmol/L TEAC/ mg protein
Alcalase	44.05a,A	0.027	33.59b,A	0.0208	35.81b	0.0222
Thermolysin	32.16a,B	0.020	31.03a,A	0.0192	34.03b,c	0.0211
Pancreatin	33.90a,B	0.021	23.61b,B	0.0146	28.21d,e	0.0175

*Assays were carried out at 1mg/ml hydrolysate concentration. Values are means for three measurements per hydrolysate. Different lowercase letters in the same row indicatesignificant different (LSD = 4.768, α= 0.05). Different capital letters in the same column indicate significant different (LSD = 4.768, α= 0.05).

Table 3.Free radical (ABTS*) scavenging activity of protein hydrolysates*.

Enzyme	Az. Higuera		Az. Noroeste		Az. Regional '87	
	% of inhibition	mmol/L TEAC/ mg protein	% of inhibition	mmol/L TEAC/ mg protein	% of inhibition	mmol/L TEAC/ mg protein
Alcalase	73.4c,A	2.781	77.2b,A	2.925	99.9a,A	3.788
Thermolysin	58.6a,C	2.223	50.2b,B	1.903	57.5a,C	2.181
Pancreatin	69.4b,B	2.632	50.2c,B	1.902	75.3a,B	2.854

*Assays were carried out at 0.25 mg/ml of hydrolysate concentration. Values are means for three measurements per hydrolysate. Different lowercase letters in the same rowindicate significant different (LSD = 1.146, α=0.05). Different capital letters in the same column indicate significant different (LSD = 1.146, α=0.05).

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Antioxidant Peptides Of Amaranth Grain As Protective Agents Against Oxidant Stress Of The Skin

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Introduction

Oxidative stress has been implicated in various skin diseases through the generation of reactive oxygen species and the depletion of endogenous antioxidant systems. The administration of antioxidants or free radical scavengers is reportedly helpful, notably in order to enhance the healing process (1). Epidemiological studies have implicated solar ultraviolet (UV) radiation in various skin diseases including, premature aging of the skin and melanoma and non-melanoma skin cancers. Chronic UV radiation exposure-induced skin diseases or skin disorders are caused by the excessive induction of inflammation, oxidative stress and DNA damage, etc. (2). On the other hand, skin assumes several important physiological functions and represents a social interface between members of society. This is the main reason of remain in the forefront of dermatological research and of the ‘‘anti-aging’’ nutricosmetic industry. This market is expanding, partly due to higher consumer demand for deliverables. As a result, bioactive peptides that are beneficial have been increasingly used to supply such activities, and more than 25 different peptides are routinely found in a vast array of skin care products in the United States alone. Many more peptides are currently in development in company pipelines worldwide that will expand not only the number of peptide ingredients but also the diversity of their application (3). Hence, the objective of this research was to evaluate the protective effect of antioxidant peptides obtained by hydrolysis of albumin-1 amaranth grain in organotypic cultures of human skin exposed to UV light.

Methods and materials

Defatted flour split grain amaranth, the albumin 1 fraction was extracted with Na_2SO_4 (5% p/v). Then, it was hydrolyzed with alcalase at pH=7.4, t=50°C and enzyme/substrate relation (E/S)=0.8 UA/g-protein, during 1, 12, 24, 36, and 48 h (TH). We determined the degree of hydrolysis (DH) through TNBS reaction (4). Antioxidant activity (AOxA) was evaluated through reduction of $DPPH^{\bullet}$ and $ABTS^{+}$ radicals and the chelating capacity of Fe^{+2} and Cu^{+3}. The antioxidant activity (AOxA) of hydrolyzates was evaluated through reduction of $DPPH^{\bullet}$ and $ABTS^{+}$ radicals and the chelating capacity of Fe^{+2} and Cu^{+3}. More AOxA hydrolyzates were applied to organotypic cultures of human skin (abdominal plasties biopsies obtained from healthy women aged 25-40 years old). For each sample (albumin 1 native and hydrolyzed 24, 36 and 48 h) was into four different treatments: a) skin without treatment (C (-)), b) skin + hydrolyzates (C (+)), c) skin + UV (H 24h, H 36h, H 48h and AN), d) skin + UV + hydrolyzates (H 24h, H 36h, H 48h and AN). The protective effect of hydrolyzates was assessed through enzymatic activity of dismutase superoxide (DSO), glutathione peroxidase (GPx) and catalase (Cat), as well as through lipoperoxidation inhibition (1).

Results and discussion

The highest DH albumin 1 (48±0.07%) was obtained from 24 h no significant differences ($p<0.05$) until 48h of hydrolysis, unlike AOxA, which increases with increase in TH. By measuring the AOxA of

hydrolysates obtained values radical reduction of DPPH$^{\bullet}$ and ABTS^{+} between 50-60% and 65-72% respectively (figure 1).

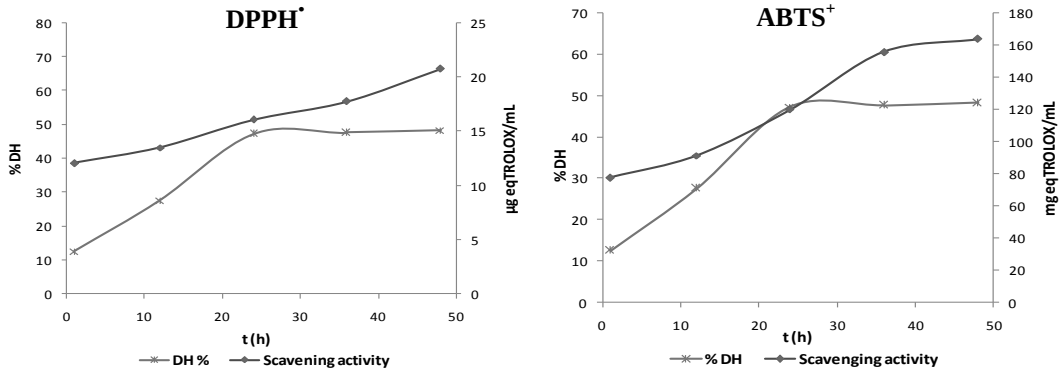

Figure 1. Comparison among albumin 1 hydrolysates AOxA assessed by several methods in order to observe their behavior by using different oxide-reduction systems.

The transition metal ions are involved in many oxidation reactions in vivo. Ferrous ions can catalyze the Haber-Weiss reaction to form the superoxide anion and subsequently form hydroxyl radicals which are very dangerous it is for nature. Hydroxyl radicals react rapidly with adjacent biomolecules and cause serious damage to them. The ferrous ion is one of the products formed in the Fenton reaction (Zhengjun et al., 2008). The chelating activity values obtained with Fe^{2+} and Cu^{3+} were 95% and 40% respectively figure 2). As these hydrolyzed avoid the formation of free radicals in the process of lipid peroxidation to be able to catch the ion Fe, placing them in the classification of primary antioxidants.

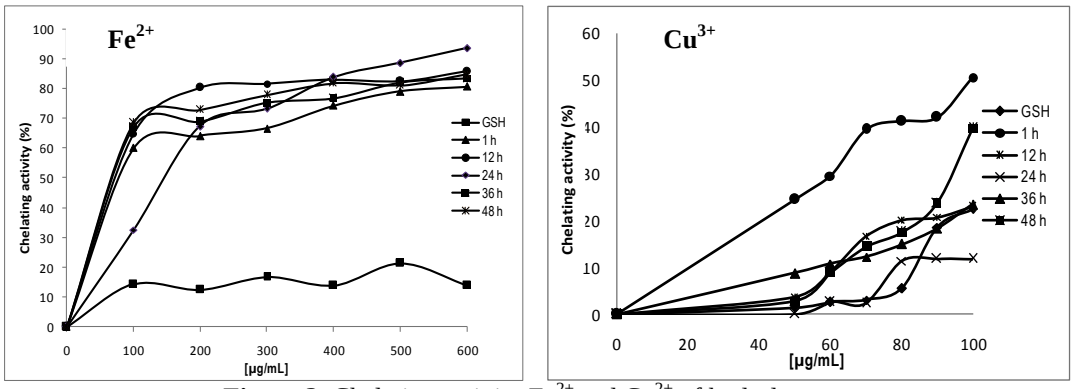

Figure 2. Chelating activity Fe^{2+} and Cu^{2+} of hydrolysates.

The enzyme activity demonstrated that the protein hydrolysates have amaranth grain protective effect against UV radiation which were subjected to human skin explants mainly against lipoperoxidation, since as shown in Figure 3 concentration of malondialdehyde decreases considerably when using the native protein and its hydrolysates. Similarly we observed no significant differences ($p < 0.05$) between treatments done to both albumin 1 native (AN) and hydrolysates with 24, 36 and 48 h.

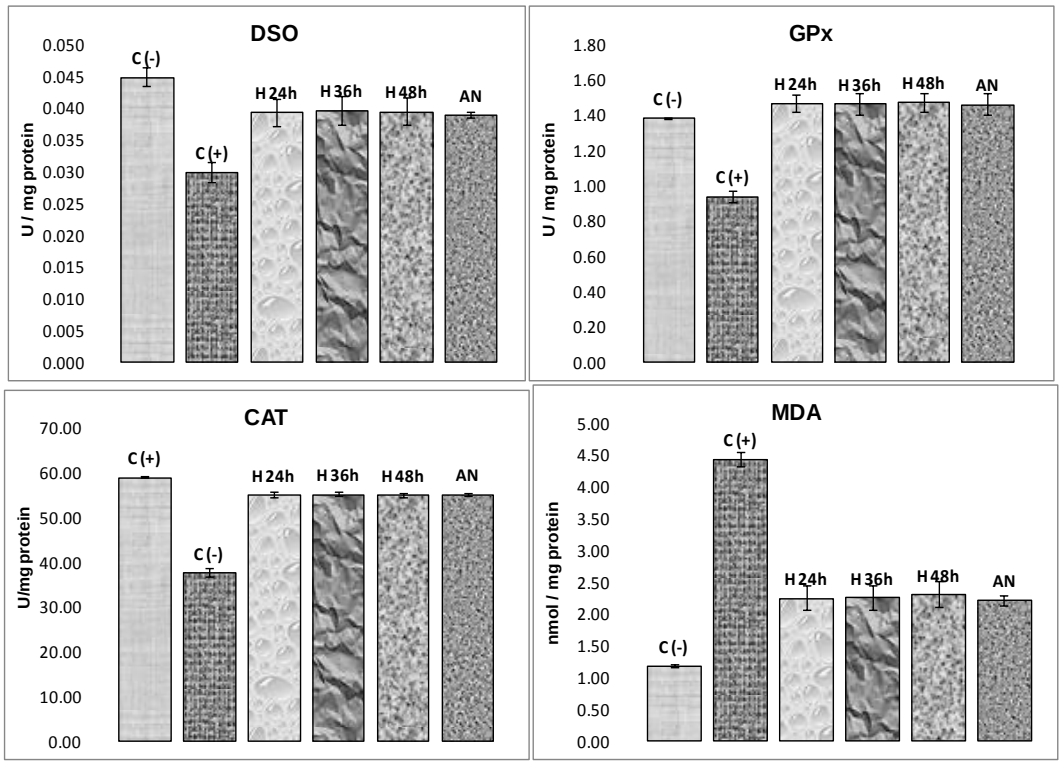

Figure 3. Photoprotective effect of albumin 1 and their respective hydrolysates in cultured human skin exposed to UV radiation.

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Production Of Enzymatic Hydrolyzates From Globulin Amaranth Seed: Biochemical And Functional Characterization

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Abstract
Globulin, the major storage protein of amaranth seed (*Amaranthus hypochondriacus* L.) was hydrolyzed by alcalase. The effects of enzymatic hydrolysis on globulin molecular weight, surface hydrophobicity, secondary structure and hipotensive activity were investigated. Moreover, the optimization of interfacial properties (foaming and emulsification) as obtained of hydrolyzates was carried out using the response surface methodology. Finally, the bitterness intensity of globulin amaranth hydrolyzates was evaluated. The results from this study demonstrated that it might be possible to produce hydrolyzates obtained from globulin amaranth seed for their application in the design of functional foods.
Keywords: amaranth grain, enzymatic hydrolysis, hypotensive activity, interfacial properties, bitterness.

Introduction
The enzymatic hydrolysis is a particularly attractive technique to modify proteins owing to its moderate temperature and pH conditions during processing, the relative easiness to control the reaction, the lack of byproducts, and maintenance of the nutritional value [(1, 2)]. It has been observed that during an enzymatic hydrolysis of proteins their molecular structure is modified, which induces changes in their techno-functional properties, such as solubility, emulsification, gelification, foaming, among others [(3)]. In addition, diverse effects on health (anticoagulant, antihypertensive, and antioxidant) have been attributed to the peptides released during enzymatic hydrolysis of proteins [(4)]. Particularly, the antihypertensive effect is related with the inhibition of the angiotensin I converting enzyme (ACE), which plays an important physiological role in the regulation of arterial pressure through the renin-angiotensin and kallikrein-kinin systems [(5)]. Thus, the study on enzymatic hydrolyzates of proteins is currently receiving great attention in the field of food and nutrition, because there is a great interest in obtaining natural origin ingredients that can be incorporated in diverse feeding systems for their application in the industrial sector, as well as for the use of alternative protein sources that have not been fully exploited [(6)].
Hence, the objectives of the present study were to evaluate the biochemical and interfacial properties, the hypotensive activity, and the intensity of bitterness of the enzymatic hydrolyzates as obtanined of globulin amaranth (*Amaranthus hypochondriacus*) seed. Besides, we aimed at establishing the hydrolysis conditions (time and enzyme/substrate ratio: E/S) that optimize the yield of foams and emulsions with hypotensive activity.

Materials and methods
Materials. Defatted flour from *Amaranthus hypochondriacus* L. seed was used. Alcalase (protease from *Bacillus licheniformis*), hippuric acid, 2,4,6-trinitrobenzenesulfonic acid (TNBS), hippuryl-L-histidyl-L-leucine (HHL), 1-anilino-8-naphthalene-sulphonate (ANS) and ACE from rabbit lung (E.C. 3.4.15.1) were purchased from Sigma-Aldrich (St. Louis, Mo, USA). All other reagents and chemicals were analytical grade. The analyses throughout this research were performed in triplicate.

Preparation of amaranth globulin hydrolyzates. Globulin (*Glob*) was extracted with Na_2SO_4 (5%, w/v) [7]. The protein was lyophilized and stored at -20ºC. The *Glob* yield were quantified by evaluating nitrogen content according to Kjeldahl´s method (%N x 5.85) [8] and protein content according to Bradford´s [9]. Hydrolysis of *Glob* was conducted with alcalase at three ratio enzyme/substrate (E/S) : 0.4, 0.8 and 1.6 AU/g protein, at pH 7.4 and 50°C [10]. After digestion, the enzyme was inactivated by immersing the reaction in boiling-water bath for 10 min and the liquid was centrifuged at 5000*g* for 20 min. The supernatant was lyophilized and stored at -20ºC until used for further experiments.

Biochemical Characterization

Degree of hydrolysis (DH). The degree of hydrolysis (DH) was determined by of free amino groups with TNBS reagent [11]. L-leucine was used as a standard. DH values were calculated using the following equation: $\%DH = \left(h/h_{tot}\right) \times 100$; where h is the number of hydrolyzed bonds and h_{tot} is the total number of peptide bonds present in *Glob*.

Molecular exclusion chromatography. Hydrolyzed protein was analyzed by gel filtration chromatography using a molecular exclusion column (Sephadex G-50). Absorbance at 214 nm was monitored and 0.5 ml fractions were collected. Standard molecular markers were used to calculate molecular weight (M_W) of the hydrolyzates.

Surface hydrophocity (So). Surface hydrophobicity was determined using the apolar fluorescente dye (ANS) [13]. The fluorence intensity (FI) of each sample was measured at 390 nm (excitation) and 470 nm (emission) using a spectrofluorophotometer (Hitachi model F93). The surface hydrophobicity (*So*), expressed as the initial slope of FI versus protein concentration was calculated by linear regression.

FTIR measurement. Mid-infrared spectra were acquired in the 4000 – 700 cm-1. Range using a Spectrum 2000, Perkin-Elmer spectrometer coupled to a Hyperion 3000 vis/IR microscope equipped with a photoconductive MCT detector and a 15x objective for transmission measumerents. A 20x Germanium ATR-crystal objective was used for ATR measumerent.

Functional Characterization

Foaming properties [14]. Foaming capacity and foaming stability were determined by preparing 1% (w/v) dispersions in 0.05 M phosphate buffer (adjusted to pH 3 – 9). The dispersions of protein fractions and hydrolysates were homogenized at 13,500 rpm for 1 min using a homogenizer (IKA T25, Ultra Turrax, IKA-Labortechnick, Staufen, Germany). The foaming capacity (FC) was calculated according to the following equation: $Foaming\ capacity\ (\%) = \left(\frac{A}{B}\right) \times 100$, where A is the foam volume (mL) immediately after mixing and B is starting volume (mL) of liquid phase. Foam stability (FS) was calculated as follows: $Foam\ stability\ (\%) = \left(\frac{C}{A}\right) \times 100$, where C is foam volume (mL) after standing for 30 min and A is foam volume (mL) immediately after mixing.

Emulsifying properties [14]. Corn oil (5 mL) and sample solution (5 mL) at 1% w/v (adjusted to pH 3 – 9) were mixed and homogenized at a speed of 20,000 rpm for 1 min. The emulsifying capacity (EC) was defined as follows: $Emulsifying\ capacity\ (\%) = \left(\frac{A}{B}\right) \times 100$, where A is height of emulsified layer; B is height of the contents of the tube. Emulsion stability (ES) was measured by recentrifugation at 3,000*g* for 5 min followed heating at 80ºC for 30 min and was expressed as follows: $Emulsion\ stability\ (\%) = \left(\frac{C}{D}\right) \times 100$, where C is height of remaining emulsion layer and D is height of original emulsified layer.

Bitterness evaluation. The bitterness intensity of the hydrolyzates were evaluated through a panel of 10 analytical judges, previously trained in the identification and quantification of the bitter taste with different concentrations of caffeine solutions (0.02, 0.025, 0.03 y 0.04 % w/v) and using a 16cm line scale. Solutions containing 3% (w/v) protein concentrations of the hydrolyzate powders were coded with 3-digit numbers and presented in duplicates to the panel in a random order using a 0.04% (w/v) caffeine solution

as the reference point. Panelists were each given 2.5 mL of the protein hydrolyzate solution and bitterness of each sample was scored in relation to that of the reference solution using 16cm line scale. Water and unsalted bread were used by each panelist to clean their palate in between evaluation of different samples.

Experimental design, modeling and optimization.

A 3^2 factorial design approach was used for optimization wherein the reaction time (X_1) and E/S ratio (X_2) were selected as independent variables and the foaming and emulsion (capacity and stability) were selected as response variables. This design permitted distinct levels to be tested: 1, 9.5 and 18 h and 1, 8 and 15 h for the reaction time for albumin 1 and globulin, respectively; 0.4, 0.8 and 1.2 UA/g protein for the E/S ratio. Optimized foams and emulsions were assayed for their ACE-inhibitory activity. All experiments were run in random order. The parameters were estimated by multiple linear regression using Stargraphics Plus v. 5.1 (Statistical Graphics, Manugistics, MD, USA). The effect of each term in the model, together with its statistical significance, for every response variable was analyzed via standardized Pareto charts.

The quadratic polynomial model proposed for each response variable, Y, took the form:

$$Y = \beta_0 + ß_1X_1 + \beta_2X_2 + ß_{1,1} X_1^2 + \beta_{2,2} X_2^2 + \beta_{1,2} X_1 X_2 + \varepsilon$$

Where: β_0 is the vertical intercept; $ß_1$ and β_2 are linear coefficients; $ß_{1,1}$ and $\beta_{2,2}$ are quadratic coefficients; $\beta_{1,2}$ is the interaction coefficient; and ε denotes the experimental error.

Results and disscusion

Alcalase hydrolysis, under the conditions of time and E/S relation used in this study, modified the molecular structure of globulin (*Glob*), obtaining hydrolyzates with: DH ranging from 2 to 30%; electrophoretic profiles that presented a progressive degradation of subunits with increasing DH; a particular distribution of peptidic species (proteoses, peptones, peptides and free amino acids) for each combination of time and E/S ratio; largest hydrophobicity surface (mainly at acid pH); and different chemical structures from than that native protein.

As a result of changes in the molecular structure of *Glob* we obtained hydrolyzates that improved the assessed techno-functional properties, presenting high solubility (≥ 70%), significantly ($p<0.05$) higher than that observed for *Glob* (34 – 64%). Hydrolyzates also presented higher ($p< 0.05$) foaming and emulsification properties than their respective protein fraction. The highest values for these properties were obtained at an acid pH (especially at pH 5 and 6). The alcalase hydrolysis of *Glob* also yielded peptidic chains with inhibitory activity for ACE; the highest activity was of 66 – 77%, obtaining IC_{50} values between 0.58 – 1.99 mg/mL.

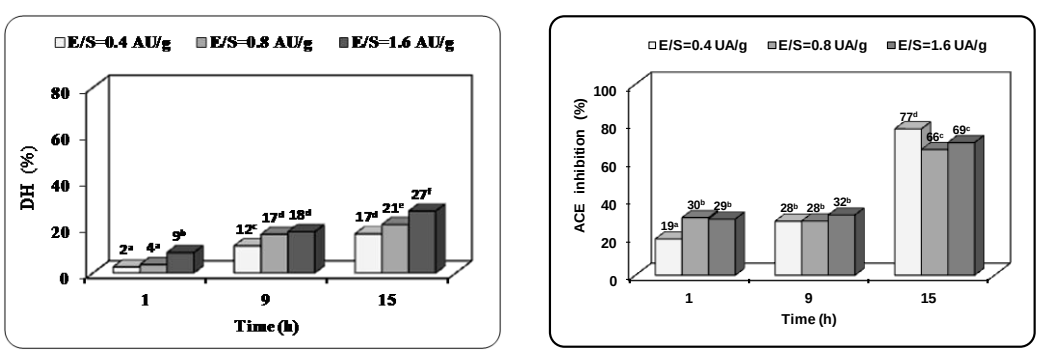

Fig 1. DH and ACE-inhibitory activity as a function of experimental conditions (time and E/S ratio) assayed for hydrolysis of albumin 1 and globulin with alcalase.

By applying the surface response design, optimal time and E/S ratio conditions were obtained for the hydrolysis of *Glob* with alcalase, which yielded greater capacity and stability for foaming and emulsification. The obtained response surfaces were adjusted to quadratic models. The fitness of the models was also expressed by the adjusted coefficient of determination (R^2 adj), which ranged from 0.721

– 0.911, which indicated that more than 70% of the variability in the final response could be explained by these models. The optimal hydrolysis conditions and their corresponding prediction by the models for foaming and emulsification (capacity and stability) as obtained of globulin are depicted in Table 1.

Table 1. Optimal hydrolysis conditions (time and E/S ratio) for globulin.

Optimal hydrolysis conditions		Response variable	Prediction
Time (h)	E/S (AU/g)		(%)
8.8	0.78	Foaming capacity	147.1
7.6	0.4	Foam stability	73.3
2.3	0.73	Emulsifying capacity	86.0
15.0	0.4	Emulsion stability	94.3

Finally, these protein hydrolyzates presented an intensity of bitterness between 2.9 and 12.3 (equivalent to 0.04 – 0.08 w/v of caffeine), classifying them as products of low bitterness (Fig. 2).

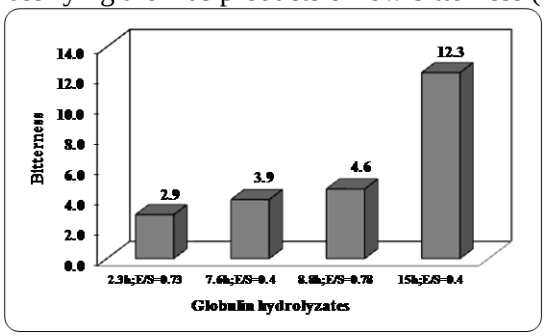

Fig 2. Bitterness intensity of enzymatic hydrolyzates determined using a trained sensory evaluation panel.

Conclussion

The present study is the first to evaluate experimentally the techno-functional properties together with the ACE inhibitory capacity of *Alb1* and *Glob* hydrolyzates of the amaranth grain (*Amaranthus hypochondriacus*). The hydrolyzates obtained from *Alb1* and *Glob* could be used in diverse food systems due to their high solubility and as foaming or emulsifying ingredients that contain antihypertensive agents for their application in the design of functional foods. In this way, an alternative use would be provided for the amaranth grain for its exploitation and thereby fostering its cultivation, since it has not reached yet a high commercial value.

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Phenolic And Cucurbitacin Compounds Of *Cucurbita ficifolia* Bouche Fruits

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Introduction

Plants form an important part of our everyday diet, and chemical constituents and their nutritional value have been intensively studied for decades. Primary metabolites, e.g. carbohydrates, lipids and amino acids, are plant constituents related to nutritional value. In addition to primary metabolites, plants are also able to synthesize a wide variety of low molecular weight compounds known as secondary metabolites (Oksman-Caldentey & Inzé, 2004). Secondary metabolites are being widely examined for their ability to provide health benefits and for its potential to be used either as food additives or as food supplements such as nutraceuticals. The health benefits of secondary metabolites are due to their can exert several physiological functions, comprising: substrates for biochemical reactions; cofactors of enzymatic reactions; inhibitors of enzymatic reactions; absorbents / sequestrants that bind and eliminate undesirable constituents in the intestine; scavengers of reactive or toxic chemicals, inter alia. Such compounds include terpenoids, phenolics, alkaloids and fiber (Dillard & German, 2000).

Cucurbita ficifolia Bouché (Cucurbitaceae), known in México as 'chilacayote', is a cultivated plant whose fruit can be used in many ways, among which can be highlighted the nutritional and medicinal uses. Immature fruits are used to prepare different dishes for human consumption, while mature fruits are used to prepare crystallized candies. Moreover, the seeds of these fruits are consumed whole, roasted, toasted or additive of dishes, and represent a considerable contribution of oils and proteins (FAO, 2012). *C. ficifolia* fruits have also been used as remedies against some health affections. The plant has been reported to cure wounds and used to treat hemorrhoids and fever (Alarcon-Aguilar *et al*., 2002). Pharmacological studies in diabetic individuals suggest that *C. ficifolia* fruit extracts exert a hypoglycaemic action (Acosta-Patiño *et al*., 2001; Alarcon-Aguilar *et al*., 2002; Hernandez-Galicia *et al*., 2002). Meanwhile, to date there are no reports that explain other curative properties of these fruits such as purgative, diuretic, bronchitis, catarrh, hemorrhoids, fever and to cure wounds, among others (Alarcon-Aguilar *et al*., 2002). In other Cucurbitaceae species, phenolic and cucurbitacin compounds possessing diverse biological activities have been identified, being them attributed for curative properties (Chanwitheesuk *et al*., 2005; Chen *et al*., 2005). Phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins) are commonly found in both edible and nonedible plants, and they have been reported to have multiple biological effects, including antioxidant activity. Flavonoids and other phenolics have been suggested to play a preventive role in the development of cancer and heart disease (Kähkönen *et al.*, 1999). Among those flavonoids are catechin, kaempherol and quercetin. Nonce, cucurbitacins constitute a group of diverse triterpenoid substances which are well known for their bitterness and toxicity. These compounds are arbitrarily divided into twelve categories, incorporating cucurbitacins A-T. The cucurbitacins are of great interest because of the wide range of biological activities they exhibit in plants and animals. They are predominantly found in the family Cucurbitaceae but there are also present in several other families of the plant kingdom. A number of compounds of this group have shown cytotoxic, hepatoprotective, anti-inflamatory and cardiovascular significant effects (Yang *et al*., 2008; Diman *et al*., 2012).

The present work was undertaken to detect phenols and cucurbitacins metabolites in fruit extracts of *C. ficifolia* in order to stand out its nutraceutical potential.

Methods and Materials

Extraction of phenols and cucurbitacins metabolites. Immature fruits of *C. ficifolia* were dried into an oven at 60°C during three days, later; 1 g of dried fruit was extracted with 200 ml of ethanol by reflux at 70°C for 2 h to obtain phenolic compounds. In the same way, dichloromethane extracts were carried out to isolate cucurbitacin compounds but reflux was at 40°C. Extracts (ethanol and dichloromethane) were separately concentrated on a rotary evaporator until reaching a final volume of 5 ml.

Determination of metabolites by preparative and analytical Thin Layer Chromatography (TLC). Concentrated extracts (ethanol and dichloromethane) were analyzed, firstly by analytical, followed of preparative TLC. In both cases, mobile phase consisted of hexane-ethyl acetate-formic acid (35:55:10). Standards of kaempherol (Extrasynthese, France) and cucurbitacin E (Extrasynthese, France) were used to get Rf values, being them used to detect those compounds in concentrated extracts. Due to the Rf values obtained of those compounds by analytical TLC were so close between them (Rf of 0.87 and 0.92 for cucurbitacin E and Kaempherol, respectively), and because although extracts were concentrated they showed being diluted, both concentrated extracts (ethanol and dichloromethane) were mixed and applied to preparative TLC. Following, the band related to Rf 0.87-0.92 was characterized by spectrophotometry.

Spectrophotometric characterization. Compounds retained in the band were dissolved in MeOH (50 mL) by agitation during 24 h, subsequently it was concentrated to 5 ml (called as TLC-KC). However, for spectrophotometrical purposes TLC-KC was diluted (1:10), so absorption was determined from 200 to 800 nm of wavelength. Also, the same spectrophotometrical analysis was carried out in the former concentrated extracts (ethanol and dichloromethane).

Total phenol content (TPC). The concentrated extracts (ethanol and dichloromethane) and the TLC-KC were used to assess the amount of total soluble phenols according the Folin-Ciocalteu method. Briefly concentrated extracts (500 μl) were mixed with distilled water (3.5 ml) and Folin-Ciocalteu reagent (250 µl). After 5 rest min, sodium carbonate saturated solution (500 µl) was added and the final volume was adjusted to 5 ml with distilled water. Samples were shaken and incubated during 30 min in dark, and were immediately read at 760 nm. A standard curve (0.94-28.22 mg l^{-1}) was built with gallic acid standard (Sigma Aldrich Co., USA) being the results expressed as equivalent mg of galic acid per gram of extract (mg GAE g^{-1}).

Results and Discussion

Results of the analytical TLC revealed the presence of phenolic and cucurbitane compounds in the ethanolic and dichloromethane extracts, respectively, especially of the flavonol kaempherol (Rf of 0.87) and the cucurbitacin E (Rf of 0.92). Moreover, the spectrophotometrical analysis of both extracts showed higher absorption (>1) from 200 to 270 nm of wavelength (Figure 1a, b), coinciding with the maximum absorption reported for kaempherol (267 and 365 nm; Extrasynthese, France) and cucurbitacin E (235 nm; Extrasynthese, France). The same pattern was observed when TLC-KC was spectrophotometrically analyzed (Figure 1c), but similar absorption values were determined when it was compared with the two concentrated extracts although TLC-KC was diluted, showing that the preparative TLC achieve the concentration of the compounds of interest. TPC were determined in concentrated extracts of ethanol, dichloromethane and TLC-KC at values of 0.30, 0.11 and 1.8 mg GAE g^{-1}, respectively. Find values were higher than those reported for other Cucurbitaceae species, *Coccinia grandis* (0.0016 mg GAE g^{-1}) and *Cucurbita pepo* (0.0002 mg GAE $g^{-}1$) (*Mongkolsilp et al., 2004*). In addition, the above results corroborated that preparative TLC accomplished higher amounts of kaempherol and cucurbitacin E.

Kaempherol is a flavonoid found in many edible plants (e.g. tea, broccoli, cabbage, kale, beans, endive, leek, tomato, strawberries and grapes) and in plants or botanical products commonly used in traditional medicine (e.g. *Ginkgo biloba, Tilia spp, Equisetum spp, Moringa oleifera, Sophora japonica* and propolis). Some epidemiological studies have found a positive association between the consumption of foods containing kaempferol and a reduced risk of developing several disorders such as cancer and

cardiovascular diseases. Numerous preclinical studies have shown that kaempferol and some glycosides of kaempferol have a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, cardioprotective, neuroprotective, antidiabetic, anti osteoporotic, estrogenic/antiestrogenic, anxiolytic, analgesic and antiallergic activities (Calderón-Montaño *et al*., 2011). This compound has been reported for different plants belonging to the Cucurbitaceae family such as *Cucurbita maxima* (Miean & Mohamed, 2001), *Momordica cochinchinensis* (Yang *et al*., 2008). Meantime, cucurbitacin E has been reported in some Cucurbitaceae plants like *Cucurbita andreana*, *Luffa echinata* and *Wilbrandia ebracteata* (Chen *et al*., 2005; Dhiman *et al*., 2012). It is a strong antifeedant for the flea beetle, and inhibits cell adhesion through a mechanism involving disruption of the cytoskeleton. Cucurbitacin E exerts potent growth inhibitory activity *in vitro* directly against prostate carcinoma explants (IC_{50} 7–50 nM in 2–6 day exposures), and the mechanism of actin disruption may underlie the toxicological activity observed from *in vivo* models (Chen *et al*., 2005).

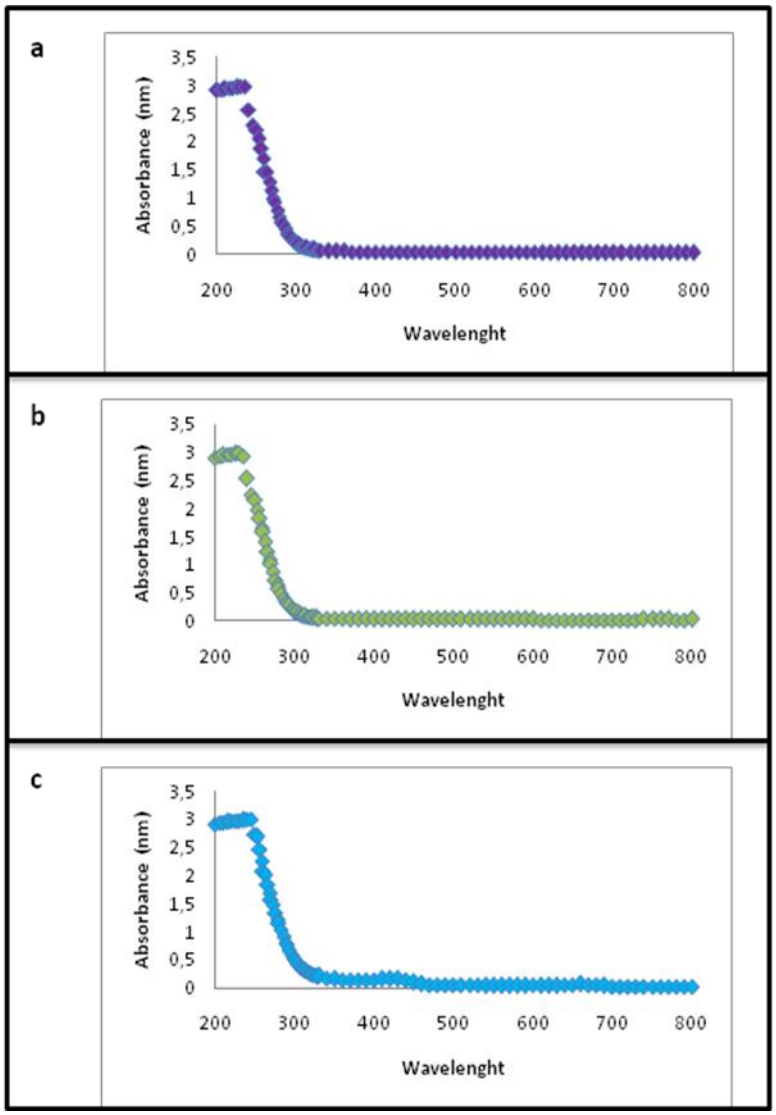

Figure 1. Spectral scan (200-800 nm) of *Cucurbita ficifolia* extracts. **a** ethanolic extract, **b** dichloromethane extract, **c** TLC-KC extract.

Further studies should be conducted in order to quantify compounds detected (kaempherol and cucurbitacin E), and to accomplish future investigations to corroborate their putative pharmaceutical effects. Above with, there will be the certainty that *C. ficifolia* fruits besides its nutritive properties and of its probed properties in lowering sugar blood levels, has the potential to help in the treatment of other illnesses and disorders, hightlighting its potential use as nutraceutical.

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Enzymatic Hydrolysis For Production Of Pectin Oligomers From Guava And Papaya

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Introduction

Oligosaccharides are increasingly attracting the interest as a prebiotic additive in functional foods. The major prebiotic oligosaccharides on the market are inulin fructo-oligosaccharides and galacto-oligosaccharides, whose benefits have been evaluated using a range of in-vitro and in-vivo methods (4). In addition, pectin oligosaccharides have currently been proposed as a new class of prebiotics, however the examination of their properties is necessary (6). Pectin oligosaccharides have been obtained with enzymes mixtures from different plant byproducts, such as chicory roots, citrus peel, cauliflowers (florets and leaves), endive and sugar beet (7, 10). Enzymatic processing may enable the recovering of valuable byproducts; the partial enzymatic hydrolysis leads to the formation of oligosaccharides. Oligosaccharides derived from pectins by either enzymatic hydrolysis or by steam injection heating under pressuring extraction showed potential prebiotics properties, as they selectively increased populations of beneficial bacteria such as bifidobacteria and lactobacilli, while decreasing harmful bacteria as clostridia (4). Several experiments suggest that application of pectin-derived oligosaccharides on healthcare seems to be carbohydrate-structure-dependent. In addition, the prebiotic activity will also be dependent on the polymerization degree and ester-substitution of the sugar moieties. (6).

With the increasing demand of physiologically functional foods, the future for products containing pectin oligosaccharides seems to be very promising, and although a good deal is known about their properties, the characterization of pectin oligosaccharides derived from novel materials is needed (4). Papaya and guava are tropical fruits of high perishability containing significant amount of pectin that were selected in this study to evaluate its potential as a source for pectin oligosaccharides production by enzymatic hydrolysis.

Materials and Methods

Papayas were bought at a local market, and guavas were collected from a local orchard. Alcohol insoluble solids (AIS) were isolated from 100 g of diced fruit flesh. Briefly, the flesh was homogenized in a ultraturrax with 300 ml of ethanol 95% to dissolve low molecular weight sugars and organic acids and the slurry was extracted subsequently, the insoluble material was filtered through glass fiber filter, recovered and subsequently washed with 250 ml of ethanol, 250 ml of chloroform:methanol (1:1, v/v) and 250 ml of acetone. The insoluble material was oven-dried at 37°C, weighed and stored in a desiccator until use. (8)

Commercial pectinases (Macerex) were used for oligosaccharide production from the AIS. The enzymatic hydrolysis was performed in sodium acetate 0.2 M, pH 4.6 and 37°C during 240 min; an aliquot of 1 ml was taken every 30 min, and enzyme activity was inactivated in water bath at 100°C for 10 min. The experiment was triplicated, the experimental unit consisted of 200 mg of AIS and the hydrolysis kinetic was profiled in terms of liberated content (mg g^{-1}FIA) of total sugars (TS), reducing groups (RS) and uronic acid (UA).

For UA determination, 200 μL aliquots were assayed as described by Ahmed and Labavitch (1977) which consisted in the following: 1.2 mL sodium borate (12.5 mM) in concentrated H_2SO_4 was added to aliquots and the mixture was kept for 5 min in a water bath at 100 °C. Coloration was produced after adding 20 μL *m*-phenylphenol (0.15 %) in NaOH (0.5 %), absorbance was recorded at 520 nm. The RS were evaluated

using the method proposed by Gross (1982); briefly, 1 mL of sodium borate (0.1 M, pH 9.0) was added to 200 μL aliquots and the mix was kept for 10 min in a water bath at 100°C, then absorbance was measured at 276 nm. Galacturonic acid was used as standard in both methods. TS content was determined by the anthrone assay (9), as follows: 400 μL distilled H_2O and 1 ml anthrone (0.2 %) in concentrated $H_2SO_4$was added to an aliquot of 100 μL, the mixture was heated for 10 min in a water bath at 100 °C; after cooling, absorbance was measured at 620 nm. The concentration was calculated from a calibration curve of glucose. All spectrophotometric methods were developed using a Cary UV-Vis 1-E spectrophotometer (Varian Inc.) and all standards used were purchased from Sigma®.

For neutral sugar and molecular size analysis, samples of 0-30, 60-90, 120-150, and 180-210 min, were combined and lyophilized. Neutral sugar composition was determined by alditol acetate method (2). Aliquots of 200 μL were hydrolyzed with trifluoroacetic acid at 121°C for 1 h, the acid was evaporated and the sugars were converted to alditol acetates with a reduction reaction using 150 μL $NaBH_4$(20 $mg \cdot mL^{-1}$in NH_4OH 1N) and later acetylation reaction with 200 μL acetic anhydride and 20 μL 1-methylimidazole as catalyst. The derivative material was suspended in 2 ml water and 3 ml chloroform. The chloroform phase was recovered; then it was evaporated, and alditol acetates were recovered in acetone for injection into a Varian CP-3800 gas chromatograph with FID (250 °C), with a DB-23 (J&W Scientific) capillary column of 30 m x 0.25 mm (210 °C) and helium as carrier gas (constant flow of 3 $ml \cdot min^{-1}$). The integration of areas was performed using the MS Workstation software v. 6.5 (Varian Inc.), and measurements were calculated from the standard curves of rhamnose, fucose, glucose, arabinose, xylose, mannose, galactose, and myo-inositol as internal standard. All standards used were Sigma®.

Size distribution profiles of the oligomers produced at different times of enzymatic hydrolysis were estimated by size exclusion chromatography (SEC) using the methodology of Rose et al. (1998) with some modifications. Briefly, 0.5 ml of sample was injected into a packed with Bio-gel P4 extra fine (Bio-Rad) column (20 x 1 cm). The sample was eluted to constant flow (10 $ml \cdot h^{-1}$) using 0.2 M ammonium acetate (pH 5.2). The eluted volume was fractionated and collected as individual fractions (collector Bio-Rad model 2110) at volumes of 0.8 ml. The uronic and reducing sugars content of each fraction was determined in order to obtain the polydispersion and chromatographic profile. The void and total volumes were estimated by elution of blue dextran and galacturonic acid, respectively (Sigma®).

Data was analyzed according to a blocked design of repeated measures over the hydrolysis time. The factor fruit at 2 levels (papaya and guava) and time of hydrolysis at 8 levels (0, 30, 60, 90, 120, 150, 180 and 210 min). Data were analyzed by ANOVA, considering this design and significance level of 95%, using Minitab version 16.

Results and discussion

The oligosaccharide production by partial enzymatic hydrolysis was prepared from the guava and papaya AIS trying to simulate the byproduct derived from juice industry. Figure 1 shows the pattern of solubilization and depolymerization of pectins produced by the enzymatic treatment in terms of soluble UA and liberation of reducing groups. The initial soluble UA in papaya was up to 4 fold more than guava, likewise, papaya pectins were rapidly liberated in contrast to the low range of solubilization occurred in guava pectins (concentration increase after 30 min).

Relative to hydrolysis, the action of pectinases during 150 min of incubation,resulted in a slow but gradual increment of RS concentration suggesting a solubilization and/or depolymerization of polysaccharides structures (5). The increment of RS production was higher in papaya AIS; moreover, the increase in RS during the guava AIS hydrolysis was slower between 60 and 90 min of incubation. On the other side, this increase occurred until the incubation time of 150 min during papaya AIS hydrolysis. The differences in the action of the pectinases suggest that there may be differences in the spatial composition of pectins of both fruits and likely the site of action of the pectinase at papaya pectin showed more accesibility than guava pectin.

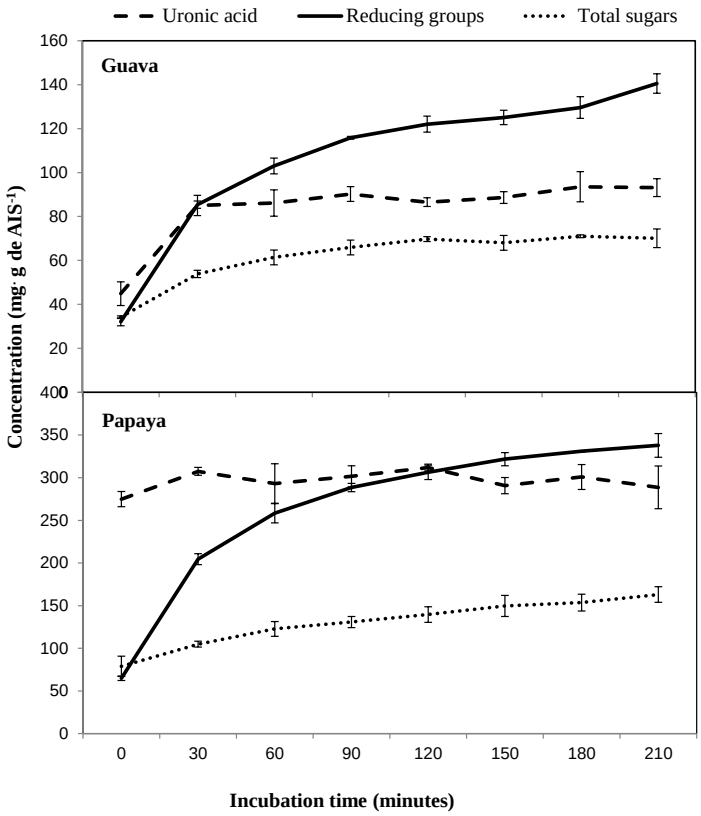

Figure1. Releasing profile of uronic acids, reducing sugars and total sugars by enzymatic hydrolysis of guava and papaya cell walls..

Table 1. Molar distribution (%) of soluble neutral sugars during enzymatic hydrolysis of guava and papaya pectins.

Time (min)	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose
				Guava			
0-30	3.89±0.02	1.08±0.00	45.54±0.76	6.03±0.11	7.03±0.08	16.01±0.81	20.42±0.16
60-90	4.28±0.09	1.18±0.02	45.66±0.10	6.48±0.03	5.32±0.02	14.95±0.15	21.92±0.40
120-150	5.11±1.6	1.23±0.04	44.37±1.22	6.41±0.45	5.08±0.24	14.85±0.26	23.82±0.01
180-210	4.82±1.5	1.23±0.02	41.61±0.96	6.74±0.42	5.02±0.22	14.61±0.08	25.30±0.02
				Papaya			
0-30	11.15±1.7	1.08±0.07	13.30±0.54	11.79±0.33	8.07±0.19	27.06±3.96	27.54±1.15
60-90	8.32±1.22	1.15±0.05	10.45±0.39	13.51±0.48	7.56±0.22	18.11±0.26	40.91±0.12
120-150	9.69±0.21	1.24±0.03	8.75±0.14	12.39±0.02	6.91±0.00	15.64±0.02	45.38±0.42
180-210	7.33±2.04	1.26±0.02	9.96±3.27	13.13±1.59	6.77±0.19	14.59±0.28	46.97±2.75

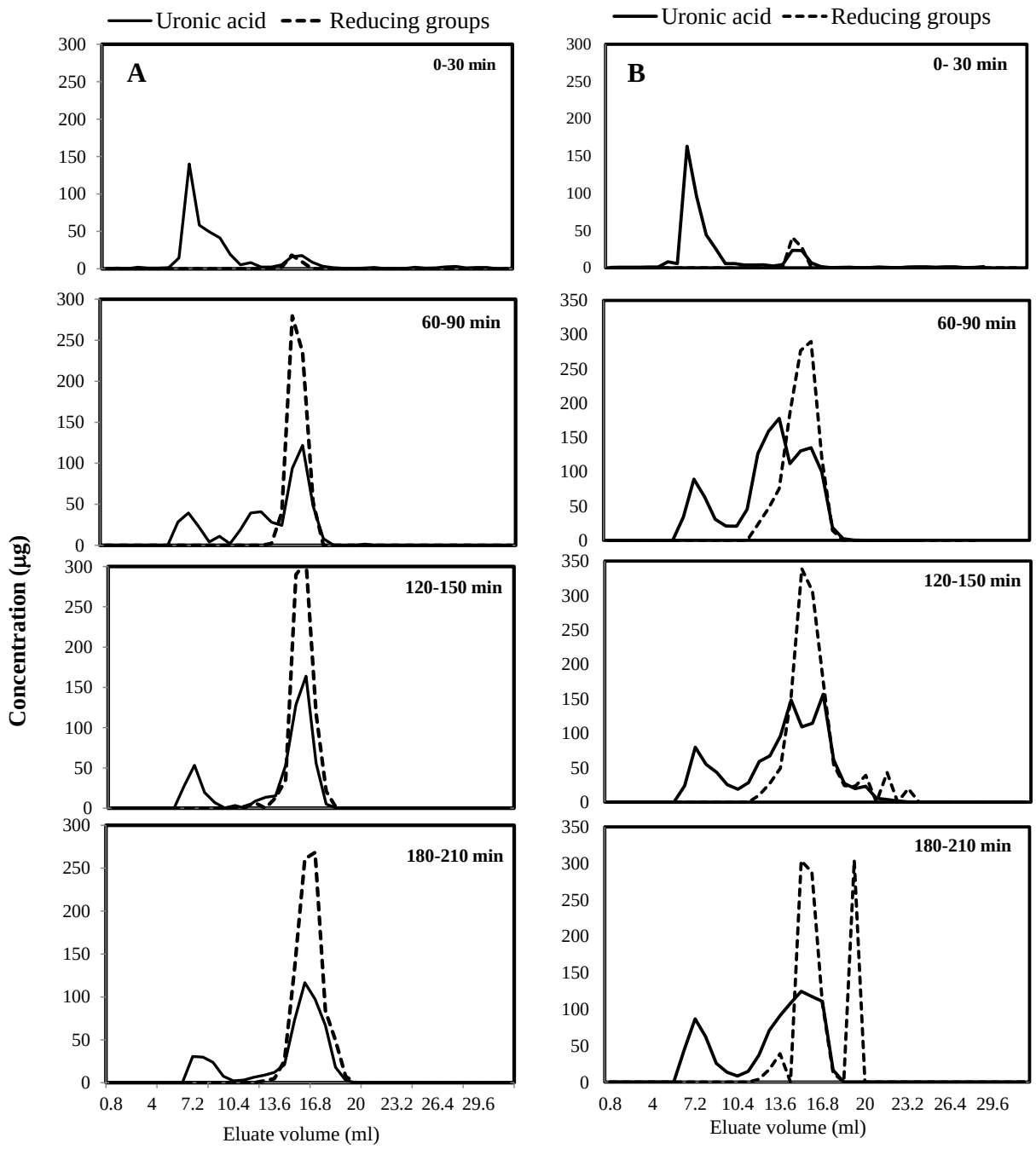

Figure 2. Changes in P4-exclusion profile based in the uronic acid and reducing groups liberated during enzymatic hydrolysis of guava (A) and papaya (B) alcohol insoluble solids.

The molar distribution of monosaccharides in the soluble pectin derived from enzymatic treatment of fruit AIS (Table 1) showed differences during incubation time. The hydrolysis of guava solids released polymers rich in arabinose starting from the 30 min, while during papaya pectin hydrolysis the changes in

sugars were dispersed, at 120 min of incubation glucose was predominant sugar followed by galactose and xylose.

At 30 min of guava AIS enzymatic maceration, the soluble pectin had the larger substitution of arabinose residues (45.5%), whereas the glucose proportion value was kept slightly increasing until 210 min (25.3%). Most of the arabinose present in the cell walls is in the form of arabinans or arabinogalactans, forming the branching structures of the Rhamnogalacturonans Type I domain (3). This result suggests that enzymatic treatment was effective to release pectins associated to the microfibrillar phase, however the treatment had low accessibility to the hemicellulosic fraction since a low content of xylose was liberated from the AIS. Xyloglucan is the main hemicellulose present in the fruits cell wall (3).

The size distribution profile showed a marked decrease in molecular size at 60 and 210 min during the hydrolysis of AIS from guava and papaya, respectively. Considering the separation of carbohydrates in order of large to small polymerization degree, the range of size exclusion of the P4 (800 to 4000 daltons) column permitted the recovering of higher molecular weight pectins (≥4 000 daltons) in the elution volume near to 8 ml whereas, pectins of lower sizes (800 daltons) were recovered at 17 ml. Figure 2 shows the accumulation of reduced size pectins over the time of AIS hydrolysis. An evident reduction of larger sizes pectins occurred at 60-90 min interval in both fruits, however a difference was observed at 180-210 min of hydrolysis in papaya where a bimodal profile in the reducing groups peaks suggested an extensive depolymerization of the soluble pectin.

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Effect Of The Addition Of Fructans In The Manufacture Of Sodium Caseinate Edible Films

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Abstract

Fructans are functional food ingredients which offer a unique combination of nutritional properties and important food technological benefits due to their health-promoting effects as prebiotics and foods additive application. They are found in *Agave tequilana* Weber var. azul. Because of its gelling characteristics, inulin allows the development of alternative edible packaging materials without compromising on taste or texture. In this research, the effect of the incorporation of fructans of *Agave tequilana* in the formulation of edible films was studied. In this case, three samples of commercial fructans were used, Agaviotica-PR95 and La Capilla from *A. Tequilana* Weber and inulin Orafti-Beneo GR. Sodium caseinate film was used as control sample. Films were developed on the basis of 10% w/**v** of sodium caseinate (SC), 20% w fructans / w SC, 3% w glycerol/ w total solid amount, and 0.1% w beeswax/ w total, and also 0.1% Tween 80/w total as emulsifier. Thickness, weight, density, color, water vapor permeability (WVP), and the mechanical properties of tensile and puncture strength were registered. All the results were analyzed by ANOVA with a significance level of 0.05. La Capilla showed 47.5 % more WVP efficiency compared to the standard, while the Orafti Beneo GR 38.7 % and Agaviotica 24.5 %. There were no statistical differences among the main attributes of color independently of the type of fructan. The addition of fructans in the films affected adversely the puncture strength of the materials, showing lower forces than in the control sample. The puncture strength of Orafti Beneo GR was higher compared to La Capilla and Agaviotica respectively. As opposed, it is observed that the addition of fructans improved the tensile strength of the films, and also showed a greater tendency than control sample except for La Capilla films. The results suggested that it is feasible to perform edible films based on fructans and sodium caseinate with sensorial characteristics acceptable to be used as bioactive coatings.

Keywords: Edible films, fructans, bioactive coatings.

Introduction

Fructans are polysaccharides with a degree of polymerization of 3 to 80, mainly consisting of fructose and some glucose residues. For technical applications as a food additive, and nutritional and soluble dietary fiber, are a major international agribusiness trend.

Chicory is the main source of linear inulin-type fructans most used in the world, but there are other sources of fructose polymers in nature. There are more than 36,000 plant species containing various types of fructans. Among these, plants belonging to the family Agavaceae reports about 310spp, and 272 of those can be found in Mexico. A. tequilana Weber Var. azul is an important crop due to the evidence of the presence of fructans.

Also, these plants have a significant content of these biomolecules branched and linear, including inulin. Studies in the characterization of agave fructans, have revealed that it is a raw material with a high concentration of fructans[5].

Furthermore, the development of edible films and coatings and / or biodegradable, has received the most attention because it contributes to the extension of the shelf life of food. First, an edible film is defined as a thin layer of edible material formed as a coating on a food or placed on or between food components[4].

As packaging, it can also function as a selective barrier against the transmission of water vapor and gases such as CO_2 and O_2, vapors and solutes. In addition, it provides mechanical integrity and it facilitates handling of food [3]. Such materials can be used as vehicles through which additives can be incorporated into the food, in order to lengthen their storage and / or physicochemical and organoleptic properties.
For the manufacture of edible coatings have been used a variety of natural polymers such as polysaccharides, proteins and lipids, either alone or in combination.

Materials and methods

Materials

The raw materials for edible coating were sodium caseinate (Sigma, lot 013K0053), Glycerol (Fermont, lot 06441), polyoxyethylene sorbitan monooleate (Tween 80, Hycel brand, lot 559) and distilled water as a solvent. Also, three commercial samples of fructans were used, Agaviotica and la Capilla from commercial A. Tequilana Weber and inulin fructans Orafti Beneo-GR donated by companies Agaviotica S. A., and S. Capilla de Guadalupe S. A.

Methods

Solutions were prepared by first adding the selected amount of Sodium Caseinate to distilled water at room temperature. Then, the solution was heated to 70°C on a hot plate stirrer and the selected amount of the commercial fructans Agaviotica, Capilla de Guadalupe and Orafti Beneo-GR, respectively, was added to prepare caseinate-fructan based solution. After incorporation, the mixture was homogenized at 13500 rpm for 1 min in an Ultra-Turrax T-25 homogenizer (IKA works, Inc., Cincinnati, OH) with a 25-F probe. Vacuum was applied to remove bubbles. A film was cast by pouring a solution onto a Plexiglas MR plate.

Characterization of films.

Thickness. Film thickness was measured with a micrometer (KafermodelF1000/30, Germany), at five random positions around the film. The mean value was used to calculate the water vapor permeability and mechanical properties.

Color properties. Color values of the films were measured with Chroma Meter CR-400 (KONICA MINOLTA). The Chroma meter color scale was used for measuring color values: L*, the lightness variable; a*, from green to blue, b*, from yellow to red, C*, chroma and °h, hue degrees.

Water vapor permeability (WVP).The gravimetric modified cup method based on standard method E96-80 (ASTM, 1989) was used to determine WVP. Test cups holding films were then inserted into the pre-equilibrated 0% RH desiccator. Steady state of water vapor transmission rate was achieved within 5 h. Each cup was weighed 8 times at 0.5 h intervals. Thee replicates of each film were tested. The WVP of the films was calculated by multiplying the steady state water vapor transmission rate by the average film thickness and dividing by the water vapor partial pressure difference across the films.

Mechanical properties. Mechanical properties were determined by a texture analyzer (model TA-XT plus Stable Micro Systems Ltd., United Kingdom) coupled to a Texture Expert software (Version 2004).

Tensile strength (TS). Standard methodD882-97 (ASTM, 1997) was used to measure tensile properties of films. Films were cut into strips with a test dimension of 70 mm by 10 mm and fitted to the tensile grips. The initial distance between the grips was 50 mm, and the crosshead speed of 0.5 mm/s. Tensile properties as tensile strength (KPa), elongation at break (%), and Young´s modulus (KPa) were calculated from the plot of stress (tensile force/initial cross-sectional area) vs. strain (extension as a fraction of original length). Five replicates of each film were tested.

Puncture strength (PS). It is used to determine the puncture or rupture characteristics of a material. Samples were cut in circles of 0.04 m diameter and were placed in cells. In this compressive test,

generally the film is compressed by a probe P/2 until the material ruptures or until an elongation limit is achieved [4]. The puncture strength was performed according to Gontard et al.(1991)5 using crosshead speed of 0.5 mm/s.

Statistical analysis. Data were analyzed by one-way analysis of variance (ANOVA) using Statgraphics® computer program (Statgraphics Software, Inc., Centurion XV.I, USA). The comparisons between groups were performed. Tukey test was used to determine the difference at $p<0.05$ significance level.

Results and discussion

Thickness

As shown in Table 1 of thickness, it was achieved to get homogeneous films according to data reported in literature, with agave commercial fructans , La Capilla and Agaviotica; the same happened with inulin-Beneo Orafti.

Table 1. Film thickness (μm), weight and density with three types of fructans.

	Thickness		Weight		Density	
Sample	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.
FA Agaviotica GR-95	104.2	7.4	0.1565	0.036	1.2931	0.049
FA Capilla de Guadalupe	80.4	15.1	0.2440	0.426	1.3324	0.063
Inulina Orafti PR-95	76.0	14.6	0.1405	0.0290	1.3325	0.104
Control	64.3	11.3	0.16	0.21	1.20	0.32

According to the color parameters reported in Table 2, the films obtained on the basis of fructans, have uniform color, since the values of the parameters are similar to the films of similar materials. The color results achieved with the CIELAB system, D65/2 °, are not different in color from the films by the type of fructan employed. There were not statistical differences among the main attributes of color independently of the type of fructan, as show in Table 2.

Table 2. Determination of the color of the films with the three types of fructans: Capilla de Guadalupe, Agaviotica, and Orafti-Beneo GR.

	L		a		b	
Sample	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.
FA Agaviotica GR-95	29.81	3.5	-0.23	0.1	0.040	0.07
FA Capilla de Guadalupe	28.03	3.2	-0.29	0.0	0.080	0.04
Inulina Orafti PR-95	28.26	1.5	-0.31	0.1	0.010	0.11
Control	28.98	2.2	-0.02	0.1	0.002	0.05

The permeability of the sample of Agaviotica was found to be 3.9 times larger than the value reported in the literature for films prepared with caseinate and glycerol. The value of La Capilla was 2.13 times and that one was added to Orafti turned out to be 2.6 times larger compared with the same reference.

Although the formulation used in this experiment differs in the amount of glycerol used. When making a comparison between the 3 fructans was found that fructan agaviótica permeability was 1.8 times higher than La Capilla. The fructan that provides increased permeability is the one of Agaviótica Company. The sample that presented lower permeability was La Capilla. The permeability value obtained is of the same

order of magnitude as that reported for films prepared from cellulose acetate and cellophane. La Capilla showed 47.5 % more WVP efficiency compared to the standard, while the Orafti Beneo –GR 38.7 % and Agaviotica 24.5 %.

Table 3. Values of water vapor permeability of the films made with the three samples of fructans.

Water vapor permeability, (g/m*s*Pa)		
Sample	Average	Std. Dev.
FA Agaviotica GR-95	1.40E-11	2.32E-12
FA Capilla de Guadalupe	9.75E-12	1.51E-12
Inulina Orafti PR-95	1.14E-11	1.93E-12
Control	1.86E-11	7.78E-12

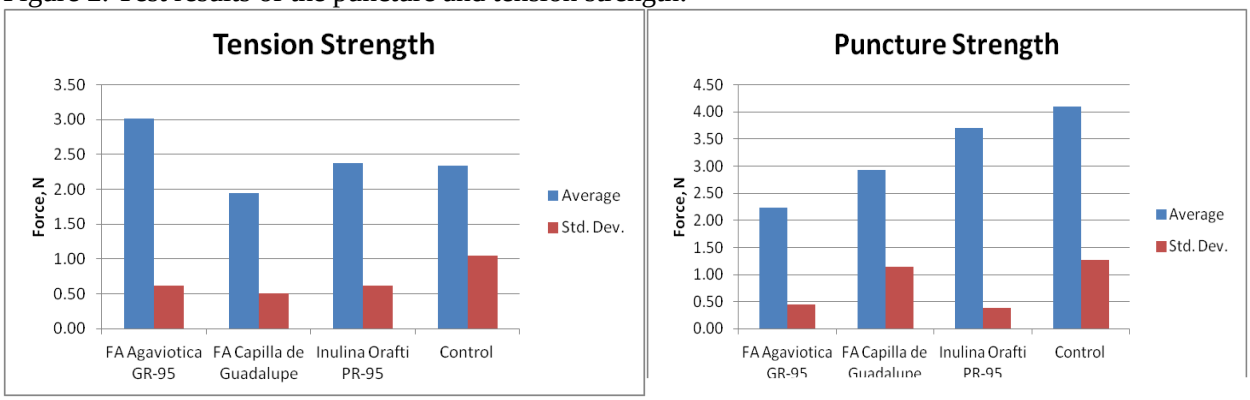
The results suggested that it is feasible to perform edible films based on fructans and sodium caseinate with sensorial acceptable characteristics to be used as bioactive coatings.

Figure 1. Films obtained from caseinate, glycerol and fructans.

The addition of fructans in the film negatively affected the puncture resistance of the material, since the value of the average penetration force at each film added with fructan is less than the control sample. The puncture resistance of Orafti was higher compared with the La Capilla and Agaviotica respectively. In contrast, it is observed that the addition of fructans enhances the tensile strength of the films, and also showed a greater tendency than the control sample, except for the films of La Capilla.

There were not statistical differences among the main attributes of color independently of the type of fructan. The addition of fructans in the films adversely affected the puncture strength of the materials, showing lower forces than in thecontrol sample. The puncture strength of Orafti Beneo-PR-95 was higher compared to La Capilla and Agaviotica respectively. As opposed, it is observed that the addition of fructans improved the tensile strength of the films, and also showed a greater tendency than in the control sample except for La Capilla films.

Figure 2. Test results of the puncture and tension strength.



Conclusions

In this paper we could demonstrate the feasibility of incorporating fructans in preparing edible films. With the addition of fructans in the formulations, there will be provide an additional benefit to this type of active packaging, since they are considered as a source of fiber, thus, it will increase the nutritional value of food. The formulation of caseinate 10% (w / v), 20% of fructans (relative to the total amount of caseinate) and 3% glycerol (with respect to the total amount of solids) was the most suitable to produce the edible coatings with fructans. The value of the water vapor permeability of the films obtained with fructans increases considerably, as discussed above, but still may be an option to consider in the application of edible coatings.

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Physicochemical Characterization Of Poro Cheese From Balancan Region Of Origin In Two Seasons Of The Year

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Introduction

A cheese can be considered traditional when it is produced locally or regionally using techniques passed down through multiple generations. These products have an important place in rural and regional food culture. Many traditional cheese types are produced and consumed locally in Mexico. However, its production only meets local demands and these cheeses are not distributed to and consumed in other regions of the country (Ercan et al., 2011). An example of these traditional dairy products is the Poro cheese, a fresh cheese characterized by its soft and pressed paste, produced with raw milk obtained from crossbreed cows.

Currently, traditional cheese producers are facing a possible extinction of their products due to numerous changes in milk production and cheese manufacture practices. For this reason, it is important to increase our knowledge about the metabolic processes involved in traditional cheese manufacturing as well as the physicochemical associated to the texture and flavor of these traditional cheeses. The final texture of cheese is affected during production and ripening. The texture is directly influenced by changesin the milk due to physically and rheologically changes during initial fermentation process (Gunasekaran and Ak, 2003). Additionalchanges occur in the cheese matrix during ripening due to waterloss and proteolysis (Beuvier and Buchin, 2004).

Likewise, cheese flavor formation depends on physicochemical parameterssuch as moisture, salt content, pH, and ripening conditions. Cheese flavor is derived from the breakdown of milk proteins, fat, and lactose by enzyme or chemical activities (Fox et al., 2000). To date, numerous studies have been conducted to demonstrate the specific characteristics of these traditional cheeses (Qian and Reineccius, 2002; Hayaloglu et al., 2008; Tarakciand Temiz, 2009; Picon et al., 2010; Hernandez-Morales, et al., 2010). However, these traditional cheeses from Mexico have not been studied. Therefore, the aim of this study was to investigate the physicochemical characteristics of milk and Poro cheeses in two seasons of the year.

Materials and Methods

Samples

Milk and cheese samples were collected in falcon tubes with preservative (Bronopol) from four dairiesin Balancan Tabasco, during the dry (march-2011, 106.9 mm rain) and rainy season (august-2011,242.5 mm rain).

Physicochemical analysis

Milk

Physicochemical analyses were performed in duplicate on all milk samples, titratable acidity was determined by the titrimetric method (method 947.05; AOAC 1995), and solids nonfat (SNF), fat and protein were measured by MilkoScan (Foss, Copenhagen, Denmark)and density was measure with a lactodensimeter.

Cheese

Samples of cheese were analysed for fat by the Gerber method (IDF, 1997), total protein by the Kjeldahl method (method 935; AOAC 1995), moisture oven drying ant 102°C(method 926.08; AOAC 1995) and ashes (method 935.42; AOAC 1995) content, pH was measured using a penetration pH meter (Sevengo SG2, Mettler Toledo, USA) and water activity (AW)was measured with an Aqualab water activity meter at 22°C. All measurements were determined in triplicate.

Statistical Analysis

Analysis of variance (PROC GLM) was performed on physical data to identify statistical differences ($P < 0.05$). Data were analyzed using SAS version 9.02 (SAS Institute, Inc., Cary, NC).Data were analyzed using SAS version 9.02 (SAS Institute, Inc., Cary, NC).

Results and discussions

Milk

The physicochemical composition of raw milk collected fromthe four dairies in dry and rainy seasons is presented in Table 1. There were not significant differences in the concentration of fat, protein, lactose, NFG, milk density and acidity of samples between the two seasons ($P < 0.05$).

Table 1.Physicochemical composition of raw milk among four cheese manufacturers (Q1-Q4)

Season	Dairy	Fat (%)	Protein (%)	Lactose (%)	NFG (%)	Density	Acidiy
Dry	Q1	3.605±0.007	3.020±0.042	4.520±0.042	8.210±0.014	1.0298±0.0	1.3±0.0
Dry	Q2	2.775±0.035	2.690±0.014	4.015±0.007	7.340±0.127	1.0326±0.0	1.5±0.0
Dry	Q3	3.460±0.014	3.475±0.035	4.585±0.007	8.905±0.064	1.0298±0.0	1.5±0.0
Dry	Q4	3.555±0.007	2.805±0.035	4.595±0.007	8.130±0.014	1.0288±0.0	1.5±0.0
Rainy	Q1	3.610±0.014	3.555±0.007	4.420±0.028	8.775±0.035	1.0295±0.0	1.6±0.0
Rainy	Q2	3.465±0.007	3.185±0.092	4.410±0.014	8.560±0.085	1.0298±0.0	1.5±0.0
Rainy	Q3	3.505±0.120	3.245±0.021	4.580±0.028	8.610±0.057	1.0298±0.0	1.5±0.0
Rainy	Q4	3.930±0.042	3.425±0.007	4.645±0.007	8.910±0.000	1.0297±0.0	1.5±0.0

NFG = non fatty solids

[a-b] Different superscript letters withn same column indicate significant differences. $P<0.005$

Cheese

Values of ash, moisture, fat, protein, lactose, pH and water activity should in the table 2.

Moisture contents ranged were between 2.69% and 3.47%. A significant difference ($P = 0.036$) was observed in moisture during the rainy season (26.93 vs. 31.28 %; dry and rainy season, respectively). This increase in moisture is associated with the environmental humidity observed in the region of Balancan (~80% H). Higher cheese moisture can result in higher levels of proteolysis, which may subsequently affect texture properties and could also result in variable rates of release of small peptides and amino acids that could contribute to secondary fermentation (Fröhlich-Wyder and Bachmann 2004).

Season of manufacturingdid not affected the Aw (0.95 dry vs 0.95 raining) of the cheese. The Aw was low compared to fresh cheese (0.97) and very similar to Añejo cheese, Bola and Cotija Cheese.Similarly, the pH of Poro Cheese (4.33 dry vs 4.25 raining) did not change between seasons of production and this value was low compared with other cheeses like Añejo cheese, Cotija, Camenbert, Roquefor, Chedar and

Gruyère. The Aw and pH are two important factors in controlling microbial growth, the development of pathogenic microorganism and the production of toxins. Yanai*et al.(1997*) reported that coliforms were found in white-brined cheese, even though they were absent from the pasteurized milk and the starter culture, but their number declined in parallel with the reduction in pH. In batzos cheese a raw ovine milk cheese manufactured in North Greece, *Enterobacteriaceae*and coliforms decreased in number gradually and, by the end of storage, the counts were lower by 2–3 log units over the initial ones; the lower pH of the cheeses manufactured during the summer resulted in lower numbers of coliforms in the cheese.

Likewise, there were no differences in fat content in cheese produced in the two different seasons. The Poro cheese has a high fat(36.70% dryvs 39.79% raining) and protein (27.93% dryvs 26.14% raining) content compared to other types of cheese.Comparable results have been reported by other research groups (Torres de la Cruz et al 2009).

Table 2. Composition physicochemical of Poro Cheese among four cheese manufacturers (Q1-Q4)

Season	Dairy	Ashes (%)	Moisture (%)	Protein (NX6.38) (%)	Fat (%)	pH	Water activity
Dry	Q1	3.81^{a}±0.42	29.32^{a}±0.08	27.41^{a}±0.57	34.94^{a}±0.63	3.94^{a}±0.05	0.94^{a}±0.01
Dry	Q2	1.38^{a}±0.39	27.07^{a}±0.61	30.17^{a}±0.97	34.78^{a}±4.53	4.38^{a}±0.03	0.96^{a}±0.0
Dry	Q3	2.12^{a}±0.17	23.51^{a}±1.19	28.01^{a}±0.67	37.00^{a}±1.32	4.46^{a}±0.05	0.95^{a}±0.0
Dry	Q4	2.57^{a}±0.37	25.40^{a}±3.81	26.13^{a}±1.00	36.22^{a}±0.63	4.48^{a}±0.08	0.94^{a}±0.01
Rainy	Q1	3.58^{a}±1.78	32.89^{b}±0.46	26.12^{a}±0.45	36.00^{a}±0.50	4.29^{a}±0.01	0.94^{a}±0.01
Rainy	Q2	2.66^{a}±0.28	30.81^{b}±0.90	28.14^{a}±0.57	37.33^{a}±1.89	4.13^{a}±0.09	0.96^{a}±0.01
Rainy	Q3	2.99^{a}±0.16	30.84^{b}±0.94	25.31^{a}±0.58	43.75^{a}±1.56	4.22^{a}±0.03	0.96^{a}±0.02
Rainy	Q4	4.26^{a}±1.10	30.60^{b}±1.26	24.77^{a}±0.58	47.67^{a}±2.75	4.32^{a}±0.03	0.96^{a}±0.01

Average of 4 dairies± standarddeviation

$^{a-b}$ Different superscript letters within same column indicate significant differences. $P<0.005$

Conclusions

These results indicate that the physicochemical composition of raw milk and cheese does not vary significantly between dry and rainy seasons. These results suggest that the manufacturing practices for this traditional product are adequate to ensure the quality and consistence of the end-product. Moreover, these results suggest that the milk and end-product fulfill regulations established by the national norm (NMX-F-700-COFOCALEC-2004).Poro Cheese wascharacterizedby containinga highfat and protein content, low moisture content, lowpH and a relatively lowAw, important factors in controlling microbial growth and the development of pathogenic microorganism.Additional studies are in progress to examine the microbial composition of this dairy product.

Acknowledgements

This work was supported by the research project “Mejoramiento de la productividad, competitividad y sustentabilidad de la cadena productive de leche en México” and funded by SAGARPA-COFUPRO-CONACYT

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Evaluation of Physicochemical Characteristics Related To Pork Quality In The Supermarkets of The Central Mexican Plateau.

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Introduction

Mexican Pork Industry has suffered a series of problems that goes from production systems, marketing schemes and final consumer acceptance, which is not to do anything, is condemned to lose its importance in national economy. It is for that reason that is required strategies that have like main objective replace pork as food of national origin, high nutritional quality, produced with hygiene and safety to achieve consumer acceptance (Rocha, 2006).

Pork market currently is suing a product demanded by consumers that satisfy a number of characteristics or factors combination, like nutritious, healthy, attractive, tasty and palatable (Eusse, 2007).

In Mexico the main pork carcasses producer states are Jalisco, Sonora, Puebla, Guanajuato, Yucatán and Veracruz, altogether generate around 70% of carcasses in the country which in average are 1, 128, 189 tons during 2005 – 2008 (SAGARPA, 2010).

Sonora produced 19.4% of the national production, followed by Jalisco with 18.80%, Guanajuato 9.00% Puebla 8.90%, Yucatán 7.70%, Veracruz 6.00%,Michoacán 3.60%, Tamaulipas 2. 90% and the rest of the country produced 23.70% (SAGARPA, 2009).

The average of pig (tons) produced statewide from 2005 to 2010 declined derivative from the increase of the grains (corn and soy) that are part of pigs' diet.

Mexicans consumes 22 million pigs annually, of which eight million are purchased abroad, mainly in the U.S. market, the shortfall in pork production is mainly due to economic factors and that the capacity exists but is required to increase efficiency (Diaz and Ortegón, 2006).

Quality is a complex subject, this means that the customer is demanding not only a high lean content in pig carcasses and especially in the most expensive parts like loins and hams, but also that the product meets a series of characteristics for a more satisfactory quality produced with the best performance (Rocha, 2006).

The concept of food quality, consists of sensory, nutritional, hygienic and technological factors. In view of qualitative requirements the strategy should be an integral production from production on the farm, through processing until the consumer (Rocha, 2006).

In Mexico the supermarkets are great pork buyers, which are characterized to sell fine cuts of meat suitable and under appropriate hygienic conditions. Although its market entailment corresponded at first to the necessity to offer a wide range of products to the consumers, at this moment one of the most dynamic sections within the shopping centers is the one of the meat. They have had a positive impact in the Mexican society because they generate a wider supply of products (variety, quality, brands and prices), but mainly a more constant supply throughout the year; also recognizes that the market systems modernization has allowed the proliferation of strip malls, letting to extend the points of sale to make the supply more constant. To sum up, this study proposes a supermarkets' classification based mainly on the socioeconomic status of the consumers. Nevertheless, the proposal of the previous classification does not determine the meat quality that is sold in each one of these commercial centers, is for that reason that was applied a methodology to determine the quality from an objective and quantitative perspective.

Methods and materials

In the current study were sampled 22 commercial centers which offer meat of pig (*longissimusdorsi*) in the central area of the Mexican Plateau (Valley of Toluca) during 3 Fall-Winter periods.

Classification of Toluca Valley Supermarkets

The classification of commercial centers that is considered for this study corresponds to a proposal of the researchers and it is sustained mainly in the socioeconomic status of the consumers; in general they have been divided in three kinds: low, medium and high, same that according to the location area were numbered from the 01 to the 22. This classification was made up of the following way, Low (were considered seven commercial centers, that is to say, 06, 08, 09, 18, 19, 21, 22), Medium (eleven shopping centers, in other words, 01, 02, 03, 04, 05, 07, 10, 11, 13, 14 and 15), and High (four supermarkets, they were, 12, 16, 17, 20). The main characteristics attributed to each classification were:

Low: It offers a wide but little deep assortment of products due to the small dimension of the establishments, gives greater weight to the sections of perishable food (butcher, fish market, poultry shop, fruits, cheeses and products with denomination of origin, among others) and essentially plays with the elements of price and service. They are stores of proximity adapted to the customer of the area. It does not have additional services to attract the clients, consequently, they cannot compete more with other more evolved. They are called also popular stores, since they realize an economic supply in price that limits products of basic purchase, their lines of products are of lower quality and price than those of a department store. It usually has hard discounts, that is to say, short assorted, many own brands, aggressive prices, very little service (Hamilton, 1989; Alonso, 1992, 2001a; Fiengenbaum and Thomas, 1990, 1995a).

Meidum: The fundamental difference with the low, aside of the size, is the existence of sections of perishable food taken care by specialized staff who realizes the own functions of the direct serve with the exception of the charge, that takes place always when coming out. They have a very aggressive policy of prices, and with a product assortment of food very similar to the low one, but that complements with sections of textile and bazaar. Are establishments catalogued like great surfaces of feeding that are dedicating more areas to the sections of nonfeeding. They work very tight margins related to food products, performing this policy of prices to hang up the consumers to go to the establishment and buy other products with higher margins in the sections of nonfeeding. It has an attractive atmosphere and it is complemented with other services offered to the customer like travel agencies, ATM, free parking. It usually has soft discounts, in other words, wide assortment, greater presence of other brands, and discount only on their brands (Hamilton, 1989; Alonso, 1992, 2001a; Fiengenbaum and Thomas, 1990, 1995a).

High: Are specialized stores, they offer a reduced but deep assortment in their small internal establishments, like greengrocers, fish market, butchers and bakery. The competitive weapon of this kind of establishments is the selection, variety and quality of a suitable assortment for the buyer. Its location is in the average attraction area, but having a proximity customer. The client pays the comfort, fast service, freedom of election and movements in the decisions of purchase and the comparison of prices and brands, although the price does not determine its decision of purchase. It usually does not have discounts (Hamilton, 1989; Alonso, 1992, 2001a; Fiengenbaum and Thomas, 1990, 1995a).

Instrumental analysis

The back pig chops (*longissimusdorsi*) were worked 24 hours after chilling and kept at 3 °C;a chop of 10ª rib was used for the measurements of marbling, subjective color (Gómez and Gómez, 1984 and Wright et al., 2005) using the scales NPPC (1999), and objective color (Colorimeter Minolta Chroma meter CR-400, measuring head CR-400, calibration plate CR-A43, view angle of 20°, made in Tokyo, Japan).Where L* indicates brightness, a* reddish tonalities and b* yellow colors. The chop from the 11th rib was homogenized with a food processor Moulinex, Ecually (France), and was used to obtain the total fat

amount considering a 2:1 chloroform-methanol mixture realized in Soxtec Foss 2055, adapted of official method AOAC #991 (Huff-Lonergan*ET to.*, 2002);the protein analysis was performed by Kjeldahl method(Honikel, 1998) and the 12th rib was used for shear force (Kgf) applying the method described by AMSA (Honikel, 1998 and Mariezcurrena, 2010).

Statistical analysis

The data were subjected to ANOVA whose linear unit corresponded completely to an experimental design at random. The study factor (treatments) was the 22 commercial centers and the three samples were considered like repetitions; also every 30 days were included three periods of sampling. The comparison of averages of treatments took control of the test of DMS concerning 5%. The variables answer were greasy (%), protein (%), humidity (%), cut force (kgf), objective color (L*, a*, b*), subjective and marbling color.These analyzes were completed by the linear correlation between pairs of variables and by the corresponding analyzes of components the main ones (the ACP) and of conglomerates.The algebraic procedures for the estimation of statistical in the three first statistical techniques were described by Gómez and the Gómez (1984) and for the multivaried analyzes are described in SAS (1988), in Sanchez (1995) and González *et al.*, (2010). The technique of simple linear correlation was described by Martinez (1988), whereas the one of the ACP it realized Sanchez (1995). With the logouts for the statistical analysis (SAS) the dendogram was obtained for commercial centers in Excel the graphic of the biplot between main components 1 and 2.

Results and discussion

Statistical analysis results are shown in Table 1, for variables better understanding, these were identified with literal as follows, X1 = subjective color, X2 = subjective marbling, X3 = L*, X4 = a*, X5 = b*, X6 = protein (%), X7 = fat (%), X8 = moisture (%), X9 = shear force (kgf).

Table 1. Means comparison results from pork loin (*longissimusdorsi*) obtained in 22 commercial centers.

Treatments (comercial centers)	Socio Economic Status	Variables								
		X1	X2	X3	X4	X5	X6	X7	X8	X9
06	Low	3.67^{a}	3.67^{ab}	46.32^{abcd}	5.91^{abcd}	4.31^{bcde}	22.41^{a}	2.86^{abcd}	70.85^{ab}	2.29^{cde}
08	Low	2.67^{abc}	3.67^{ab}	52.22^{a}	5.51^{bcd}	5.81^{abc}	21.73^{a}	2.69^{bcd}	72.60^{ab}	1.86^{e}
09	Low	3.33^{ab}	2.67^{bcd}	50.49^{abc}	7.57^{abc}	6.84^{a}	22.22^{a}	2.66^{bcd}	71.63^{ab}	2.21^{de}
18	Low	3.33^{abc}	3.00^{bcd}	44.92^{bcd}	4.22^{d}	3.72^{de}	22.49^{a}	3.07^{abc}	70.97^{ab}	3.21^{abcd}
19	Low	3.67^{a}	3.00^{bcd}	45.19^{bcd}	5.97^{abcd}	4.93^{abcd}	22.27^{a}	2.17^{cd}	73.94^{a}	3.41^{abc}
21	Low	3.00^{abc}	3.00^{bcd}	49.19^{abc}	5.84^{abcd}	4.31^{abcde}	22.96^{a}	2.12^{cd}	71.66^{ab}	3.54^{a}
22	Low	2.66^{abc}	2.66^{bcd}	48.02^{abcd}	6.18^{abcd}	5.20^{abcde}	21.56^{a}	3.60^{ab}	70.93^{ab}	3.42^{abc}
Mean		**3.19**	**3.09^{x}**	**48.05**	**5.88**	**5.03**	**22.23**	**2.73**	**71.79**	**2.85**
01	Medium	3.00^{abc}	2.67^{bcd}	44.26^{cd}	6.75^{abcd}	4.88^{abcde}	22.38^{a}	2.69^{abcd}	68.33^{ab}	3.49^{ab}
02	Medium	2.66^{abc}	2.00^{d}	46.38^{abcd}	6.42^{abcd}	5.45^{abcd}	22.20^{a}	3.15^{abc}	72.86^{ab}	3.23^{abcd}
03	Medium	2.67^{abc}	3.00^{bcd}	48.14^{abcd}	6.90^{abcd}	5.84^{abc}	21.84^{a}	2.58^{bcd}	72.05^{ab}	2.75^{abcde}
04	Medium	2.33^{bc}	2.33^{cd}	46.15^{abcd}	5.23^{bcd}	4.22^{cde}	21.63^{a}	2.21^{cd}	71.71^{ab}	2.57^{abcde}
05	Medium	3.33^{ab}	2.67^{bcd}	46.96^{abcd}	7.23^{abc}	6.41^{a}	20.56^{a}	2.07^{cd}	72.73^{ab}	2.93^{abcde}
07	Medium	2.67^{ab}	2.33^{cd}	48.46^{abcd}	8.37^{a}	6.60^{a}	22.63^{a}	2.63^{bcd}	71.04^{ab}	3.22^{abcd}
10	Medium	2.00^{c}	2.33^{cd}	50.90^{ab}	6.03^{abcd}	6.20^{abc}	21.51^{a}	1.98^{d}	69.01^{b}	2.30^{cde}
11	Medium	2.67^{abc}	2.33^{cd}	51.24^{ab}	6.70^{abcd}	6.27^{ab}	21.29^{a}	2.74^{bcd}	70.79^{ab}	2.84^{abcde}
13	Medium	3.00^{abc}	3.33^{abc}	49.95^{abc}	5.49^{bcd}	5.31^{abcde}	21.86^{a}	2.58^{bcd}	73.83^{a}	3.06^{abcd}
14	Medium	2.33^{bc}	2.00^{d}	50.55^{abc}	4.84^{cd}	5.43^{abcd}	22.37^{a}	2.34^{cd}	72.83^{ab}	3.43^{abc}
15	Medium	3.67^{a}	2.67^{bcd}	44.36^{cd}	6.13^{abcd}	4.38^{bcde}	21.28^{a}	2.20^{cd}	73.59^{a}	2.24^{de}
Mean		**2.75**	**2.51^{x}**	**47.94**	**6.37**	**5.54**	**21.77**	**2.49**	**72.01**	**2.91**
12	High	3.00^{abc}	3.67^{ab}	42.36^{d}	5.06^{bcd}	4.89^{abcde}	21.06^{a}	3.82^{a}	72.35^{ab}	2.37^{bcd}
16	High	2.67^{abc}	2.33^{cd}	50.27^{abc}	7.74^{ab}	5.96^{abc}	22.09^{a}	2.20^{cd}	73.43^{ab}	2.89^{abdce}
17	High	3.67^{a}	4.33^{a}	45.13^{bcd}	6.71^{abcd}	5.59^{abcd}	21.98^{a}	2.97^{abcd}	71.72^{ab}	2.73^{abcde}

20	High	3.67[a]	3.33[abc]	41.99[d]	6.13[abcd]	3.35[de]	22.29[a]	2.39[cd]	73.13[ab]	3.39[abc]
Mean		**3.25**	**3.41[y]**	**44.94**	**6.41**	**4.95**	**21.85**	**2.84**	**72.65**	**2.84**

X1=Subjective Color; X2=Subjective Marbling; X3=L*; X4=a*; X5=b*; X6=Protein (%); X7=Fat (%); X8=Moisture (%); X9=Shear force Kg_f

[a,b,c,d,e,] Means with different superscript within each column denote significant differences (Tukey, p≤0.05).

[x,y] Means with different superscript in a column for commercial center groups (Low, Medium, high) are statistically different (Tukey, p≤0.05)

As it is appraised in Table1, an analysis of variance (P ≤ 0,05) for all the variables (X1-X9) was realized according to the established classification in groups low, medium and high; and the results indicated that significant differences for any of the variables except for X2 (subjective marbling) did not exist, to which a mean comparison Tukey's test was applied that indicated that two statistically different groups were formed, in the first and with the lowest mean were the low and medium level commercial centers, that is to say that fat presence in loin eye is smaller in meats from high level commercial centers. In addition to the above, a means comparison between all the commercial centers was realized, in order to determine if different sub-groups with similar characteristics were formed, nevertheless, this was not thus.

For *subjective marbling (X2)*, data shown in Table 1, arithmetic means obtained from tested samples ranged from 2.0 to 4.33. Wright (2005), stated that quality pork chops are those that reaches marbling scores of 3 or higher in NPPC scale, higher veined index indicates a greater presence of intramuscular fat which influences directly meat sensory attributes such as juiciness, tenderness and flavor and determines consumer acceptability (Brewer, 1999). The color and marbling scores according to NPPC chart (1999), which were recorded in this part of the current research, were the basis for other analyzes as objective color determination, establishing L * (lightness), a * (red intensity) and b * (yellow color intensity) values.

In the case of *L * (X3)*, results shown in Table 1, arithmetic means were in the range of 42.36 (12) 52.22 (08), the range of data obtained in this study, coincided with that reported with Brewer (1999) and Alarcon (2006), who stated that if there are values above 57 are presented PSE meat which is meat with a lower water retention capacity, caused by partial proteins denaturation, due to its higher acidity, this meat releases more water, so that its surface refracts more light and it looks brighter, increasing the values of L *, these authors propose that values closer to 51 indicates good quality meats as they have a reddish pink color, are also of firm texture and exhibit little or no exudate, in contrast to values below 38 represent DFD meat which is characterized by having a strong reddish color and dry that impinging directly on a greater water retention capacity. The NPPC score chart (1999), reported a PSE meat when it had values between 55 to 61, being an optimum quality meat, which was in the range between 43 to 49 and finally a DFD meat is presented when recorded values between 31 and 37.

For values of *a * (X4)* shown in Table 1, range displayed was between 4.21 and 8.37. In July, was detected the highest arithmetic mean (8.37). Alarcón (2006), proposed that better color characteristics are presented with higher values of a * (in green color threshold by registering negative numbers or red if positive numbers are registered) and lower values of b * (color thresholds in negative in blue and positive in yellow).

In the case of *Protein (X6)* arithmetic means obtained from evaluated samples were statistically equal, varied from 20.55% (05) to 22.96% (21). Aguilar (2002); Eusse (2007); Hennet (2007) and Scheffler (2007); coincided in mention that the optimal protein content desired in pork meat is considered from values greater than 20.0%, since lower values could indicate a PSE meat, so that proteolytic enzymes that degrade myofibrillar proteins. The optimum range for this parameter is from 20.0 to 23.0% depending on factors such as breed and feeding type.

For the variable *Fat (X7)*, results presented in Table 1, arithmetic means obtained from tested samples ranged from 1.97 to 3.82%. 12 with the highest arithmetic mean was statistically equal to 01, 02, 06, 17,18 and 22 (means of 2.87, 3.15, 2.85, 2.96, 3.06, and 3.60%, respectively) but differed significantly from the rest of the commercial centers. Galián*et al*., (2007), Daszkiewicz *et al.*,(2004) and Laack*et al*., (2001) indicated that IM fat values between 2.0 and 3.0%, are ideal in relation to the sensory meat quality

because sensory evaluations conducted in several laboratories in the presence of specialized committees, showed that the optimum IM fat varies from 0.8 to 3.0%. Furthermore, Fernandez *et al*. (1999) showed that an increase in the values of IM fat increases sensory meat quality in some measure. However, values above 3.5% caused a consumer rejection.

For *moisture (X8)* data observed in Table 1, arithmetic means obtained from tested samples showed no significant differences for this characteristic since the percentages varied from 69.0% to 73.82%. 13 with the highest arithmetic mean.

Finally, for *shear force Kgf (X9),* results shown in Table 1, the range of variability of tested samples was 1.86 to 3.54Kgf. 19 with the highest arithmetic mean. Wright *et al.,* (2005) and Galián (2007) mentioned that shear strength values lower than or equal to 2.27 kgf pressure means tender meat. Values between 2.27 to 3.63 kgf represents fairly tender meat over 5.44 Kgf represents tough meat. Overall, we can say that shear force showed tenderness (or softness) of meat so that it becomes a decisive factor in assessing the acceptance, in other words, consumer decision to continue buying a product. Laack*et al*., (2001); Fortomaris*et al.,* (2005) and Wright *et al.,* (2005), stated that tenderness is a complex attribute, which involves various factors such as content and fiber density in the muscle, amount, type and connective tissue arrangement, intramuscular fat content, farm work conditions, animal stress, until product preparation before consumption. Recent studies showed that shear force values for meat quality is the one found to values between 3 and 5 kgf (Laack*et al*., 2001; Fortomaris*et al.,* 2005 and Wright *et al.,* 2005).

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Phenolic Compounds Extracted From Agro-Industrial Waste And Its Effect On The Biological System Of *Artemia salina*

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Introduction

In the plant kingdom can be found a variety of compounds with several biological properties, one of this is the antioxidant capacity that is present in some compounds of vegetal origin (1). The polyphenols are the principal antioxidant natural compounds that have been studied for its application in the food industry (2). The phenolic activity has been studied due to consumer demand of food with high nutritional value (3).

The antioxidant property of phenolic compounds refers to the cellular protect capacity against the peroxidation process caused by reactive oxygen species (ROS) (4). Several phytochemicals have gained recognition in the prevention and management of chronic-degenerative diseases, as the case of cancer (5). Cancer is a major health problem in the world with over six million of deaths reported per year. In Mexico is estimated that exist between 50 and 80 cases of cancer per 100,000 people (6). Although, the natural antioxidants constitute *a priori* an alternative healthier and security than synthetic antioxidants, the safety of this compounds not be taken for granted, so that is necessary studied the toxic effects of natural substances isolated and used as pure compounds (7).

Regarding this, in the present study was evaluated the biological activity of phenolic extracts from pomegranate (*Punica granatum*) and pecan nuts (*Carya illinoensis*) peels on *Artemia salina* larvae, as the preliminary test for subsequent cytotoxicity assays. Pomegranate and pecan nuts are fruits cultivated in Coahuila state and were used in the elaboration of typical products. However, the wastes materials as peels and shells are not used in others productive activities. According to, the polyphenols extraction from these natural sources represented an alternative for the use of shells considering as waste.

Methods and materials

Plant material. Were used red pomegranate (*Punica granatum*) of "Wonderful" variety and pecan nut (*Carya illinoensis*) of "Riverside" variety. All assays were performed in triplicate.

Sample preparation. Pomegranate and pecan nut shells were dehydrated at 60 ºC and subsequently pulverized in a commercial grinder (8). The powder obtained was screened for determinates the particle size and this was stored in hermetic plastic container.

Polyphenols extraction. The extraction of pecan nut polyphenols (PNP) was carried out in a ratio of 1:4 w/v (100g powder /400 mL water) in a reflux system at 60 ºC during 8 h. The process was monitored at 2, 4, 6 and 8 h (11). For the extraction of pomegranate polyphenols (PGP) was used a ratio of 1:5 w/v (100 g powder/500 mL water) at 60 ºC into heating stove with manual agitation during 30 min (12). Finished the extractions, the supernatants was filtered and centrifuged at 3,000 rpm during 15 min. The obtained extracts were preserved at -4 ºC.

Polyphenols quantification. The hydrolyzed tannins quantification was performed although the Folin-Ciocalteu method (13). 400 µL of extract sample was added with 400 µL of Folin-Ciocalteu reagent; this was shaken and reposed during 5 min. Subsequently were added 400 µL of 0.01 M Na_2CO_3 this was shaken and reposed during 5 min also added 2 mL of H_2O to read the absorbance at 725 nm in a UV spectrophotometer. The concentration extracts was calculated according to the standard curve (400 ppm) and reported as Gallic acid equivalents (EAG). The condensed tannins quantification was carried out using the HCl-Butanol method (12). 500 µL of extract sample was added with 3 mL of HCl-Butanol reagent

(1:9) and 100 µL of ferric reagent. The tubes were closed with screw caps and these were heating during 1 h at 100 ºC. The samples absorbance was read at 460 nm. The concentration extracts was calculated according the standard curve (500 ppm) and reported as catechin equivalents (EC).

Fractionation of extracts and tracking of polyphenols. The aqueous extracts of pomegranate and pecan nut were fractionated although a liquid-liquid separation by dispersion, used hexane (Hx) and ethyl acetate (AcOEt) in a stirring system at room temperature. The separation was performed in a separator funnel begin with Hx and recovering the organic phase in a rotary evaporator. This process was repeated with the AcOEt. For the tracking of polyphenols used a phenolic hydroxyl test (13). 50 µL of obtained fractions was placed in contact with 50 µL of $FeCl_3$; a blue to black coloration indicates the polyphenols presence in sample. Was used Gallic acid as positive control and ethanol as negative control.

Determination of biological activity. Eggs of *A. salina* were hatched in artificial sea water (37 g NaCl/ L) in optimum temperature (25 ºC) and aeration conditions. The microdilution method in ELISA plates was used; 1000, 500, 250, 100 and 50 ppm concentrations were probed on 10 nauplii of *A. salina* in a total volume of 200 µL. The $K_2Cr_2O_7$ reagent was used as positive control and 10 nauplii in sea water as viability control. The bioassay was performed at 25 ºC during 24 h (14).

Results and discussion

Polyphenols extraction. In the case of PNP extraction, the powder particle size and extraction time was determined preliminarily. The extraction was performed with the full powder and two defined particle size: 149 µm and 841 µm. According the hydrolyzed and condensed tannins quantification, the maximum extraction time was observed at 2 h, with the three types of powder used, however the greatest accumulation of polyphenols was presented with a powder of 149 µm particle size (Figure 1 and 2). With these results found that the particle size is an important factor affecting the amount polyphenols extracted. This occurs due to when the particle size is smaller, the diffusion distance between solute and solvent is reduced and increases the concentration gradient and so that the extraction rate increased too. Kossah *et al.* (15) obtained high concentrations of phenolic compounds using a small particle size this reduced of 5 h to 1 h regarding the extraction time. This occurs due to when the particle size is smaller the solute distance of solute is shortened into solvent (16).

In agreement with the results of preliminary PNP extraction, the final extraction from pecan nut shells carried out with a particle size of 425 µm (medium particle size) to not affect the yield of extraction and a time of 2 h. The PGP extraction was performed using the same particle size and the established time by Ascacio-Valdés (2010). Subsequently, the polyphenols quantification carried out and the results showed in table 1.

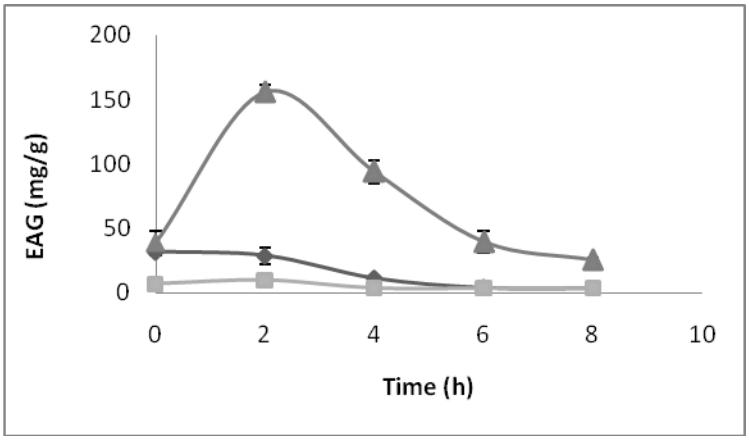

Figure 1. Time and particle size effect on hydrolyzed tannin extraction from pecan nut shells powder. Full powder (◆); 841µm (■); 149 µm (▲). EAG (Gallic acid equivalents).

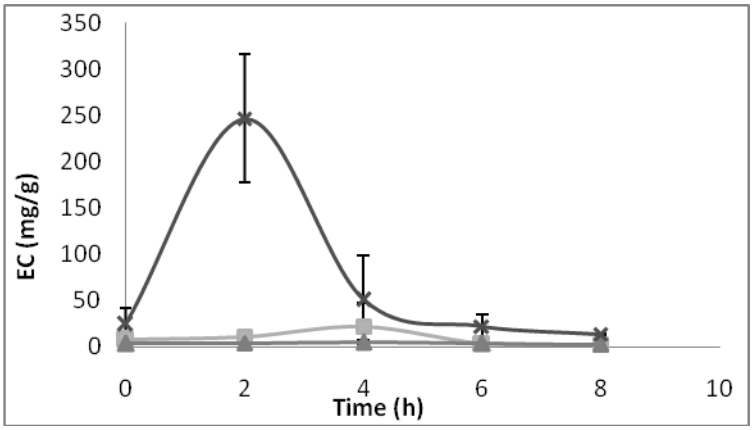

Figure 2. Time and particle size effect on condensed tannin extraction from pecan nut shells powder. Full powder (△); 841µm (■); 149 µm (X). EC (Chatequin equivalents).

Table 1. Total polyphenols quantification of pecan nut and pomegranate shells extracts.

	Hydrolysable tannins EAG (mg/g)	Condensed tannins EC (mg/g)	Total polyphenols (mg/g)
PNP	106.3 ± 8.164	248.5 ± 9.827	354.80
PGP	34.0 ± 1.923	17.3 ± 2.032	51.30

Was obtained 106.3 mg/g EAG (hydrolysable tannins) and 248.5 mg/g EC (condensed tannins) of PNP, that represent a high yields extraction considering that in others investigations the concentrations rages between 0.5-24 mg/g EAG in dry weight (17, 18) and of 13-47 mg/g EC depending of nut type used (19, 20). According these results, the polyphenols extraction conditions used in the present study increased the interest compounds yields.

In the case of PGP quantified, was obtained 34 mg/g EAG that is an accepted concentration compared with the Pedriali *et al*. in 2010 (21), however, the concentrations reported by Wang *et al.* in 2011 with 179 mg/g (18) are higher than concentrations obtained in the present study, but using different extraction conditions (40 ºC/4 h), which can be explain the polyphenol concentration difference. However, was obtained 17.3 mg/g EC, which is expected result due to the hydrophilic nature of compounds presents in pomegranate shells (22).

Fractionation of extracts and tracking of polyphenols. The phenolic hydroxyl test was performed with the whole extracts and its fractions in solution at 2,000 ppm. Was observed a positive reaction in the whole extracts as well as the aqueous fractions of both plant presented a black-blue coloration. This response is due to the chloride ion attack on hydrogen of hydroxyl group of phenolic compounds presents in the extracts, this cause a bond rupture and the union of phenoxide group to iron, which origin a colored complex characteristic (23). The AcOEt fractions presented a positive reaction too, however, this showed a lower intensity, that can be explained by the phenolic compounds presence in a low concentration. Also, the Hx fraction of pomegranate was the only fraction that presented a negative reaction.

Determination of biological activity. In this test was determined the lethal concentration (LC_{50}) of extracts and its fractions on *A. salina* larvae, shows in table 2.

Table 2. Lethal concentration of pomegranate and pecan nut polyphenolic fractions (WE: whole extract).

Pomegranate extract			Pecan nut extract		
Fraction	**LC_{50}**	**Lethality**	**Fraction**	**LC_{50}**	**Lethality**
WE	211.7 ± 2.4	moderate	WE	> 250.0	moderate
Hx	No effect	----------			
AcOEt	464.2± 14.3	low	AcOEt	No effect	----------
Aqueous	85.0 ± 2.4	high	Aqueous	< 200.0	High-moderate
$K_2Cr_2O_7$ (+): 13.9 ± 0.5					

The aqueous fraction of pomegranate extract was presented the greater toxicity with a LC_{50}= 85 ppm compared with the whole extract (LC_{50}= 211.7 ppm) and AcOEt fraction (LC_{50}= 464.2 ppm), considering this a high lethality according with the levels reported by Sanabria *et al.* (24). Krishnaraju *et al*. (25) reported a LC_{50}= 45 ppm from hydroalcoholic extracts of pomegranate shells. This information demonstrated that water is a best extraction solvent of phenolic compounds from pomegranate by its hydrophilic nature, due to increases the biological activity of extract. In the case of pecan nut extract, the aqueous fraction presented a higher level of lethality with a LC_{50}< 200 ppm compared with the whole extract (LC_{50}< 250 ppm) and AcOEt fraction that not present toxicity. In others studies where measured the lethality of different nut variety (*Araucaria bidwillii*) on *A. salina*, not found anything toxic effect (26). Agreement with the previous information, the variety of fruits or plant as well as the type of extraction and solvent used influenced significantly on final concentration of polyphenols, which is directly related with its biological activities.

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Influence Of Acetic Acid Bacteria On The Acidity Of The Cocoa Beans During Fermentation

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Introduction

Cocoa (*Theobroma cacao*) is a crop that has a long tradition in the history of Mexico (1). Currently, from cocoa was obtained four intermediate products (cocoa liquor, cocoa butter, cocoa mass and cocoa powder and chocolate. However, for these products can be exploited, the cocoa beans should be treated by a cured process. The stages of this process are: harvest, fermentation, drying and storage. Fermentation is the principal stage, because the changes that originate during this stage are fundamentals for the appearance of the typical precursors of aroma and flavor of cocoa (2, 3, 4, 5, 6). The succession of microorganisms involved in fermentation begins with the yeast followed by lactic acid bacteria (LAB), acetic acid bacteria (AAB) and finally bacilli (7). However, in some fermentations is observed that yeasts, LAB and AAB exhibit the same behavior since the start of the fermentation (8, 9, 10). The concentration of each of these microorganisms varies during the days it takes for the fermentation and between each fermentation batch. The selection of each one of it, is mainly due to the resistance and adaptation developed during this stage (11). During the fermentation step, increasing the aeration of the fermenting mass and the temperature reaches 37°C, which favors the growth of AAB and start to be the dominant microorganisms (12). These are responsible for the oxidation of ethanol to acetic acid, which is later oxidized to carbon dioxide and water (13, 14). The accumulation of acetic acid in the dicotyledon is slow in the early hours but increases after the third day (8, 10, 11) maintaining a ratio to the end of fermentation. Furthermore, the diffusion of acetic acid into the grain and consumption of citric acid which surrounds the grain causing pH changes (11, 15) which vary according to the variety, location and type of fermentation (16). In Mexico, not enough is known of AAB species involved in fermentation and the characteristics that contribute to the fermentation of cocoa. The aim of this study was to isolate and identify molecularly the AAB present during the traditional fermentation of cocoa and quantify the production of acid in the grain and the produced by each of the AAB *in vitro*.

Materials and methods

Raw material

The cocoa bean used in this study was provided by the "Asociación Local Agrícola de Productores de cacao" of the municipality of Huimanguillo, Tabasco. The cocoa beans contained in the industrial fermenting boxes (1 ton approx.) were mixed every 24 h by the beneficiaries. The sample recollection was performed at three different sites of each box (surface, middle and bottom), every 12 h, the grains were placed in sterile bags.

Microbiological analyzes

Sample preparation

In sterile bag was placed one cocoa grain by each 10 mL of NaCl (0.85%) and the bag was massaged until the pulp was completely removed of the grain. Subsequently, dilutions were made (10^{-4} a 10^{-6}) with the same physiological solution (NaCl, 0.85%) for each of the samples. The dilutions were dependent on the microorganism and the number of CFU reported (10, 17).

Growth conditions for AAB isolated during the traditional fermentation of cocoa

250 µL of the samples diluted were taken every 12 h (as mentioned in the previous section). For the isolation and quantification of the AAB, were used the Acetobacter/Gluconobacter (A/G) and modified CARR media. To observe the growth and isolating the microorganisms, was used the scanning method with handle glass. Subsequently, the Petri dishes were incubated at 37°C for 24 h. The test was performed by duplicate.

pH determination

10 g of mucilage, shell with remains of mucilage, dicotyledon and whole grains were taken. Dicotyledon and whole grains were frozen with liquid nitrogen and pulverized in a coffee grinder (Krups model F203). The samples were diluted in 90 mL of distilled water, placed in thread flasks were carried and boiling during 15 min, subsequently, the samples were cooled to room temperature and the measurement was performed using a potentiometer (OAKTON pH500 S).

Acetic acid extraction

Approximately 20 g sample of cocoa previously pulverized with liquid nitrogen and 100 mL of distilled water were placed in a 125 mL Erlenmeyer flask. The extraction was performed in an ultrasonic bath (Westprime Systems model LR84943), during 30 min. The methanol extract obtained was filtered with Whatman paper No. 4. Acetic acid was quantified by gas chromatography (10).

DNA extraction, PCR and sequencing and molecular characterization of AAB

DNA Extraction of microorganisms isolated were performed as described previously Romero *et al*. in 2012 (18). A region of nuclear DNA, containing the ITS1-ITS2 regions and the 16S gene, was amplified by PCR using 6 different pairs of primers and amplification conditions (18). The resulting products were purified with the Geneclean® II kit (Bio101 Inc., California, USA) according to the manufacturer's protocol. DNA samples were sequenced by the Instituto de Biotecnología, Cuernavaca, Mor., Mexico.

DNA sequences from each AAB strain were assembled by use of Chromas 1.45 (School of Health science, Griffi th University, Gold Coast Campus, Southport, Queensland, Australia), and then aligned using the ClustalX inference package (19). The sequences were subjected to the basic local alignment search tool (BLAST) available at http://blast.ncbi.nlm.nih.gov/Blast.cgi against sequences available in GenBank. Phylogenetic analysis of the aligned sequences was done by Neighbour-Joining analysis with 100 bootstrap replicates and was supported by bootstrap method. Unrooted phylogenetic tree was constructed with help from the PhyloDraw program 0.8 (20).

Quantification of acetic acid produced by the AAB

For quantification of the acetic acid, were used 50 mL of sample, was titled with NaOH 0.1 Normal using phenolphthalein as indicator (21).

Results and Discussion

Evolution of the AAB during fermentation of cocoa

During development of the traditional cocoa fermentation was observed the same behavior in the three boxes studied, although each one started the fermentation at different time. The AAB showed their maximum growth from the second day of fermentation, but decreased around the third day, this growth could be related to the ventilation (17).

Evolution of acetic acid production and pH during the cocoa fermentation

The results clearly shows that the acetic acid increased after 48h; however, the maximum concentration obtained from the cocoa bean was lower than that reported by other authors (10, 17), who reported a decrease immediately after reaching the maximum concentration of this acid. The high concentration of acetic acid, at the end of fermentation, can be related to the species or strain of the AAB found at this stage. According to some authors (21), not all strains of the same species have the ability to oxidize acetic acid to CO_2 and H_2O.

Figure 1, shows clearly the pH decrease in the different samples after increased acetic acid. The pH of the mucilage beginning about 4, while the mucilage with shell started with a pH value of 3.5. The pH value of mucilage correspond to the same value reported by García *et al*., in 2005 (22) of a cocoa fermentation from Jalpa de Mendez, in Tabasco, México.

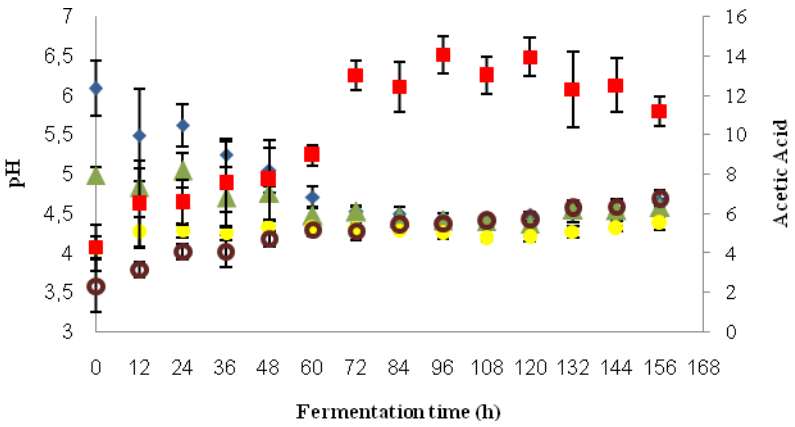

Figure 1. Evolution of acetic acid production and pH during the cocoa fermentation. ■ Acetic acid. ♦ Dicotyledon. ▲ Grain. ● Mucilage and ○ Mucilage with head.

However, these data vary with those reported by Ardhana and Fleet (11), this difference may be due because the cocoa beans used in this study contained less citric acid, which is reported to be consumed by microorganisms at the beginning of fermentation (7). With the progress of fermentation was observed a slight increase in pH in the mucilage. This change in pH is attributed to yeast, as are the main degraders of citric acid (23). As shown, although the shell is very thin, retains a degree of acidity, since the pH of the mucilage with shell was slightly more acidic than the mucilage at the beginning of the fermentation, but starting from 60 hours all samples followed the same behavior. In the case of the dicotyledon and the whole grain the pH decreased from 6.5 and 5, respectively to 4.5. The decrease in pH in the dicotyledon is mainly due to the diffusion of metabolites produced by microorganisms, which are absorbed by the dicotyledon among those that stand out acetic acid and lactic acid produced by the AAB and LAB (24).

Molecular characterization of isolates AAB

Specific amplification of the intergenic sequence spanning the rDNA of the 16S gene was achieved by using the primers ITS1 and ITS2. Two fragments were amplified of 750 and 678 bp for ITV75 and ITV114 strains, respectively. The sequences were deposited in GenBank bases with the accession number JF930132for strain ITV75 and JF930134 for strain ITV114. There was no amplification for strains ITV55, ITV61, ITV82 and ITV98. The lack of amplification of the ITS sequences from these strains is likely due to the lack of recognition of the primer ITS2 (25). The BLAST search for ITS sequences of strains ITV75and ITV114, showed homology (98%) with the sequence of strain LMG 1663 of *Acetobacter tropicalis* (FR716480) reported in the NCBI database. The homology of the strains in comparison to other sequences was less than 70%. For identification of the strains ITV55, ITV61, ITV82, ITV98 and to confirm the identification of strains ITV75 and ITV114, the DNA sequences of the 16S gene were amplified by PCR using primers AC1 and AC3. Obtaining the following fragments: 414 bp (ITV55), 836 bp (ITV61), 819 bp (ITV82), 834 bp (ITV98), 407 bp (ITV75), and 412 bp (ITV114). The sequences were deposited in GenBank bases with the following accession numbers: ITV55 (JF930135), ITV61 (JF930136), ITV75 (JF930133), ITV82 (JF930137), ITV98 (JF930138) and ITV114 (JF930145).

BLAST analysis of the region 16S-23S of the sequences of the strains ITV75 and ITV114 revealed the highest homology (98 to 100%) with strains of *A. tropicalis*, which is shown in the phylogenetic tree generated in this study (Figure 2).

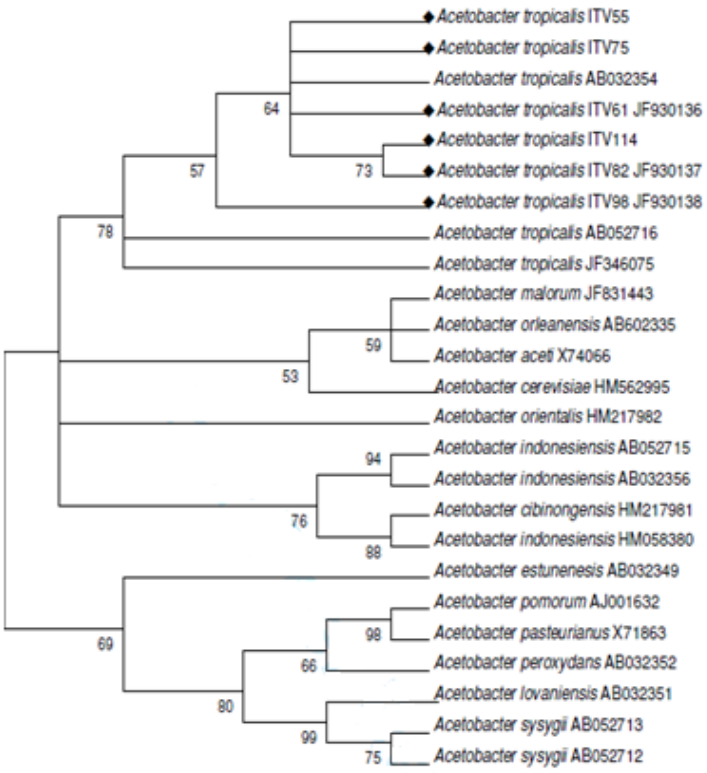

Figure 2. Phylogenetic tree based on 16S rDNA sequence showing the relationship of strains isolated from cocoa fermentation with *Acetobacter tropicalis.*

Phylogenetic analysis revealed that *Acetobacter* strains isolated in this study are aligned close to the strains of *A. tropicalis* from GenBank (AB052716, JF346075, AM748710, and AB03254). *A. tropicalis* is often found in the fermentation of cocoa beans (26). The strains identified in this study differ with the strains identified in the fermentation of cocoa (*Acetobacter pasteurianus*, *A. ghanensis*, *A. senegalensis* and *A. lovaniensis*) made in Indonesia (9). This difference could be related to the diversity of microorganisms and environmental conditions.

Acetic acid production during the traditional fermentation and produced by the strain ITV61

Comparing the acetic acid produced during cocoa fermentation and the produced by the strain ITV61, the increase of acetic acid during the traditional fermentation was after 60 h, however, the acetic acid production by *A. tropicalis* (ITV61) increased after 48 h (0.2% to 2.41 ± 0.11%) under the conditions used (18). However, this strain was able to oxidize it to CO_2 and H_2O (21). Although *A. tropicalis* has been reported as the most predominant microorganism fermentation in Ghana (27), there is insufficient information about the production of acetic acid of this species in the cocoa bean.

In conclusion the results obtained, allow us to propose that in the future, one could select strains that are most important during the process of fermentation and inoculated to avoid compromising the quality of fermented cocoa beans.

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Use Of Pulque And Xaxtle (Sediment Of Lactic Acid Bacteria And Yeasts Of The Vats Of Pulque) In Traditional Mexican Bakery

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Introduction

The use of lactic bacteria and yeasts in bread was established in Italy [1], Germany [2], Spain [3], and France[4]. Bread fermented with lactic bacteria and yeasts, examples include the famous Italian products associated with Christmas, Panettone, which had its origin in Milan [5], Pandoro originally from Verona [6] or his counterpart Colomba, that traditionally have been baked in the Easter [5]. French bread fermented in San Francisco, USA [7], and the crackers [8] are two additional examples of wheat products that rely on the process of sour.

In Mexico the bread was introduced by the Spanish, this traditional process was learned by the natives, but they developed it handmade. They baked a product fermented with bacteria and yeasts called "pulque bread", using pulque as a substitute for the water and the xaxtle like a biological agent that spans the volume of bread*;* xaxtle is a residual product of the fermentation of pulque, it is sediment at the bottom of the vats [9]. The xaxtle is a word derived from "xachtle" of voice Nahuatl it means "residue that remains after threshing, seed" also aztequismo "xmachtle" which means "residual product of the fermentation of pulque" [10]. In the xaxtle there are lot microorganisms, such as yeasts and bacteria, so it can be much more viscous than pulque fluid [11]. Pulque is a fermented drink which have been identified and isolated various microorganisms responsible for the alcoholic fermentation (genus *Saccharomyces* and *Zymomonas spp*.), viscose (genus *Leuconostoc*), and acidity of the pulque (genus *Lactobacillus*) [12, 13]. In this research the main objective is to use the xaxtle and pulque in order to bake bread of pulque and get it back to use as in the past.

Materials and methods

2.1 Raw

Collection of the xaxtle took place in the community of Huiloapan, which is located in the East of the State of Tlaxcala, Mexico;the xaxtle was freeze-dried (Freeze-dryer SMH-50 computer at 230 volts) in order to obtain a homogeneous batch.

Pulque was brought in a ranch located in Nanacamilpa, Tlaxcala, and it was transferred to Mexico City, under temperature of cooling (4 - 7) °C.

2.2 Design experimental

Design of experiments with two independent variables and five levels of variation of amount of xaxtle (Xg_Lper 100 g of flour) and fermentation time (min) were used to prepare bread of pulque and xaxtle. Using the Design Expert Plot version 7, the program was based on thirteen experiments, with five repetitions of the central point, four factorials points and four axial points [14].

Table 1.Independents variables and its variation levels in the process of bread of pulque and xaxtle.

Independient Variables	Levels of variation				
	-1.41421	-1	0	1	1.41421
xaxtle (g/100 of flour)	1.55	3	6.5	10	11.44
Fermentation time (min)	17.57	30	60	90	102.42

2.3. Studies *in vitro*

The content of total starch (TS) was determined by the method of [15]. The content of total resistant starch (TRS) was determined by the method of [16]. Bread products were tested *in vitro* to determine the rate of hydrolysis of starch [17].

2.4 Scanning electron microscopy (SEM)

A stainless steel blade was used to cut cubes (3 to 4 mm per side) from the center of the samples using a method by [18]. Dried samples were covered with gold using an ionizer (Desk II Denton Vacuum, Moorestown, NJ, USA) for 500 s at 100 mTorr and 20 mA. Samples were examined at 1000X and 2000X in a JSM 5800LV SEM (Jeol Inc., Peabody, MA, USA) at 15 kV and 0 Pa, equipped with an integrated program for digital image capture.

Results and discussion

The optimal area for making bread with pulque and xaxtle, corresponded to the overlap of the graphics from outline of specific volume of bread ($cm^3 \cdot g^{-1}$), texture (kgf) and Luminosty (RRU) of its crumb. This was achieved with a volume specific bread of pulque and xaxtle, from 1.23 to 1.51 $cm^3 \cdot g^{-1}$; texture from 1.73 and 1.85 kgf, and a luminosity of the crumb of 0.6 RRU with fermentation time from 84 to 99 minutes, and xaxtle content from 2.37 to 3.40 Xg_L per 100 g of flour content.

Bread with pulque and xaxtle got smaller 51.84 pGI, and conventional bread 56.15 value, resulting in a reduction of 4.31 units, the decline of the pGI was due to the use of the xaxtle, which contains bacteria lactic acid and yeast [11], necessaries to increase the organic acids, as lactic and acetic acid, produced by bacteria which transform the available sugars in the mass, this has been shown to lower the Glycemic Index [19].

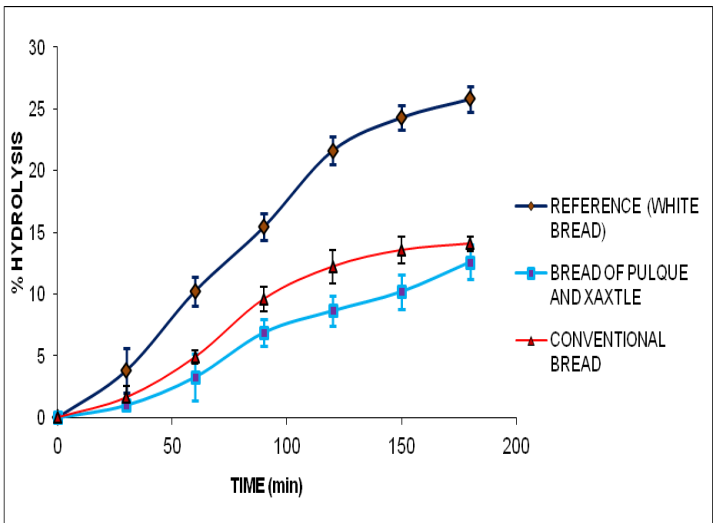

Fig 1.Hydrolysis percentage determined after chewing, incubation with pepsin and incubation with α-amylase in bread baked with pulque and xaxtle, conventional bread and used as a reference white bread.

Microstructures of starch granules of different sizes were observed (SG), as well as a thin gluten film (GF) which was due, among other factors, to the kneading and fermentation of the dough (Figure not show). The bread structure, that was developed during the fermentation, was affected by the pulque and xaxtle, and it had a more compact crumb due to the interaction between lactic acid bacteria with yeasts, which yield a small amount of CO_2 [20].

Conclusions

Response variables of texture and luminosity in pulque bread were influenced by the content of xaxtle, and the specific volume was influenced as fermentation time as content xaxtle, being their optimum conditions 90 minute of fermentation and 3 g xaxtle/100 g of wheat flour. The use of the xaxtle and pulque in a product of baking does not significantly alter its chemical composition; favors the reduction of the pIG because of decreasing DS content, so pulque bread is considered a product with low glycaemic index. The use of the xaxtle as a leudante agent increases the nutritional and sensory properties of bread producing a quality higher than the conventional product.

Acknowledgements

The study was financially supported by CONACyT-Mexico, SIP-IPN and COFAA IPN.

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Casein Proteolysis During Storage Of Oaxaca Cheese Manufactured By Direct Acidification

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Abstract

During storage of Oaxaca cheese, proteases of several sources hydrolyze caseins into peptides and even amino acids that could give a bitter taste to cheese. One option to reduce proteolysis is to manufacture cheese by direct acidification.

Oaxaca cheese was manufactured by direct acidification using organic acids (lactic, citric and hydrochloric). A control cheese was made using lactic acid bacteria (*Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. c*remoris*). Chemical composition (moisture, fat, pH and protein) was measured. During storage (4°C, 22 days) proteolysis as NS pH 4.6 and NS TCA 12 %, and casein (α_S and β) degradation by SDS-PAGE were determined.

Chemical composition (average) of direct acidification cheeses was 54.2 %, 22.9 % and 20.1 % in moisture, protein and fat content, respectively. On cheese control these parameters were 52.1 %, 22.3 % and 21.5 %. pH was lower on control cheese (5.2) than in direct acidification cheeses (5.5 average) and few changes were observed during storage.

At the end of the storage (22 days), proteolysis was lower on direct acidification cheeses than on control cheese. General proteolysis measured as NS pH 4.6 was 9 % (average) on direct acidification cheeses, while on control cheese was 12 %; NS TCA 12 % was 4 % and 6 %, respectively. The higher caseins degradation on control cheese, mean that additional proteolysis was carried out by lactocepin and peptidases of acid lactic bacteria, besides plasmin and chymosin proteolysis. SDS-PAGE showed a progressive hydrolysis of α_S and β caseins in all cheeses during storage.

Introduction

During storage, a great series of physicochemical changes carry out on cheese components. The most important of them is proteolysis (24). After coagulation, the primary stage of proteolysis corresponds to the casein hydrolysis by remaining chymosin, native milk protease (plasmin) and proteases of the lactic acid bacteria (LAB) that generate high molecular weight peptides. Secondary proteolysis converts these peptides in low molecular weight peptides and free amino acids. Further transformations drive to the production of other savory substances as amines, thiols and thioesters (11, 24).

In manufacturing of unripened cheeses it has been observed that not addition of lactic acid bacteria (and their enzymes) decreases proteolytic activity and initial characteristics of the cheeses remain during more time (9, 10, 5). In Mozzarella cheese manufacturing, Feeney *et al*. (2002) reported that the degradation of casein as1 and ß was lower in cheese manufactured by direct acidification than in control cheese, where lactic bacteria were added (7). Sheehan *et al*. (2004) evaluated the effect of preacidification of milk and preacidification-lactic culture mixture on proteolysis of Mozzarella cheese during storage. They found a higher proteolysis of casein α_{s1} than β in cheeses with added lactic bacteria to preacidificated milk. Proteolysis was greater on control cheese (acidification with lactic acid bacteria only) (21). Colín-Cruz *et al*. (2011) studied the effect of fat content (15, 21 and 25%) on proteolysis during storage of Oaxaca cheese. Results showed that after two weeks of refrigeration storage over 50% of caseins had been hydrolyzed; proteolysis increased as fat content in cheese rose (3). Garcés (2010) manufactured Oaxaca cheese at 34 and 37 °C and determined proteolysis during storage. Casein degradation was greater in cheese manufactured at lower temperature though the growth of lactic bacteria was similar to that obtained at 37 °C (8).

Acidification of milk on manufacturing Oaxaca cheese is achieved by lactic acid bacteria. Survival bacteria in the cheese hydrolyze caseins and it might contribute in the shortening of the shelf life. So, the

aim of this study was to manufacture Oaxaca cheese by direct acidification and to evaluate proteolysis during storage (22 days, 4 °C).

Materials y Method

1. Cheese manufacture

Oaxaca cheeses were obtained in 15-kg batches using standardized milk (3.2 fat g 100 g^{-1}). Milk was pasteurized (72°C, 15 s), and cooled at 5 °C. Acidification of milk was performed with 88.5% lactic acid (JT Baker, USA), 50% citric acid (JT Baker, USA) or 37.5% hydrochloric acid (Fermont, México) until pH reached 5.4. Acidified milk was heated at 34 °C. Diluted pure chymosin (0.22 mL kg^{-1} milk, dissolved 1:20 in distilled water, Hansen) was added to the acidified milk. The formed gel was cut with stainless-steel wire knives into 1-cm cubes and it was gently stirred for 30 min at 34 °C. The whey was drained and the curd was kneaded twice by hand in a volume (20 % of the initial milk volume) of warm (80 °C and 75 °C) water. The hot curd was stretched and allowed to cool, then strands were dry salted (2.0 % salt, w/w) and rolled into ~500-g balls. The cheese balls were packed in polyethylene bags and placed in cold storage at 4 ºC. Control cheese was manufactured by a standardized process (4) inoculated with a 1 % (v/v) mixture of *Lc. lactis* ssp. *lactis* and *Lc. lactis* ssp. *cremoris* (Hansen). Each experiment was performed in triplicate.

2. Chemical Analysis

A representative sample of milk from each batch was used to determine the following: protein by the Kjeldahl method (15), fat using the Gerber method (14), and pH with a potentiometer (13).

A representative sample of cheese was ground and moisture content was determined in an atmospheric oven (Richardson, 1985); protein content by the Kjeldahl method (15) and fat content using the Gerber method (Richardson, 1985). A pH meter was used to measure the pH directly from a ground sample.

3. Microbiological Analysis

Total, coliform and psychrotropic bacterial count, were assessed by the plate count method (17, 18).

4. Proteolysis

At days 1, 8, 15 and 22, the level of cheese nitrogen soluble in acetate buffer at pH 4.6 and in 12 % TCA were measured (Bynum and Barbano, 1985). SDS-PAGE electrophoresis was used to monitor the proteolysis of α_S and β caseins (Verdi *et al*., 1987).

5. Statistical Analysis

A block design with two factors: type of acidification and storage time was used. Each experiment was performed in triplicate. Analysis of variance (ANOVA) was conducted on data results.

Results and Discussion

Chemical composition of cheeses

The chemical composition of direct acidification and control Oaxaca cheeses is given in Figure 1.

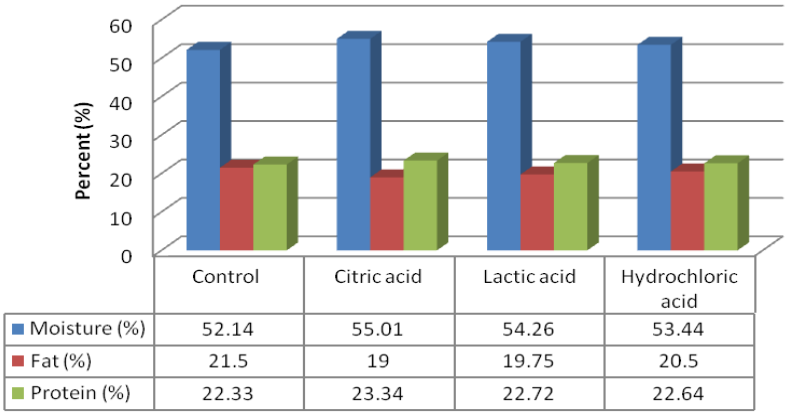

Figure1. Chemical composition of Oaxaca cheese manufactured by direct acidification.

The differences in chemical composition between direct acidification cheeses and control cheese were mainly in moisture that could be attributed to processing time. Stirring time was 30 minutes in direct acidification processing which wasn't enough for releasing whey from curd resulting in high moisture in cheese. Furthermore, since pH didn't change during process, new interactions sites were no formed, that could limit syneresis (6). In control cheese curd acidification was slow until to reach pH ~5.2 and stirring time was 120 minutes, so syneresis was adequate (22). Differences in moisture were 2 % average (Figure 1). Fat content was lower in cheeses manufactured by direct acidification, particularly because curd obtained was fragile and released fat during stirring. With regard to control process, losses in fat were five times major in whey in acidified cheeses. In addition, because of the weakness of the curd losses of fat during kneading was almost twice than in control cheese.

Proteolysis

The evolution of proteolysis during storage of the cheese is shown in Figure 2. pH 4.6 NS was higer in control cheese since day 1 suggesting that there was hydrolysis of casein (about 2%) during the process of manufacture and storage prior to analysis (~ 18 h). This proteolysis was certainly originated by reminant chymosin and mainly by the cell wall preteinase (lactocepin) of lactic acid bacteria inoculated to milk (24). Increasing of proteolysis was higher in control cheese. However, biggest differences were observed on day 1, as stated before, which could be ascribed to the high cell number of lactic bacteria that decreased during kneading. Garcés (2010) reported that during kneading lactic bacteria cell count decreased from 10^9 to 10^7cfu (8). Besides lactic acid bacteria in control cheese, reminant chymosin and plasmine were the agents responsible of the casein hydrolysis in all cheeses. At the end of the storage (day 22) 12 % and 9 % (average) of caseins were hydrolized in control and acidified cheeses, respectively.

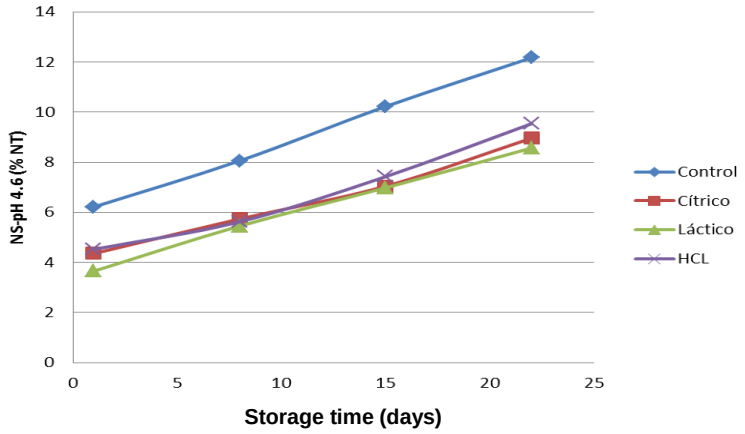

Figure 2. pH 4.6 NS evolution of direct acidification Oaxaca cheese during storage.

As high molecular weight peptides are released (measured as pH 4.6 NS) secondary phase of proteolysis carry out. Essentially microbial proteases hydrolyze the former to generate low weight molecular peptides and amino acids that were measured as TCA 12 % NS (Figure 3). In control cheese, an increase more accentuated of TCA NS was observed to day 10. I may be due to progressive lysis of lactic bacteria and releasing of endocellular peptidases involved in the hydrolysis of high molecular weight peptides (22). Garcés (2010) found that at 2 weeks of storage population of lactic bacteria had decreased to $4.9x10^6$ cfu (from10^7 at day 1) (8). At day 22 TCA NS had reached up to 5.9%. In direct acidification cheeses a similar behavior was observed but less accentuated. In these cheeses increasing of TCA NS cannot be attributed to lactic acid bacteria since they are absent. It could be associated to the presence of "wild" lactic acid bacteria and psychrotrophic bacteria that polluted the milk or cheese during processing. In direct acidification Oaxaca cheese, Portilla (2011) reported that polluant bacteria population increased from 10^2 at day 1 to 10^8 ufc/g at day 22 (17).

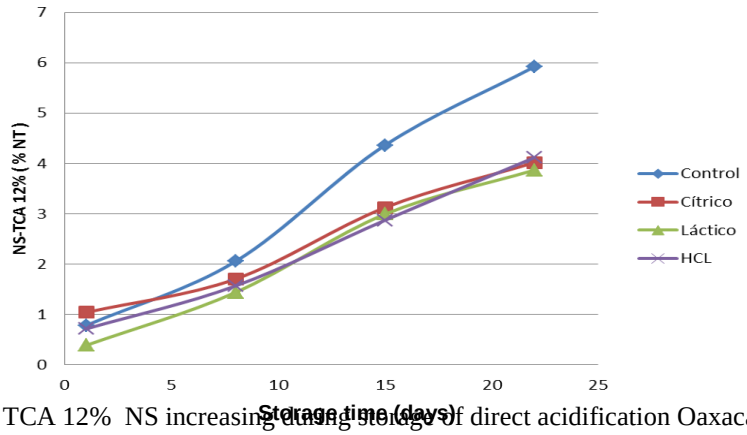

Figure 3. TCA 12% NS increasing during storage of direct acidification Oaxaca cheese.

Statistical analysis indicates that there were significant differences between the use of acids and the use of lactic acid bacteria on casein proteolysis measured as pH 4.6 NS and TCA 12% NS.

SDS-PAGE electrophoretograms of the patterns (α_S and β casein), control cheese and direct acidification (acid citric) Oaxaca cheese are shown in figure 4. In lane 1 the first band corresponds to caseins α_S (α_{S1} y α_{S2}) with molecular weights of 23.545 Da and 25.226 Da, respectively. The band below the caseins α_S corresponds to β casein having a molecular weight of 23.983 Da. Casein κ appears below with a PM 19.006 Da. Lanes 2 and 3 shows the patterns (α_S and β casein). In all lanes thin bands observed at the top are surely some minor whey proteins. In the electrophoretogram of control cheese, a decrease of thikness of α_S casein band is observed as storage time advanced, and at the same time it seemed that β casein band became thicker. During storage α_{S1} casein is hydrolyzed by remaining chymosyn and a big peptide is released (24). It is possible that this big peptide accumulate just below α_S casein band during electrophoresis separation and provoked β casein thickening. At the end of the storage (day 22) the α_S casein band became thinner and several bands (at the level of κ-casein band) were observed. These results agree with the obtained ones in the quantification of pH 4.6 NS. Electrophoretograms of direct acidification Oaxaca cheeses showed similar. In these ones, a comparable behavior was observed: α_S casein band became thinner and β casein seemed more ticker but thin bands were more separated (Figure 4).

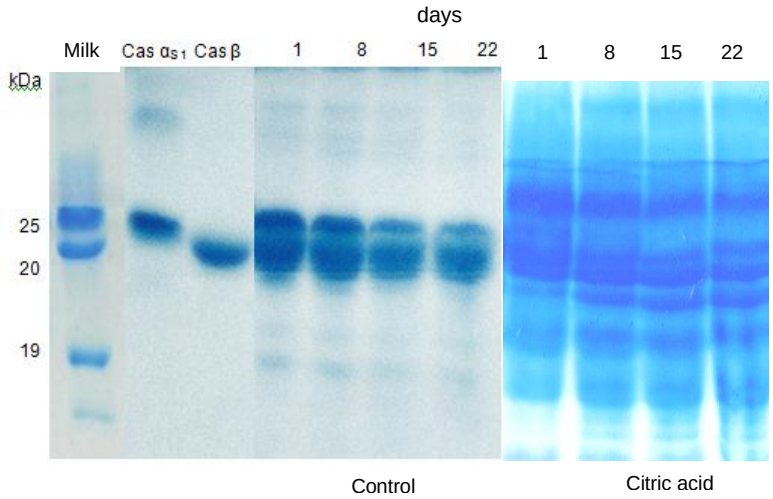

Figure 4. SDS-PAGE electrophoretograms of control and direct acidification (acid citric) Oaxaca cheeses, during storage. Lane 1 = milk; lane 2 = α_S casein pattern; lane 3 = β casein pattern. 1, 8, 15, 22 = days of storage.

Conclusions

The use of organic acids in cheese making give to Oaxaca cheese different physicochemical characteristics to those of a traditional cheese. Direct acidification cheeses are higher in moisture and lesser in fat content. Inoculating lactic acid bacteria to milk produces a higher proteolysis during storage than in direct acidification cheeses. The fact that a lesser proteolysis was observed could mean that manufacturing Oaxaca cheese by direct acidification could extend shelf life of the cheese. Additional studies are necessary to know the impact on sensory characteristics and microbial population on direct acidification Oaxaca cheese processing.

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Evaluation Of Germinated And Malted Oats As Substrates For Probiotic Bacteria In Non-Dairy Beverages

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Abstract. Probiotics are widely recognized for their health benefits about 43% of this market is represented by dairy products. Nevertheless, these have the disadvantage of not being suitable for lactose intolerance consumers or those with high cholesterol. Oat has been previously used in the formulation of non-diary probiotic food. Germination and malting are processes that can be applied to oats to increase their nutritional value and nutrient bioavailability. The aim of this work was to assess the potentiality of germinated and malted oat based substrates as novel media to design non-dairy probiotic drinks. The probiotic strains *Lactobacillus casei* 431 and *Lactobacillus rhamnosus* HN001 were single cultured in oat based media. Fermentations were carried out at different settings of substrate concentrations, temperature, agitation and headspace. Cell viability was evaluated throughout the fermentation assays. Malted oat was the best media to support the growth of the bacteria at a concentration of 3 % (w/v) with added sugar (1.5 % w/v). Final cell concentrations were of 1.4 x 10^8 which is within the probiotic levels. The main factors that influence the growth of the probiotic cultures were type of oat substrate, the addition of sugar and the interaction of temperature and agitation. Higher cell viability was observed a low temperature (20-25 $^{\circ}$C) and high agitation levels (150-180 rpm).
Keywords: Oat substrates, malting, germination, probiotic, fermentation.

Introduction

In recent years, cereals and its ingredients are accepted as functional food because they provide dietary fibber, proteins, energy, minerals, vitamins and antioxidants required in human health maintenance. Also, cereals can be used as fermentable substances for the growth of probiotic microorganisms (1). Oats have high content of soluble and insoluble fibres resulting in positive effects on blood cholesterol levels (2). Strains of several *Lactobacillus* species, used as probiotics, exert a range of health promoting activities such as immunomodulation, enhancement of resistance against pathogens and reduction of blood cholesterol levels. Growth and product formation during fermentation by microorganisms can be affected by medium composition, presence of oxygen, pH and product concentration (3). The probiotic market is represented by the milk based products (43%) but lately products that aid lactose intolerance has increased so cereal based products could have a huge potential either to fulfil the consumer demand for non-dairy functional products (4). On the other hand, during the germination the biochemical activity produces essential compounds and energy for the formation of the seedling. Hydrolytic enzymes are activated and these decompose large molecular substances, such as starch, non-starch polysaccharides and proteins, to small molecular compounds (5). Malting is a process that confers better sensorial characteristics, enhanced flavour and colour to the grain, through germination and a controlled heating process where the Maillard reactions have place (6). The aim of this work was to assess the potentiality of germinated and malted oat based substrates as novel media to design non-dairy probiotic drinks.

Materials and methods

2.1 Cultures

Lactobacillus rhamnosus HN001 was isolated from a synergic mixture of Danisco. *Lactobacillus casei* (L. casei 431) was obtained from Chr Hansen®(Chr. Hansen, Milwaukee, Wis., U.S.A).The cultures were maintained at – 20° C in a cryopreservative fluid (50% glycerol and 50% MRS broth). The inoculums 1% (v/v) of the cultures were re-suspended in a fresh MRS-broth (Difco, USA)and incubated at 37°C for 10 h. Afterwards, the cultures were centrifuged (2000 rpm, 10 min, 4 °C), washed twice in phosphate buffer and re-suspended in the corresponding oat media.

2.2 Oat substrates

Oat (*Avena sativa*) var. Karma cultivar was purchased from a local market. The preparation of germinated oat substrates followed the method reported by Moongngarm and Saetung (2010) and the malted oat was prepared followed the method reported by Salmerón (2010). After the treatments oats (including oat without treatment) were ground separately in a hammer mill (Pulvex, U.S.A.)

2.3 Preparation of probiotic oat beverages

To prepare the oat based beverages, oat flours (oat, germinated oat and malted oat) were mixed separately at 3, 5 and 7% (w/v) and water, then heated at 90 °C for 1 h and cooled at 50 °C for 30 min. The mix was centrifuged (5702 R Eppendorf, U.S.A.) at 4000 rpm, 15 min and 25°C. The obtained media was sterilized at 121 °C for 15 min. Each media was inoculatedat 3% (v/v).

2.4 Fermentation

2.4.1 First stage

The effect of probiotic culture (*L. casei* and *L. rhamnosus*) and sucrose addition (1.5% w/v) in malted oat media at 7% (w/v) on final cell growth were evaluated by optical density (595 nm) and the Miles and Misra (1938) method. Afterwards fermentations were done to evaluate the effect of media (oat, germinated oat and malted oat) and oat concentration (3, 5 and 7% w/v) on final cell growth. These were performed with *L. casei* and added sucrose (1.5% w/v). From this assays the best media formulation was obtained.

2.4.2 Second stage

In order to optimize the fermentation parameters (temperature, agitation and headspace) a response surface central composite design was applied (see Table 1).The cell growth (after 14 h) was selected as the dependent variable.

2.6 Statistical design

A one way analysis of variance (ANOVA) and a Tukey´s test with a 95% confidence level was used to select the best bacteria, oat substrate and substrate concentration where maximum cell growth was used as the response variable in each stage. On 1st stage the results were analyzed using a factorial design 2^2 to choose the bacteria culture and sucrose addition. Afterwards a factorial design 3^2 was used to choose the oat substrate and its concentration. In the 2nd stage in order to optimize the fermentation parameters a response surface central composite design was applied (see Table 1). Using Minitab (version 16, U.S.A.).

Table 1. Levels of independent variables, temperature, agitation and headspace used for response surface central composite design.

Independent variable	Levels		
	-1	0	+1
Temperature (°C)	25	31	37
Agitation (rpm)	50	100	150
Headspace (mL)	83	125	167

Results and discussion

3.1 First stage fermentation

The growth kinetics of *L. casei* and *L. rhamnosus* was evaluated in a malted oat drink (7% w/v). Also the sucrose addition at 1.5% (w/v) as reported by Angelov et al.(2005), was studied. Results are shown in Figure 1. Concerning the time it takes to reach the stationary phase *L.casei* need less time (12 h) than *L.rhamnosus* (13 h). In this figure the addition of sucrose did not show any difference.

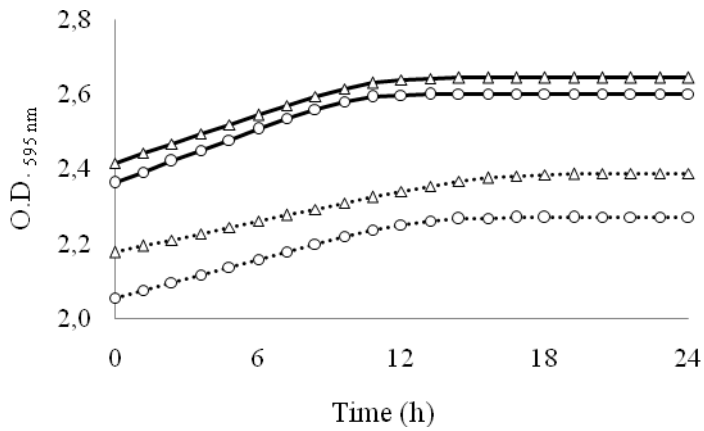


Figure 1. Fermentation growth kinetics on malted oat media at 7% w/v carried out in a microreader (at 595 nm, 37 ° C for 24 h, every 30 minutes a reading was taken). *L. rhamnosus* (……), *L.casei* (---), sucrose addition (Δ) and without sucrose addition (O).

In the growth of *L. rhamnosus* (Table 2) there was not any significant difference by the addition of sucrose. The probiotic strain *L. casei* did show a better growth with sucrose as reported by Gupta et al. (2010), who evaluated the growth of a lactic strain in an oat media with added sucrose. Due to the better growth performance of *L. casei* was selected to carry out the selection of the media formulation. Sucrose concentration remained fixed 1.5 % w/v.

Table 2. Analysis of variance of the CFU/mL of *L. casei* and *L. rhamnosus* in malted oat drink at 7% w/v with and without sucrose addition at 1.5 %, at the begging (t_0), after 24 h (t_{24}) of fermentation and the Net Growth at 37 °C.

Bacteria	Sucrose	CFU/mL*		
		t_0	t_{24}	Net Growth
L. casei	-	$4.6x10^6 \pm 1.5x10^6$ a	$16.3x10^6 \pm 8x10^6$ a	$11.7x10^6 \pm 7.8x10^6$ a
L. rhamnosus	-	$33.3x10^6 \pm 23x10^6$ a	$53.3x10^6 \pm 25.2x10^6$ ab	$20x10^6 \pm 10x10^6$ a
L. casei	+	$16.7x10^6 \pm 2.5x10^6$ a	$76.3x10^6 \pm 9.6x10^6$ bc	$59.7x10^6 \pm 8x10^6$ b
L. rhamnosus	+	$70x10^6 \pm 10x10^6$ b	$96.6x10^6 \pm 11.5x10^6$ c	$26.6x10^6 \pm 5.8x10^6$ a

*Numbers represent mean values of three independent replicates ± SD. Different letters indicate statistically significant differences between the means ($p < 0.05$) for CFU/mL.

Results of the formulation selection assay are shown in Table 3. Better growth was observed in the malted oat at 3% and oat at 7%. Even though they have different substrate concentrations there is not significant different because as already mentioned the malting process confers more bioavailability of nutrients. In germinated and malted oat mediums there was a growth decay when the substrate concentration was increased. On the other hand malted oat confers more body and flavour to the beverage, hence the sensory acceptance could be better. Therefore on this basis malted oat was selected.

Table 3. Analysis of variance of the CFU/mL of *L. casei* in oat drinks at different substrate concentrations with sucrose addition, after 24 h of fermentation at 37 °C.

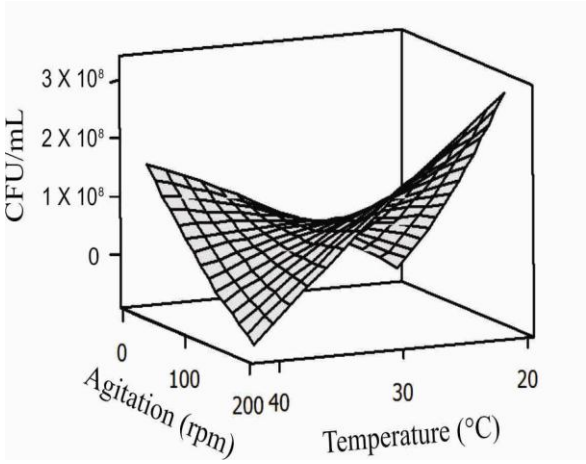
Substrate	Substrate concentration (% w/v)	CFU/mL*		
		t_0	t_{24}	Net Growth
Oat	3	$8.7x10^6 \pm 2.3x10^6$ c	$10x10^6 \pm 1.7x10^6$ a	$1.3x10^6 \pm 0.5x10^6$ a
Oat	5	$13.3x10^6 \pm 2.3x10^6$ cd	$64.3x10^6 \pm 5.1x10^6$ c	$51x10^6 \pm 6x10^6$ b c
Oat	7	$2x10^6 \pm 1x10^6$ a	$87.3x10^6 \pm 2.1x10^6$ d	$85.3x10^6 \pm 3x10^6$ d
Germinated	3	$13.3x10^6 \pm 2.3x10^6$ cd	$64.3x10^6 \pm 5.1x10^6$ c	$51x10^6 \pm 6x10^6$ b c
Germinated	5	$7x10^6 \pm 4x10^6$ ab	$14.3x10^6 \pm 2.5x10^6$ a	$7.3x10^6 \pm 5.5x10^6$ a
Germinated	7	$1.3x10^6 \pm 0.6x10^6$ a	$15x10^6 \pm 4.4x10^6$ a	$13.7x10^6 \pm 4.6x10^6$ a
Malted	3	$2x10^6 \pm 1x10^6$ a	$87.3x10^6 \pm 2.1x10^6$ d	$85.3x10^6 \pm 3x10^6$ d
Malted	5	$4.7x10^6 \pm 1.2x10^6$ ab	$46.3x10^6 \pm 3.5x10^6$ b	$41.7x10^6 \pm 3.8x10^6$ c
Malted	7	$14.7x10^6 \pm 0.6x10^6$ d	$79.3x10^6 \pm 9.5x10^6$ d	$64.7x10^6 \pm 9.5x10^6$ b

*Numbers represent mean values of three independent replicates ± SD. Different letters indicate statistically significant differences between the means ($p < 0.05$) for CFU/mL.

3.2 Second stage fermentation

The optimization of the fermentation parameters was carried out in order to obtain the best cell growth of *L. casei* on the malted oat media 3% (w/v) with sucrose (1.5%) (Figure 2).The results show that the interactions between temperature and agitation had a significant ($p<0.05$) effect on cell growth. High agitation levels seemed to have more influence on cell growth as the objective of the agitation is to confer an adequate mass transfer. Although lactic acid bacteria have an optimum temperature of 37 °C, this study shows the significance of agitation and temperature on the growth of the probiotic culture in the oat medium. It was observed that the oxygen concentration (based on the initial headspace) did not have any significant effect on the final cell concentration. Gokavi et al. (2005) and Angelov et al (2005) did not use agitation during the lactic fermentation of an oat-based substrate and the temperature was maintained at 37 °C in such experiments they obtained cell populations around the 7 log CFU/ml that were lower than the results obtained in this work (8.1 log CFU/ml). Our results are comparable to those obtained by Gupta et al. (2010) and Kedia et al. (2008) were they cell populations of 10.4 log CFU/ml and 8.5 log CFU/ml, respectively. From this study it is observed that at 25 °C (or lower) and with an agitation of 150 rpm (or higher) *L. casei* can achieve a cell growth within the probiotic levels during the fermentation of a malted oat media at 3% (w/v) with sucrose (1.5% w/v).

2a



2b

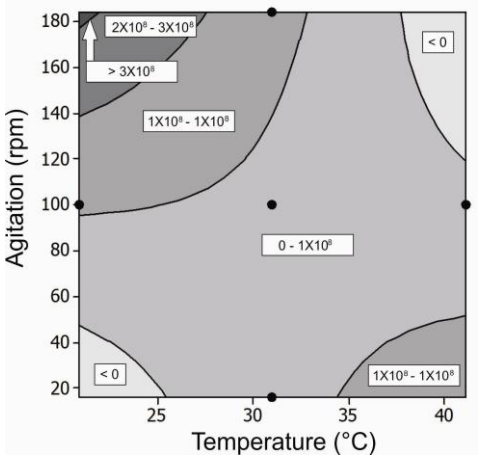


Figure 2. Fermentation of *L. casei* on malted oat media. 2a. Surface Plot of CFU/mL vs Agitation and Temperature. 2b. Contour Plot of CFU/mL vs Agitation, Temperature.

Conclusion

During the formulation of probiotic oat beverages the treatment applied to the oat substrate, sucrose addition, and the factors of temperature an agitation play a significant role on the probiotic culture cell growth. In further research it is recommend to explore the fermentation parameters at higher levels of agitation and lower temperatures settings.

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Molecular Monitoring of a Mixed Culture DuringFermentation of *Agave duranguensis* Juice for Mezcal Production

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Introduction

Mezcal is an regional alcoholic beverage obtained by *Agave* fermentation. The fermentation is carried out primarily by the action of yeasts native naturally and inoculated strains; finding different yeast genera and in the first stages of fermentation, which include the genera *Saccharomyces*, *Kluyveromyces*, *Torulaspora*, *Hanseniaspora*(1,2). It is produced traditionally leaving high concentration residual sugars, ethanol variable, and formation of undesirable compounds such as methanol. To ensure fermentation and product quality, to the fact that a large number of variables involved in spontaneous fermentation, has favored the use of active dry yeast, and favoring a more rapid onset of fermentation (usually reduces the lag phase) and a total consumption of fermentable sugars, thus reducing potential problems of fermentation and microbial allows greater control, which is not possible in spontaneous fermentations (3, 4). This has led to a mixed culture of native strains to improve the fermentation process in the production of mezcal. This study was followed up by mtDNA and δ elements to demonstrate that fermentation is actually carried by strains inoculated.

Materials and methods

Cultive medium

- YDP Medium
  1% yeastextract
  2% dextrose
  2 % peptonecasein
  2% agar
- *Agave duranguensis*juice

Biologymaterials

- *S. cerevisiae* (ITD-00185)
- *T. delbrueckii* (ITD-00014)

Oligonucleotide

In this study we used a set of oligonucleotides which were applied to the identification of species yeast, for the characterization of strains within the same species or for their specific detection.

- ITS 1 TCCGTAGGTGAACCTGCGG. (5)
- ITS 4 TCCTCCGCTTATTGATATGC. (5)
- NL-1[a] GCATATCAATAAGCGGAGGAAAAG. (6)
- NL-4[a] GGTCCGTGTTTCAAGACGG. (6)
- δ12 TCAACAATGGAATCCCAAC(7)
- δ21 CATCTTAACACCGTATATGA-3 (7)

Metodology

Fermentation kinetics.Two fermentations were performed. A spontaneous fermentation carried with 6 L of*Agave duranguensis* juice in a stainless steel tank, which was incubated at 28°C for 42 hours. A second fermentation was performed at the same conditions but this was inoculated with a mixture of 75% *S. cerevisiae* and 25% *T. delbrueckii*to reach an inoculum concentration of 10^7 cells/mL. Samples were taken every 6 for quantification of dextrose, fructose and ethanol by HPLC(8).

*Isolation of yeast and DNA extraction.*The isolation of yeast was performed by the method of Harrigan(9), while DNA extraction was performed by the method described by Querol(10,11).

*Amplification of 5.8s-ITS region of ribosomic DNA.*The region which includes the gene 5.8S and intergenic regions ITS1 and ITS2 was amplified using the oligonucleotides ITS1 and ITS4 (5). The cells from one colony were resuspended in the PCR mixture. The amplification conditions were described by Esteve-Zarzoso et al. 1999 (12). The reaction mixture was prepared in a final volume of 50 µL, 0.2 µL of Taq polymerase (TaKaRa Biotechnology, Dalian), 5 µL of 10x reaction buffer, 1 µl deoxynucleotide (dTTP) and 2µL of the sample DNA. Amplification was performed in a thermocyclerProgene (Techne, Cambridge, UK). The PCR products were visualized by electrophoresis in 1.5% agarose stained with Gel Red 10, 000X (Biotium, Glowing Products for Science, Hayard, CA.). The size of the fragments was estimated by comparison of their mobility with standard marker (100bp Invitrogen).

Enzymatic digestion of amplified of region.5.8 s-ITS. The amplified DNA obtained by PCR of the region5.8s-ITS was digested with the restriction enzyme CfoI, HinfI and HaeIII (Roche Molecular Biochemicals, Mannheim, Germany). The restriction fragments were separated by electrophoresis on 3% agarosegel with buffer TAE 1X (40 mMTris-acetate, 1 mMEDTA, pH 8) and visualized with UV light after stained with Gel Red 10,000X (Biotium, glowing Products for Science, Hayard, CA.). As molecular of weight marker was used DNA ladder 100 + 50bp (Invitrogen).

*Enzymatic digestion of mitochondrial DNA.*The genomic DNA of isolated strains of inoculated fermentation were digested with the restriction endonuclease HinfI (TaKaRa Biotechnology, Dalian) and Dde I (Roche Molecular Biochemicals, Mannheim, Germany) according to protocol described by Querol*et. al*(11). The reaction mixture contained 15 µL of genomic DNA, 2 µl RNAse 500 µg/mL (Boehringer Mannheim), 1.5 µL of enzyme and 2.5 µL of buffer 10X. The mixture was incubated at 37°C overnight. The restriction fragments of mtDNA were separated in 1.5% agarose gels in buffer TAE 1X (40 mMTris-acetate, 1 mMEDTA, pH 8) at 90 V. After electrophoresis, gels were stained with RED Gel 10.000 X (Biotium, Glowing Products for Science, Hayard, CA). The fragments were visualized with UV light. The size of the fragments was estimated visually with the marker of lambda DNA digested with PstI.

Amplification of the domains D1 and D2 of the 26s ribosomal gene. PrimersNL-1 and NL-4 were used for amplification of the 26s gene. The reactions were performed with final volume of 100 µL that contained 10 µL reaction buffer10x, 0.2 mM of oligonucleotides ITS1 and ITS4, 0.2 mM of dNTPs, and 4 µL of DNA. Amplification conditions were 94°C for 3 minutes and 99 cycles of 10 minutes at 96°C, 5 min at 50°C and 4 min at 60°C, using a GeneAmp 5700 thermocycler Sequence Detection System (PE Applied Biosystems). The amplified fragments were analyzed by electrophoresis on 1.4% agarose gels. The gene purification was performed with High Pure PCR Product Purification kit (Roche Appliied Science Mannheim Germany). After obtaining the sequences, errors are eliminated by comparison of both strands of DNA using the software Chromas V 1.43 (Brisbane, Australia). The sequences were aligned using the ClustalX software, a version of Windows ClustalW(13), then the sequences were searched in the BLAST database.

Results and Discussion

Figure 1 shows that fermentation inoculated with the mixture of selected strains was carried out faster than spontaneous fermentation. Inoculated fermentation showed total sugar consumption at 30 h of incubation, producing amaximum ethanol concentration of 67g/L. Spontaneous fermentation showed a maximum ethanol production of 42 g/L and leaving 35 g/L of residual sugars, despite having been incubated to 48 hours. Thus, inoculated strains were able to prevail on the native microflora,showing greater competitiveness in the number of cells, faster consuming sugars and presenting higher performance (14).

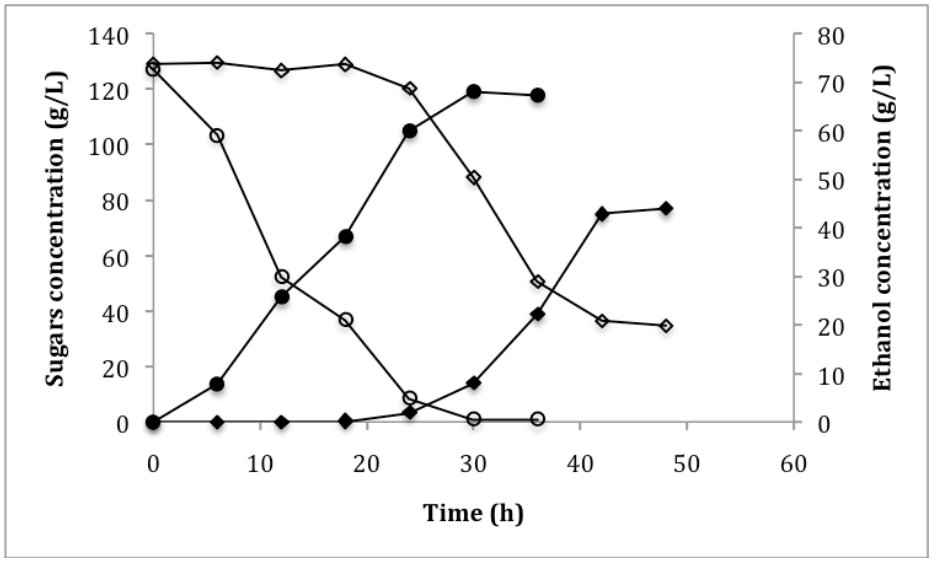

Fig.1 Kinetics of fermentation of *Agave duranguensis* juice: spontaneous fermentation (●, ○) and inoculated fermentation (◆, ◊).

A total of 170 colonieswere isolated from bothfermentations: 100 colonies corresponded to spontaneous fermentationand 70 colonies from inoculated fermentation. Amplificationof the DNA region ITS-5.8s was performed in ordet to determine the variability of the species during the fermentation. Spontaneous fermentation showed only two different amplified. One of 720 bp, which prevailed throughout the fermentation. The other amplified had a size of 380 bp, which was found only at 18 and 24 h of fermentation (Fig. 2). Digestionsby restriction endonucleasesCfo I, Hinf I and Hae III were performedto the domains of D1/D2 region and the 26S ribosomal gene and 5.8s-ITS region gene,following ofsequencin. In the identification of species was found 99% similarity to strains of *Kluyveromycesmarxianus* (corresponding to amplified of 720 bp) and *Clavisporalusitaniae* (corresponding to amplified of 380 bp).

Molecular characterization was conductedby mtDNA andδ elements, in order to know the behavior of strainsinoculated. In the inoculated fermentation, we found two amplified sizes of 850 and 800 bp. The first prevailing until the end of fermentation, and the second was found up to 18h of fermentation. According to the database Yeast-ID, these amplified corresponding to strains *S. cerevisiae* and *T. delbrueckii*, respectively, which werethe strains inoculated. We identified two mitochondrial patterns, the pattern I is the strain of *S. cerevisiae* and the pattern II corresponds to the strain of *T. delbrueckii*.This study demonstrated that*S. cerevisiae* inoculated at the beginning of the fermentation was the responsible of increased consumption of sugar during fermentation.This work demonstrated that the restriction analysis of mtDNA and δ elements allows to monitor strains of fermentations inoculated, identifying differences between strains of the same genus, mostly in *S. cerevisiae*, as it is considered responsible for alcoholic fermentation (11, 15, 16).

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Physicochemical Characterization Of The Coconut Palm Sap (*Cocos nucifera L.)* For *Tuba* Production

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Introduction

The *tuba* is a refreshing drink with ancestral roots in Colima, is obtained from the coconut palm sap (*Cocos nucifera L.*) through a cut in the inflorescences in its early stage, when they are still covered by the spathe. The coconut (*Cocos nucifera Linn*) is a member of the family Arecaceae (palm family). It is an ornamental tree grown in villages and towns. The coconut is native to the littoral zone of Southeast Asia (Malaysia, Indonesia, Philippines) and Melanesia (1). *The Cocos nucifera L.* known as the "tree of life", is a multipurpose plant, being in the 12th place in the list of species of food plants important to man, besides being a of the most beautiful in the world (2). Another uses from the *C. nucifera L*. are the Plant medicine formulas that are pounded and placed in the fiber and squeezed or rubbed over the injury, allowing the sap of the plant to drip on the skin. The syrup from the inflorescence of *C. nucifera* is tapped and made into an alcoholic beverage known as *tuba*.(3)

The tuba one traditional drink of the Pacific Coast, which is produced from the fermentation of the sap extracted from the inflorescence of the coconut palm. This drink has years of residing in Colima where the process of obtaining the tuba is transmitted from generation to generation, and has its origin in the Philippines. This drink came to Mexico under the Manila Galleon travel in the sixteenth century. The commercialization of the tuba was very important so it began producing other derivatives of it like wine coconut being one of the most exploited in the region even to surpass the fine Spanish wines. So in 1612 the Viceroy of New Spain ordered the felling of several coconut decision was not executed by the arguments presented by the neighboring Colima. In Mexico, the tuba is extracted only in certain places, such as the coast of Colima, Michoacan and Guerrero, but not on a large scale, with the exception of Cihuatlán, Jalisco, where there are 300 000 coconuts for obtaining the sap or juice palm.

The process first step is to collect the sap that flows freely from the severed inflorescence, which remains with a length ranging from 45 to 75 cm. The sap start to flow after three or four days since the inflorescence was cutted off. The sap is recollected twice a day from the pot, jar or bamboo tied to the extreme. The sap is recollected twice a day from the pot, jar or bamboo tied to the extreme.

Methods and Materials

In this work, a palm sap physicochemical characterization was performed. Two different regions Colima's downtown (CD) and Asmoles area (AA) were studied over the year; the sap of twenty six palms was collected in the morning and afternoon. In both regions, the palms were cultivated under identical conditions of irrigation and fertilization, and all the analysis were performed in triplicate.

- Reducing sugars

Reducing sugars were determined by the dinitrosalicylic acid (DNS) reducing sugar assay (4). The DNS assay was performed under the following procedure: samples of palm sap were diluted with distilled water

in order to fit with the standard curve. Then, 250 µl of the diluted solution was pipetted into a 22-mm screw-cap culture tube to which 750 µl of DNS reagent was added. Later, this mixture was placed in a boiling water bath for 15 min, cooled, and diluted with 4 ml of distilled water. The absorbance at 550 nm of the reacted mixture was compared with a calibration curve prepared using glucose standard solutions. All reagents were purchased from Sigma-Aldrich (St. Louis, MO) with purity over 99.0%. The measurements were doing in a Spectrophotometer Perkin Elmer; model LAMBDA 25 & 35 UV/Vis System.

Potential of Hydrogen

Measurements were made on a pH meter Denver Instrument model 215.

- Titratable Acidity

The general method to determine titratable acidity (TA) in fruit juices is based on the titration of the sample with 0.1 mol/L NaOH, using phenolphthalein as indicator or potentiometric detection in the case of heavily colored samples (5). 10 ml of the sample was degassed by Sonicator Elma S130H Elmasonic for 15 min at 35 ° C. Thereafter was added 10 drops of 1% phenolphthalein and titrated with a solution of 0.1 N sodium hydroxide to observe the color change from white to purple. The calculation expresses the titratable acidity in terms of g/L of H_2SO_4

- Assimilable Nitrogen

Once the measurement has been registered total acidity proceeds to add 20 ml of 37% formaldehyde at pH 7 and stirred vigorously for color change from purple to white. Following the protocol of total acid added is added through a burette 0.1 N sodium hydroxide until observe the color change from white to purple and recorded milliliters of 0.1N sodium hydroxide used. The results of assimilable nitrogen **(AN)** are expressed in mg / l.

- Carbohydrates Content

The carbohydrates content were determined for the method proposed by Anderson et al. 2000 with some modifications. The Thin Layer Chromatography (TLC) was performed using the coconut sap *Tuba*. The *Tuba* was centrifuged during 15 min at 11 000 rpm and 4°C. Then, 1 µL of sample, was loaded onto a HPTLC plate silica gel analytical-60 with aluminum support (20 x 10 cm, Merck), with a spaced of 1 cm each one and a distance of 1 cm from the origin on the base plate. The carbohydrates used as standard were: glucose (DP1), sucrose (DP2), 1-kestose (DP3) nystose (DP4) and kestopentose (DP5). HPTLC plates were developed five times in a solvent system, butanol: propanol: water (3:12:4 v/v/v). Then the HPTLC plates were sprayed with a revealing reagent (aniline 4% (v/v) and diphenylamine 4% (w/v) dissolved onto a solution of acetone and phosphoric acid 85% (v/v) in a 5:5:1 (v/v/v) relation. Finally, the plates were baked at 100°C during 5 min and the carbohydrates were visualized.

- Total Soluble Solids

Samples of palm sap were centrifuged at 11, 000 rpm for 10 min a 4°C. Thereafter, total soluble solids were measured using an ATAGO refractometer (model PAL-1, ATAGO, Japan) with a scale ranging between 0 and 45 degree Brix (°Bx) and with an accuracy of ± 0.1 °Bx. The soluble solids content were reported as °Bx.

Results and Discussions

The tuba analysis of the two regions shown that the total soluble solids slightly decrease, (15.04 ± 1.91 to 14.16± 1.416 °Brix in CD and 16.45 ± 0.53 to 14.10 ±0.28 °Brix in AA), from the cold season to hot season respectively. Further analysis of reducing sugars in the morning and the afternoon in AA showed an increase 16.45 ± 0.53 to 14.10 ±0.28 g/l from respectively. In contrast in the CD zone we obtained measurements to 15.98 ± 1.78 to 16.87 ± 0.54 g/l in the morning to the afternoon respectively. The results shows that slightly increase during the day In contrast the titratable acidity (TA) and assimilable nitrogen (AN) increased from the 1.76±0.18 to 3.13±1.33 g H_2SO_4/L and 216.65±24.45 to 722.27±29.59 mg/L in the AA. Whereas, in the CD, the TA and AN present no significant changes, ranging from 1.24± 0.70 to 1.40± 0.48 g H_2SO_4/L and from 208.74± 45.45 to 209.33± 14.98 mg/L, respectively. The Figure 1 and 2 show the identification of fructooligosaccharides by thin layer chromatography.

Figure 1 shows a thin-layer chromatography 20 palms collected in the morning found in the area of AA. Based on the chromatogram can observe the presence sucrose DP2 and glucose DP1. The sucrose is found in high concentrations in comparison to glucose which is almost imperceptible. This explains the values obtain by the technique of reducing sugars

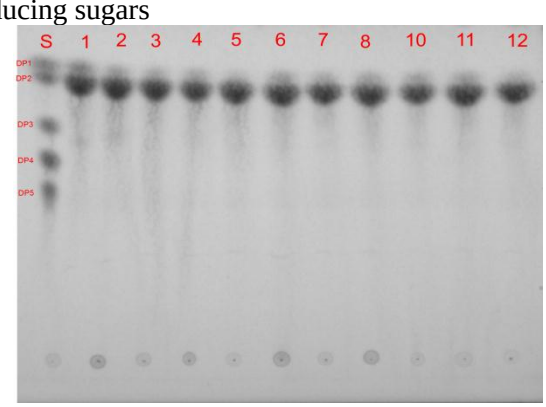

Figure 1. TLC of the not-fermented sap obtains from the AA. The picture demonstrates then presence of sucrose (DP2) and glucose (DP1).

In contrast in Figure 2 shows a chromatogram of the tuba collected in the afternoon in the area AA. Based on the chromatogram can say that the concentration of sucrose significantly decreases disappearing in some samples. In the afternoon samples the glucose increases significantly which correlates with data previously obtained by the measurements of reducing sugars.

The results of TLC would indicate the presence of invertase which have the ability to hydrolyze the glucose-fructose bond. Invertases were found and reported in organisms such as bacteria, fungi, higher plants and animals. (6, 7)

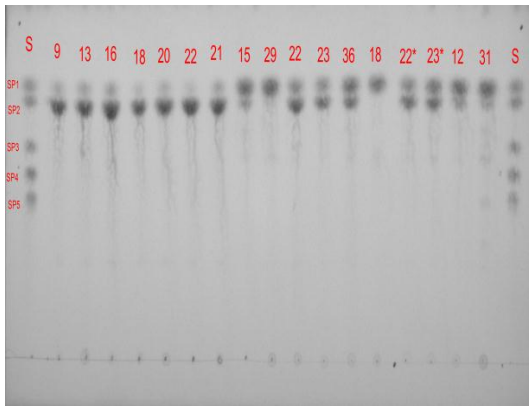

Figure 2. TlC of the sap fermented and not fermented of the AA collected in the afternoon and morning respectively. The not fermented saps are the samples from the 9 to 21 and the fermented samples are the 29 to 31. In this TLC include a fermented sample (15) collected in the morning.

The presence of invertase tells us that a fermented tuba or fermentation process has a higher bacterial load evidenced by assimilable nitrogen concentration has a much greater amount of invertase. A reasoning for this process would be: under normal conditions the sap has high concentrations of sucrose and the low levels of glucose and fructose. Once the fermentation process increases the production of invertase produces the breakdown of sucrose to glucose and fructose which leads to a considerable increase in the levels of these metabolites. Subsequently these metabolites decrease due to consumption of these sugars by microorganisms present in the sap. The results obtained by determination of reducing sugars using DNS technique show that the sap, obtained recently and unfermented plant has low levels of glucose and fructose in free state.

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Evaluation Of Phenylpropanoids And Isolation Of Endogenous Microorganisms In Fermentation Of *Capsicum annuum L.*

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Introduction

Fermentation of food has been used for a long time as a preservation technique. In order to optimize the beneficial effects of a specific fermentation,the biochemical changes and microorganisms involved are subject of investigation.

Since they are exposed to the environment, fresh vegetables contain a variety of microorganisms, such as fungi, yeast, Gram positive and Gram negative bacteria. Aerobic organisms are mainly from the genus *Pseudomonas, Flavobacterium, Bacillus* and some molds. Some anaerobes are also present, including the genus *Klebsiella, EnterobacterE.coli* and yeast such as *Candida, Saccharomyces, Hansenula, Pichia* and *Rhodotorula.* Present in minor amounts are the lactic acid bacteria (LAB) *Lactobacillus, Weisella, Leuconostoc, Pediococcus, Streptococcus* and *Enterococcus*, among others [1].LAB can proliferate in vegetable fermentations in appropriate conditions, inhibiting the growth of pathogenic bacteria by the decrease of pH and organic acid production [1].

On the other hand, phenolic acids such as ferulic, caffeic and *p*-coumaric were found in chili peppers. [2] These compounds have shown antimicrobial and antioxidant properties in their free form [2, 3]. The increase in free phenolics in pickled peppers due to hydrolysis of the compounds to which they are bonded has been reported [4]. So it is likely that a fermentation process may also release some of the bound phenolics from the peppers matrix.

Objective

The objective of this work is the evaluation of free phenolic compounds during fermentation of jalapeño pepper as well as the identification of endogenous microorganisms involved in the fermentation process.

Materials and methods

Raw material and experimental design

We used fresh jalapeño pepper (*Capsicum annuum* L.) about 18 to 20 weeks (post-anthesis). Peduncles were removed for processing and the peppers were cut into slices of uniform size. A central composite design 22with five replications of the central point was chosen to optimize the fermentation process and to establish the influence of the most relevant factors. An optimization trial was carried out using the Design-Expert® Version 7.0.3 software (Stat-Ease, Minneapolis, MN).

Acidity and pH assay

The acidity measurement was conducted by the AOAC method 16,276 (1984)and reported as percentage of lactic acid. The pH was measured using a direct meter Milwaukee SMS110 Smart monitor.

High-Performance Liquid Chromatography Assay

The analysis of capsaicinoids was carried out with reverse phase HLPC, using a C18 column in isocratic regime 50:50 with 100% acetonitrile and 10% acetonitrile and a flow of 1 mL/min at 280 nm. The injection volume was of 10 μL.

The capsaicin standard curve was made with solutions of 0.1 to 0.05 mg/mL of methanol. The dihydrocapsaicin standard curve was carried out with 0.22 to 0.055 mg/mL of methanol, with five intermediate points, respectively [5, 6].

Free Phenolic Quantification

Two grams of sample were added to 8 mL of 80% ethanol and moderately agitated for 10 minutes. Afterward, the samples were centrifuged at 3700 rpm at a temperature of –4°C for 10 minutes. The supernatant was then recovered for analysis.

Quantification of phenolics was carried out as follows:100 μL of the extract were added with 750μL of Folin reagent, kept for 5 minutes, then neutralized with 60% sodium bicarbonate, and kept in the dark for 90 minutes. Afterward, the absorbance is read at 750 nm and interpolated in a ferulic acid curve previouslymade [7].

Antioxidant Activity

The antioxidant activity was determined using the ABTS radical technique. A 2.45 mM solution of ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) is added with 0.66 g of sodium persulfate and kept in the dark for 12–16 hours. Five hundred microliters of this solution is then transferred into a beaker, and the volume is taken up to 50 mL with 80% alcohol, adjusting the absorbance to 0.7 ± 0.2 at a wavelength of 734 nm. Nine hundred ninety microliters from this adjusted solution is mixed with 10 μL of the sample in order to determine the difference in absorbance from reaction time 0 to 5 minutes[8].

Isolation of microorganisms

The isolation was madein the chili as well as in brine in different times of fermentation. The samples were inoculated in MRS (Man Rogosa-Sharpe) and YEPD (Yeast Extract, Peptone and Dextrose) mediums, with serial dilutions in order to isolate the different morphotypes present in the fermentation.

The samples were incubated for 24 hours at 32 °C, and a differential count of the morphotypes was performed. Main morphological characteristics were described of the selected colonies and replanted by cross-streak in their respective media until purification. The colonies were kept frozen at -80°C with glycerol in a proportion of 50% (v/v). Isolated colonies were observed with an optical microscope with Gram stain for microscopic description.

Results and discussion

Kinetics of the fermentation process

The optimal conditions for the fermentation of jalapeño pepper were obtained at a salt concentration of 8.42% and a temperature of 31.5°C, which yield a theoretical pH of 4.27.

Under these optimal conditions, the fermentation follows the predicted behavior regarding the production of lactic acid and pH reduction (Figure 1).

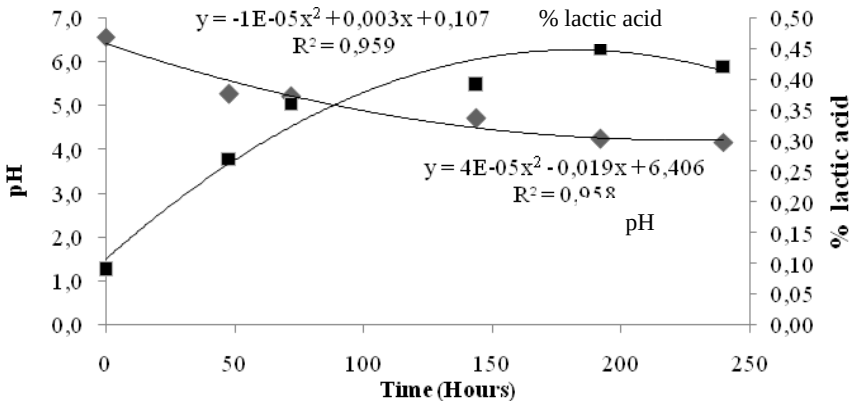

Figure 1. Changes observed during fermentation of jalapeño peppers (*Capsicum annuum* L.) on pH and lactic acid production.

The products inside the brine show a transfer of fluids, minerals, and nutrients by osmosis until reaching the equilibrium, resulting in products with very peculiar flavors, great stability, and high nutritional qualities.

Phenolics and capsaicinoids

The macerated extract of chili pepper shows an increase in phenolics and capsaicinoids after 72 hours. The same isobserved for freephenolics, which show a higher concentration and antioxidant activity at 72 hours (Table 1).

Table 1. Changes in antioxidant activityduring fermentation of jalapeño peppers (*Capsicum annuum*L.)

Time (hours)	**Antioxidant activity (mM Trolox/g dry weight)**			**Free phenolics concentration/g dry weight**
	Capsaicinoid extract	Macerated extract of chili pepper	Free phenolics extract	
0	11.21±0.08	12.65±0.3	70.46±0.6	7.92±0.02
72	6.61±0.7	49.47±0.4	169.74±0.2	51.81±0.04
240	3.51±0.4	16.15±0.1	159.52±0.3	17.02±0.01

The quantification of free phenolics (Table 1) shows that after 72 and 240 hours there is a higher concentration than the initial. These changes in concentration may be due to the changes in microbial populations. Furthermore, it has been reported that some phenolic compounds are more active than others, which depends on their particular chemical structure and their concentration on the extract along the fermentation process.

Along the fermentation process, the antioxidant activity given by capsaicinoids is lower than the total antioxidant activity, and gradually decreases until reaching a minimum at 240 hours. This may be due to the activity of LAB on these compounds, either using or transforming them, or bydiffusivityinto the brine (Figure 2).

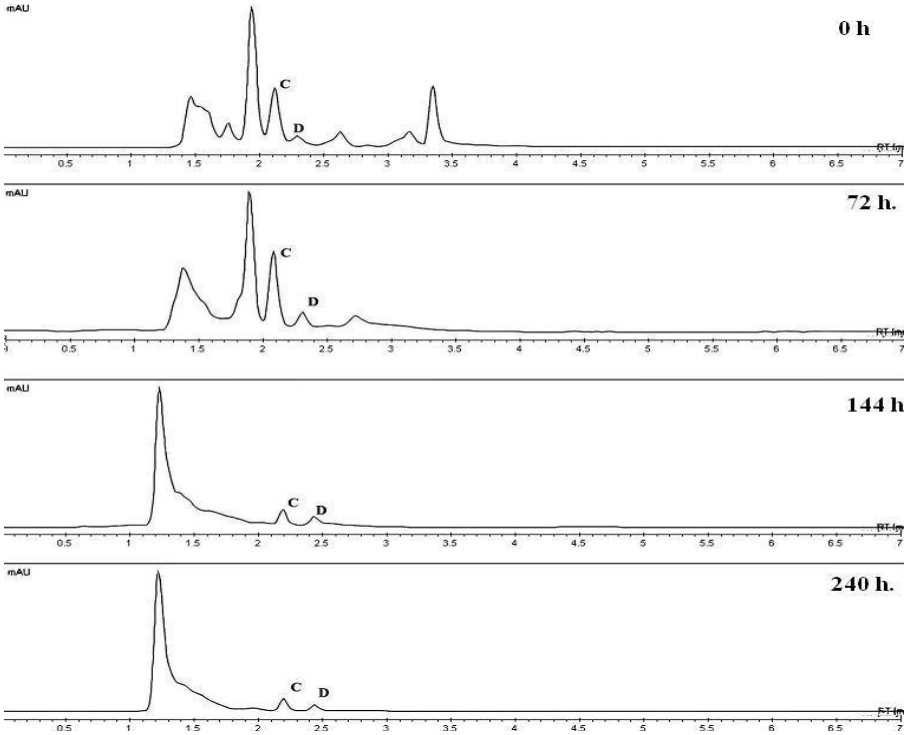

Figure 2. Changes in concentration of capsaicinoids during fermentation of Jalapeño pepper.(*Capsicum annuum* L.)C: capsaicin; D:dihydrocapsaicin.

Isolation of microorganisms

Were obtained 21 strains in MRS medium for the identification. The strains were observed and classified into six different morphotypes. The colonies present characteristics of lactic acid bacteria. They are Gram positive, nonsporulating bacilli,1-2mm long and 1mm wide (Figure 3).

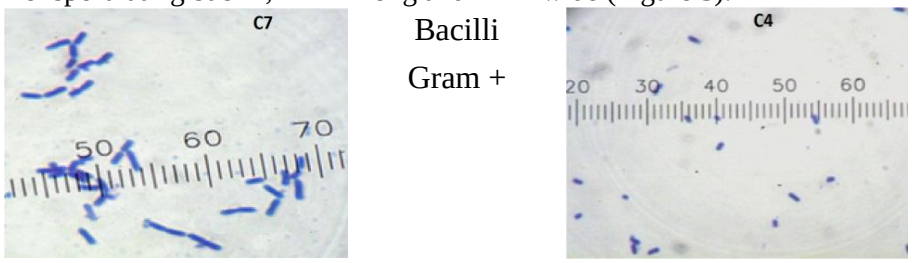

Figure 3.Lactic acid bacteria present in the fermentation of Jalapeño pepper (*Capsicum annuum* L.)

In YEPD medium were isolated 11 morphotypeswhich includes Gram negative bacteria's, yeast and Gram positive bacteria's.Gram negatives are abundant in the first stage of fermentation, until 12 hours. But they stop growing because the LAB changed the medium and proliferated in the rest of the process. The yeast were present in low proportion when the pH down, but their growing is not considerable (Figure 4).

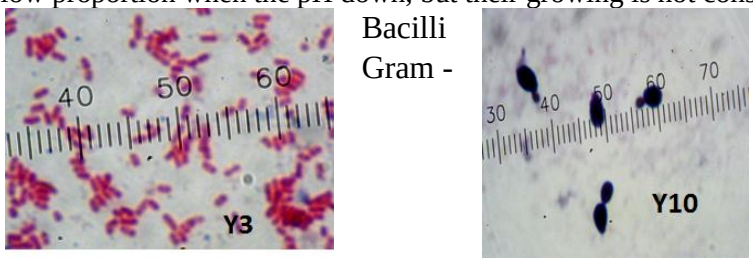

Figure 4. Isolation from fermentation of Jalapeño pepper in YEPD medium.

The results indicate that a succession in microbiotatakes place in the fermentation of chili peppers. During the first hours of the process endophytes are present, but later they are inhibited by the LAB and antimicrobial compounds liberated from the matrix of the pepper during the fermentation. These factors contribute to the selection of LAB over other endophytes such as yeast and Gram negative bacteria.

Conclusions

- Chili pepper fermentation promotes the release of compounds from the chilli matrix, making them more available.
- The antioxidant activity of phenolics compounds is higher than that of capsaicinoids because the phenolics have hydroxyl groups in their structure.
- The decrease in the amount of capsaicinoids suggests a diffusion phenomenon into the brine, or that the microbiota is using these compounds in their metabolism.
- A succession of morphotypes in the fermentation was observed,however, lactic acid bacteriapredominate along the process.

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Phenolic Composition And Physicochemical Analysis Of Merlot Red Wine Matured During Three Months In Wood Barrels

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Introduction

Keywords: cask aging, red wine quality, phenolics

Oak barrels have been widely used for the ageing of both wines and spirits, as it has positive effects for the beverage such as color stabilization, clarification and flavor enriching and blending. Oak composition and the conditions during maturation are very important to the final quality of the product.
Every cask has a life span period, based on the quality and quantity of the extractable compounds initially present in the wood. This life span is related to the number and length of the extractions or refillings that was subjected, and the composition of the wine or spirit that was placed in the barrel. Both the supervision of the wine behavior and the time that is in contact with the wood, are very relevant as some compounds increased along maturation time, while some others suffer biochemical and physicochemical transformations [6].
Wine flavor can be improved after wood maturation as some of the extracted compounds have fragrant properties, but do not override the whole wine aroma [8].
Wood ageing in wine is carried out more frequently in reds, but it also takes place in some white wines. The operation is performed after fermentation and before bottling. This practice is an old tradition used to improve sensory quality of wine [4].
Phenolic compounds extraction has also an important role during wine maturation in wood barrels. The diffusion kinetics of this extraction has been shown to have a high initial rate, as at the beginning there is a big difference of wood phenolics concentrations between the oak body and the wine. As the concentrations difference decreases, the extraction rate is also reduced. Previous studies have shown this behavior for some relevant wood extracted compounds such as vanillin [9].

Methods and materials

For this research, changes in phenolic compounds and selected physicochemical analysis were determined along three months of ageing in French oak barrels, for Merlot Red Wine from Baja California, Mexico. Barrels were new and had a medium toasting treatment; maturation was carried out in a winemaker cellar under temperature and humidity conditions commonly used for industrial production. Wine samples were taken after 1, 2, and 3 months keeping also a simple with no wood contact. A completely random design was used were the factor of study was the maturation time, using one way analysis of variance as the statistical method. Physicochemical analyses were carried out according to the methods of NORMEX 2005 [7].

Phenolic compounds were analyzed by HPLC, using 11 standard compounds for identification according to the adapted method of Delgadillo [3]. It was performed on an Agilent HPLC series 1200 attached to a DAD detector. For separation, it was used a RP-18 column (LiChrosphere) 250 x 4,6 mm, 5µ (Varian, Palo Alto, CA.USA), flux was 0.8 mL/min and the injection volume of 10 µL. Detection measurements

were carried out at 260, 280, 420 and 520 nm; while temperature was kept at 25 °C. Eluents were A) water /acetic acid 98:2 (v/v) and B) methanol. Gradient started at A at 90 % and was kept constant for 4 min, then from 4 to 40 minutes B increased at 60 %, and during the last 10 minutes B decreased at 10%. Total analysis time was 55 minutes.

Results and discussion

Table 1. Results of physicochemical analysis of Merlot red wine before aging in barrels compared with those given in NORMEX2005.

Testing	Results	
	Initial conditions	Values as NORMEX 2005
Total acidity (tartaric ac. g/L)	3.45	3.5-8
Volatil acidity (acetic ac. g/L)	0.42	1.5 (maximum)
Free sulfur (SO_2 mg/L)	64	50 (maximum)
Total sulfur (SO_2 mg/L)	102.4	350 (maximum)
Total phenols (ác. gálico mg/L)	592.5	190-1300
Alcohol (% v/v)	11.72	10-14
Residual sugar (g/L)	3.5	0-5 (dry wine)

Physicochemical analysis are essential to explore the conditions present in wines as they can revealed the virtues or faults from winemaking process, the same as providing information to know if the wine is adequate for barrel maturation.

Table 1 shows the results of 7 different tests that were carried out to wine before starting ageing. It showed that samples have the typical parameters of a dry red wine: total acidity (3.45 g/L tartaric acid) and volatile acidity (0.42 g/L acetic acid) are low, total sulfurous was 102.4 SO_2 mg/L (under the permitted levels), ethanol strength 11.42 % v/v, and residual sugars were 3.5 g/L.

Free sulfurous was above the maximum limit, according to NORMEX 2005 [7], but this values are established for wines already for sale. In this case, this level was measured at the beginning of the maturation and it is expected to be reduced afterwards. Moreover, it will protect the wine from a faster and/or excessive oxidation during the ageing time [1].

Wine that will be directed to wood maturation must accomplish minimal physicochemical conditions to assure an adequate evolution and to avoid excessive oxidation, as the conditions in barrels promote slow oxidation process [5].

Phenolic composition of wine depends on the raw materials and the vinification process. Processing influence the physical phenomena such as seeds and peels solids diffusion towards the must. Wood compounds extraction also influence the physicochemical and biochemical phenomena where phenolics are also implied (oxidation, degradation and condensation).

Phenolic quantity of 11 selected compounds related to wood maturation [2], are shown in Table 2 (identified and calibrated with standards). As wine is maturing some of them decrease due to degradation process, or their combination, either direct or indirect, with ethanol [10]. This could be the case of caffeic acid, p-coumaric acid, and ferulic acid. On the other hand, syringic acid increased, which implies this compound was extracted from wood.

Table 2. Retention time of the standards and their concentration in wine during aging in oak barrels.

Standars:	Retention time (min):	Concentration (mg/mL)			
		0 months in barrel	1 month in barrel	2 months in barrel	3 months in barrel
Gallic ac.	6.953	0.3403	0.4075	0.3495	0.3987
Quercetin	11.569	0.9514	1.5683	2.9661	1.6810
P-hydroxybenzoic ac.	16.527	1.2744	1.4053	1.5473	1.4609
Caffeic ac.	17.129	1.8905	1.6574	1.7982	1.3668
Epicatechin	21.387	0.4780	0.6042	0.5160	0.5576
p-Coumaric ac.	22.151	0.3234	0.0666	0.0751	0.1209
Ferulic ac.	22.892	0.1381	0.0884	0.1282	0.0825
Sinapic ac.	27.149	0.0512	0.0837	0.0849	0.0791
Syringic ac.	29.023	0.1187	0.1337	0.1402	0.2189
Resveratrol	34.626	0.6449	0.6682	0.6947	0.7049

Conclusions

Merlot wine from Baja California showed adequate conditions for barrel maturation. Some Wood phenolics were extracted along the storage time that has been explored, such as syringic acid, while other compounds from grape have change composition due to the physicochemical changes under barrel conditions.

Acknowledgments

We appreciate the collaboration of Vineyard Casa de Piedra, Posgrado en Biotecnología of Universidad Autónoma Metropolitana and Instituto de Ciencia y Tecnología del Distrito Federal.

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Preliminary Results Of Sensory Evaluation And Physical Chemistry Of Raspberry And Cranberry Nectar

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Introduction

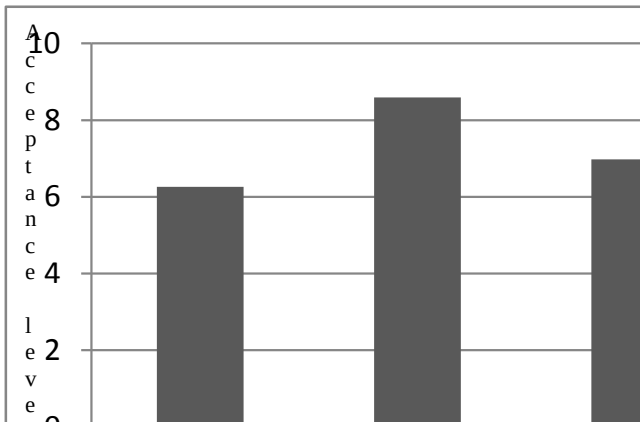
Raspberries and cranberries are fruits that are consumed all around the world on their fresh state or as processed products. They contain phenolic compounds, specially benzoic acids and cinnamic, anthocyanins, flavonoids, catechins, and tannins which can be kept in the fruit's elaborated products. (1). Another outstanding characteristic about these fruits is that they are rich in fiber with average values of 4.7 g/100 and low caloric input that they provide, 35 Kcal/100 g and 56 Kcal/100 g, respectively (2, 3, 4). Particularly, the raspberry contains excellent antioxidant as the ellagic acid; which helps to reduce cancerous lesions (5). Meanwhile, the cranberry highlights its' antibiotic, antioxidant and desinflamatoria action. Because of their organic acids concentration, which act in disintegrating the uranic tract deposits, the cranberry decreases urinary infections risks, controls cholesterol, vascular diseases, strengthens immune system, keeps in good conditions the blood circulation and helps to maintain a skin hydration(2). The raspberry and cranberry's antioxidant capacity, when kept for more than 8 days at 0, 10, 20, 30 °C, stays stable and increases the anthocyanin's value therefore, the antioxidant capacity presents an ascorbate decrease (6). Due to their health benefic properties, it is highly important, to use these fruits as raw material to create functional food such as jam, dietary fibers and even drinks as nectars; which help to preserve individuals' good health or on the contrary, use fruits as adjuvants to counteract some pathologic symptoms(1). For such reason, the study's purpose is to develop a formulation to create a functional nectar with sensory and acceptable physicochemical characteristics.

Materials and methods

This study was divided into two stages: the first stage was about the development of three nectar formulations raspberry and cranberry based, where some formulations were added sweetener quality. Later, the formulation that gained greater acceptance for its organoleptic characteristics was selected by a panel of untrained judges. The second stage consisted in selecting the one formulation of greater approval and proceeded with its reformulation; from which was obtained two new formulations (F4 y F5). It was added agave inulin to the first one as sweetener and agave syrup to the other one; both from the same commercial brand (it is important to mention that any of the nectars were added conservatives). Once the nectar was obtained, 10 ml from each formulation were stored in 10 Corning tubes (five covered with foil and the remaining without it) under refrigeration. Another 10 tubes with nectar (five covered with foil and the remaining without it) under room temperature. Sampling of each of the formulations was performed in the following chronological order: basal, one, five and seven weeks after its preparation, in order to observe changes that might present according to the type of sweetener used and the storage conditions in each formulation. A potentiometer from the Hanna Instruments brand, model HI9812-5 was used for pH values and an ATAGO brand, model-T Master refractometer, which allowed to measure brix degrees of each formulation. Statistical analysis was performed using the program Excel Officce 2007 by ANOVA test with a P value of <0.05 and preliminary results are described as average ± standard deviation.

Results

No statistically significant difference was observed between formulations (F1-F3), but it was decided to choose F2 since acceptance level was higher compared to the rest of the other two formulations. And the results of sensory evaluation of the attribute; "flavor acceptance" is shown in Figure 1.



Graph 1. Preliminary results of sensory evaluation of the attribute: "flavor acceptance", the nectar of raspberry and cranberry.

Preliminary results obtained in the second stage of the study are shown in Tables 1 and 2, of which the collection of information about certain changes in physical-chemical characteristics such as pH and brix degrees through time.

Table No. 1. Preliminary results of the evaluation of the physicochemical formulation F4, a nectar-based raspberry and cranberry.

Sampling	Formulation F4							
	Environment				Refrigeration			
	Light (n)=5		Without/Light (n)=5		Light (n)=5		Without/Light (n)=5	
	pH	° Brix	pH	° Brix	pH	° Brix	pH	° Brix
Basal day 0	4.4±.39	72.0±3.3	4.4±.43	72.0±2.6	4.4±.22	72.0±2.1	4.4±.45	72.0±2.1
7 days	3.8±.39	68.0±3.3	3.8±.43	68.0±2.6	3.8±.22	70.5±2.1	3.8±.45	70.0±2.1
35 days	3.3±.39	65.3±3.3	3.3±.43	66.0±2.6	3.3±.22	67.0±2.1	3.4±.45	67.5±2.1
49 days	2.8±.39	63.0±3.3	3.3±.43	65.0±2.6	3.0±.22	67.0±2.1	3.2±.45	67.0±2.1

1 Average ± standard deviation of pH and Brix of the F4. (n): sample size (number of tubes). ANOVA test was performed with a P value <0.05.

Table 2. Preliminary results of the evaluation of the physicochemical formulation F5, a nectar-based raspberry and cranberry.

Sampling	Formulation F5							
	Environment				Refrigeration			
	Light (n)=5		Without/Light (n)=5		Light (n)=5		Without/Light (n)=5	
	pH	° Brix	pH	° Brix	pH	° Brix	Ph	° Brix
Basal day 0	4.4±.16	72.0±3.6	4.4±.16	72.0±3.5	4.4±.27	72.0±3.0	4.4±.49	72.0±3.0
7 days	3.5±.16	70.0±3.6	3.5±.16	70.0±3.5	3.5±.27	70.0±3.0	3.7±.49	70.0±3.0
35 days	3.3±.16	64.8±3.6	3.3±.16	65.0±3.5	3.3±.27	65.0±3.0	3.2±.49	65.0±3.0
49 days	3.0±.16	63.0±3.6	3.0±.16	63.3±3.5	3.3±.27	65.0±3.0	3.2±.49	65.0±3.0

2 Average ± standard deviation of pH and Brix of the F5. (n): sample size (number of tubes). ANOVA test was performed with a P value <0.05.

Discussion

Stage 1.

The results of sensory evaluation showed no statistically significant difference between the formulations (F1-F3). This means that any of them could be elected to proceed with Phase 2 of the study. However, the

selection criteria for the reformulation was decided on the basis of which he earned the highest score in the sensory evaluation as seen in Figure 1.

Stage 2.

In physicochemical studies is important to note the changes in pH and brix degrees, as the factors that help to identify decomposition of food as well as weak or ineffective processes. On the other hand you may not have found statistically significant differences in the samples stored at different conditions as would be expected to change in the samples at room temperature, though, although this may be due to the time of year the study was conducted spanned time in late winter and early spring, where the ambient temperature in the city has an average of 20-25 ° C. It is also important to note that the physical space where the samples were stored air-conditioning at an average temperature of 15 ° C, while for subsequent studies take into account all these variables bias. It is noteworthy that after 35 days the tubes study samples exhibited sedimentation and the presence of gases. This was probably due to fermentation was not added because no preservatives.

Finally, it may be useful to add a preservative as part of the formulation to prolong the shelf life of the nectar.

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Effect Of Fermentation Conditions In The Production Of A Pineapple Wine (*Ananas comosus (L.) Merrill*)

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Abstract

Pineapple (*Ananas comosus* (L.) Merr.) is a tropical fruit native and domesticated in South America. It is mainly consumed as a fresh fruit but also as juice, canned fruit and jam. In Mexico, there is an overproduction of pineapple with not much industrialization and thus it is important to search for alternative means of processing and commercialization. This work focuses on developing a pineapple wine from the golden sweet pineapple (MD2) juice fermentation, and the evaluation of the fermentation process on its physico-chemical, nutritional, functional, and sensory properties. Golden sweet pineapple juice was previously conditioned with sucrose, ammonium sulfate, adjusted to pH 3.5 with citric acid and pasteurized to make the pineapple must. Fermentations were carry out using must at two levels of fermented sugar (25 and 30 ºBx) and three different yeast strains of *Saccharomyces cerevisiae*, Sc326 and Sc327 (yeast from distillery process) and Sc718 (yeast from mezcal fermentation), at 25°C for 144h. Aliquots were withdrawn every 48 h to monitor pH, ºBx, reducing sugars concentration, yeast growth and ethanol production. Wines were also evaluated for titratable acidity, ascorbic acid, polyphenols and antioxidant activity at the end of fermentation. After 144h, fermentation process was completed, cells were removed by filtration and the products were stabilized under refrigeration during four days. Finally, the wines were sensory evaluated by a classification scale for attributes. Kinetics fermentations showed significant differences ($p<0.05$) between yeast strains for all the monitored parameters. The ascorbic acid concentration ranged from 15.37 to 39.1 mg/100 mL, whereas the antioxidant activity ranged from 1.80 to 3.02 mmol TE/L. These variations seem to depend on the homogeneity of the juice and do not depend on the yeast strain used for fermentation. On the other hand, ethanol production was dependent on the yeast strain, with yields of 100 to 140 ml/L (10.3-14.5 °GL) with a maximum of 220 ml/L (22.4 °GL) for SC326 at 30°Bx. In general, the sensory evaluation showed that judges accept moderately pineapple wines obtained. The wine that scored the highest note of general acceptance was obtained with strain SC326 at 30 °Bx, which coincides with the highest concentration of ethanol obtained.

Keywords: golden sweet pineapple, MD2, pineapple wine; kinetic fermentation.

Introduction

Mexico produces diverse and abundant tropical fruits, which in addition to providing a nutritious diet, are an important contributor to the Mexican economy (1). Papaya, mango, banana, and pineapple occupy the first place in national production. Nevertheless, some of these fruits, particularly pineapple, often saturate the national market because of an overproduction of the fruit, a lack of industrial uses, and a lower quality of the product (2). One option to industrialize pineapple is exploring the potential of fermented products like wine. Given that the juice is rich in sugars, as well as vitamins and minerals, one can expect that it would be a good substrate for fermentation of alcohol. In the literature there are several reports about fruit wine production, but there are slight information available about pineapple wine technology nor its physico-chemical and sensory characteristics. For this reason, the objective of this work was to evaluate the potential production of pineapple wine using the golden sweet pineapple variety (MD2), and the effect of the fermentation process on the physico-chemical, nutritional, functional, and sensory properties.

Materials and Methods

Raw material. Golden sweet pineapples (MD2), produced in the south of Veracruz state, were selected for uniform size, maturity, and absence of physical or pathological damage. The selected fruits were washed and peeled, and the juice was extracted by pressing. The juice was filtered through a cotton cloth, homogenized, and freezing until needed.

Yeast strains. Three strains of yeast *Saccharomyces cerevisiae* (CDBB-L-326, CDBB-L-327, and CDBB-L-718, called Sc326, Sc327, and Sc 718, respectively) were obtained from "Colección Nacional de Cepas Microbianas y Cultivos Celulares del CINVESTAV". Sc326 and Sc327 were isolated through the distillery process, while Sc718 was isolated from the production of mezcal. The strains were maintained in agar papa dextrose (PDB) under refrigeration with monthly re-inoculations in the same medium.

Inoculum preparation. From the pure cultures kept under refrigeration, a pre-inoculum was prepared in 10 ml of PDB broth at 25°C for 36 hours. A volume of must was then inoculated with 1 mL (1 x 10^6 UFC/mL) of the pre-inoculum and incubated under the same conditions. This was the inoculums for the fermentations.

Fermentation procedure. The must was prepared by adjusting the pineapple juice to 25 and 30 °Bx with sucrose, and as a nitrogen source 0.67 g/L of ammonium sulfate was added (3). The pH was adjusted to 3.5 with a 50% citric acid solution. Must was inoculated with 4 mL of inoculum and fermentations were carried out at 25°C for 144 h without agitation. Each treatment was done in quadruplicate. During the fermentation, the musts were sampled each 48 h and evaluated for pH, °Bx, reducing sugar content, viable yeast content, and ethanol concentration. At the end of the fermentation time, the musts were filtered and refrigerated at 5°C for four days, after which they could be called wines. The wines were also evaluated for the titratable acid content, ascorbic acid, polyphenols, and antioxidant activity. In addition, wines were sensorially evaluated as described below.

Viable cell counting. The viable cell count was made by the method of counting in Neubauer chamber (Hausser Scientific) where viable cells are painted in blue with methylene blue dye (4). The count was done manually using a binocular Nikon microscope.

Analytical methods. The pH was measured with a digital potentiometer (Oakton, model 510) following standard methodology. The titratable acidity was determined by titration with NaOH 0.lN. The soluble solids (°Bx) were determined with a digital refractometer (Sper Scientific, model 300035). The ascorbic acid content was evaluated by the titration method using 2,6-dichloroindophenol (5). The fermentable sugar content was quantified using the dinitrosalicilic acid (DNS) method (6). Total polyphenols were determined with the Folin-Ciocalteu technique using gallic acid for the calibration curve (7). The antioxidant activity was determined by the DPPH method using Trolox for the calibration curve (8). All of the spectrophotometric readings were taken with a UV-Vis spectrophotometer (Jenway 6305). Finally, ethanol concentration was enzimatically quantified with a commercially available alcohol dehydrogenase (Enzyme kit L3K, DCL).

Sensory evaluation. Sensory evaluation of wines was carry out with a panel of 20 untrained judges using a descriptive test with classification scales of attributes. For That, the panelists indicated their level of liking on a graphic horizontal scale anchored on the edges (9). The parameters evaluated were flavor and odor, using the attributes of the pineapple, sweetness, alcohol, and liking, as well as overall liking of the wine.

Statistical analysis. All determinations were done in triplicate and were analyzed statistically ($p < 0.05$) using Statistica software (StatSoft, v7.0).

Results and Discussion

Physico-chemical parameters of pineapple juice. According to the preliminary tests (data not shown) performed on the two varieties of pineapple grown in Veracruz (cayena and MD2), MD2 was selected for having better physico-chemical properties. In general the juice of MD2 pineapple obtained presented values of pH and °Bx (Table 1) similar to those reported by other authors (10, 11), but with a titratable acidity (0.54 g citric acid/L) slightly inferior to those reported (0.68 – 0.98) (10). Nevertheless, this

resulted in a large concentration of polyphenols (30 times more) and ascorbic acid, even though the antioxidant activity of the pineapple juice was similar to that reported by Mullen *et al.* (13). These results correspond to the juice obtained from pineapples at the optimum state of ripeness to be used for fermentation.

Growth kinetics. During the kinetics it was observed that the yeasts demonstrated a behavior related to the consumption of sugar independent of the initial concentration in the must. After 48 hours and until the fermentation ended, strain Sc327 showed a lower speed of consumption (μ = -0.02), while strains Sc718 and 326 showed similar speeds (μ = -0.06 and μ = -0.07, respectively). For the growth of the yeasts observed, all grew exponentially during the first 48 hours and entered a stationary or dead phase in the remaining time. Strain Sc327, the slowest in growth, and showed the greatest drops in velocity of growth at μ = -7.86 x 105 during the last 96 and 144 hours of fermentation.

Table 1. Physicochemical parameters of golden sweet pineapple juice (MD2).

Parameters	MD2	Reported Value	References
pH	3.84±0.09	3.46 - 3.63	10
Titratable acidity (g citric acid/ L)	0.54±0.001	0.68 - 0.98	10
Bxº	13.5±0.33	14	11
Reducing sugar (g/ L)	32.10±0.27	--	--
Ascorbic acid (mg/ 100 mL)	53.51±3.03	45.0	11
Polyphenols (mg GAE/L)	1093.33±5.62	36.16	12
Antioxidant activity (mmol TE/ L)	2.21±0.10	3.0	13

Ethanol production kinetics. In the production of ethanol, Sc327 at 25 °Bx during the first 48 hours with a production rate of μ = -1.90 ml yielded a concentration of 91.29 mL of ethanol/L (Figure 1). While during the remaining 96 hours of fermentation the Sc 326 strain, adjusted to 30°Bx, demonstrated the greatest speed of production for ethanol with a velocity of μ = -1.55 mL, and was the strain that produced the greatest quantity of ethanol during all the fermentation, with a product of 224.66 mL, and 54% more than 25°Bx. On the other hand, Sc718 and Sc326 produced 103.99 and 135.99 mL ethanol/L, respectively.

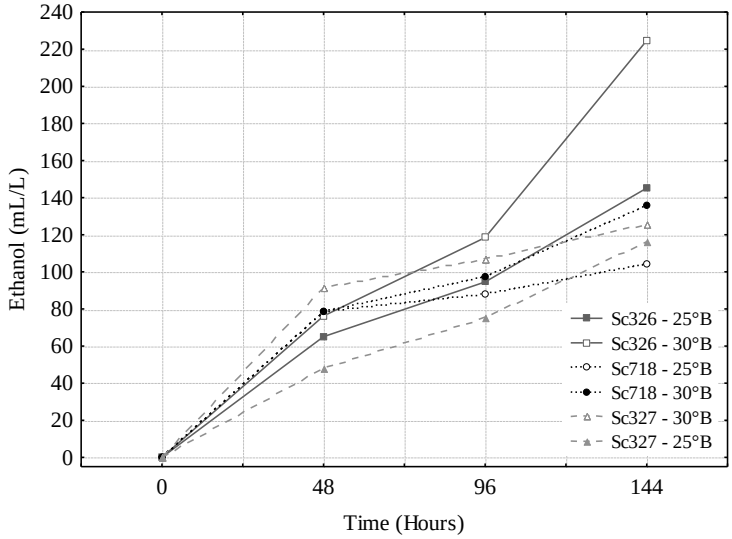

Figure 1. Ethanol production of *S. cerevisiae* 326, 327 and 718, to 25 °C in must adjusted to 25 and 30°Bx.

Physico-chemical parameters of the wine. The physico-chemical analysis of the pineapple wines (Table 2) demonstrates that wines made from pineapple juice are similar to red and white wines in measures of pH and °Bx, and agrees with the information reported by Alén-Ruiz *et al.* (14). The red and white wines had pH values that varied generally between 2 and 4 units, while their °Bx varied from 6 to 30 units. With respect to the alcoholic grade, NOM-142-SSAl-1995 (15) indicates that the alcoholic drinks could contain from 2 to 55 °GL; therefore, all samples of the pineapple wine would be acceptable according to the specifications. Mukamal *et al.* (16) reported values of 0.3 and 1.8 g/L of polyphenols in white and red wines, respectively, while Alen *et al.* (14) reported that the antioxidant activity is the same in white and red wines and varies from 0.5 and 4.0 mmol ET/L. The quantity of polyphenols and antioxidant activity found in the wines made of pineapple juice were found to coincide with those reported in the literature. Finally all the wines showed significant differences ($p<0.05$) among them.

Table 2. Physicochemical parameters of wine from golden sweet pineapple juice.

Parameters	Concentration of solids (ºBx)					
	25 °Bx			30 °Bx		
	Sc326	Sc718	Sc327	Sc326	Sc718	Sc327
pH	3.61±0.01	3.53±0.01	3.68±0.02	3.69±0.01	3.55±0.01	3.65±0.01
ºBx	7.3±0.17	9.77±0.31	9.30±0.20	15.3±0.20	15.83±0.25	16.43±0.31
Titratable acidity (g citric acid/ L)	17.85±0.43	18.29±1.46	18.04±0.57	18.91±1.87	18.47±0.60	17.66±0.22
Ethanol (ºGL)	14.5±1.20	10.3±1.41	11.6±0.40	22.4±1.20	13.5±1.67	12.5±0.92
Ascorbic acid (mg/ 100mL)	39.1±0.1	29.08±0.2	15.37±0.1	31.03±3.39	27.12±3.39	17.03±0.3
Polyphenols (mg GAE/ L)	1.10±0.02	1.05±0.04	0.86±0.01	0.96±0.03	1.02±0.01	0.96±0.03
Antioxidant activity (mmol TE/ L)	3.02±0.12	2.85±0.03	2.08±0.12	2.19±0.13	2.99±0.04	1.80±0.08

Sensory evaluation of the wines. A scale of categories was used to evaluate the odor, flavor and overall acceptability of the wine with responses to individual attributes in the categories of pineapple, sweetness, alcohol, and overall agreeableness. Table 3 provides the grades obtained for each wine. According to the results obtained, the wines with the highest ratings are those in which the must was adjusted from the beginning to °Bx of 30 units. This can be a consequence of sweetness of the wines, that is, a large quantity of sugar at the beginning resulted in the quantity of residual sugar.

The wines with the greatest amount of residual sugar received the best ratings. The results obtained through the evaluations of the six wines indicate that the wine with the best acceptance in all of the attributes was made with a combination of Sc326 and 30°Bx, except for the attributes of pineapple flavor and sweetness, where they obtained a major probability (19 and 25%) the combination Sc327 and 30°Bx. The wines were significantly different ($p<0.05$) in the attributes of general acceptability and flavor, except for the "alcohol" flavor. However, that wasn't a significant ($p<0.05$) difference in the attributes of aroma.

Table 3. Sensory evaluation of wines produced with golden sweet pineapple (MD2).

	Odor				Flavor				Overall liking
Wine	Pineapple	Sweet	Alcohol	Liking	Pineapple	Sweet	Alcohol	Liking	
Sc326-25	6.7±2.8	4.8±2.2	6.0±2.2	6.5±2.1	6.6±2.3	3.4±1.9	6.6±2.6	5.7±2.5	5.4±3.0
Sc327-25	7.0±1.5	4.4±2.0	5.8±1.8	6.5±1.9	5.9±1.6	4.3±1.1	6.6±1.8	5.9±1.8	6.0±2.1
Sc718-25	5.7±2.2	4.0±2.0	5.9±2.6	6.5±2.4	5.7±1.9	3.5±2.2	5.9±2.7	6.3±2.0	5.9±2.0
Sc326-30	7.1±1.6	5.3±2.4	7.1±1.8	7.8±2.0	7.4±2.1	5.7±2.7	7.4±1.5	8.3±1.6	8.6±1.5
Sc327-30	6.5±2.0	5.8±1.9	5.8±1.0	6.9±1.7	7.8±1.9	7.6±1.8	7.0±1.6	7.8±2.1	7.9±2.1
Sc718-30	7.0±1.9	5.2±1.5	6.5±2.2	7.3±1.7	7.2±1.8	5.8±1.8	6.6±2.1	7.4±1.8	7.3±2.0

Conclusions

With respect to the physico-chemical analysis of the pineapple juice, the results show that the majority of the parameters were inside those reported in the literature, especially the quantity of polyphenols, although there are no previous reports of the analysis of the MD2 or golden sweet. Comparing it with other varieties, the concentration of total polyphenols present in the pineapple juice was 30 times greater than that reported in the literature. With relation to the fermentations, a greater concentration of initial sugar produced a greater quantity of ethanol. The fermentations with the yeasts Sc326 produced a greater quantity of ethanol than those produced with Sc718 or Sc327. The wines with greater acceptance were those that started with 30°Bx, the ones most acceptable being made with strain Sc326, whose alcohol content was the greatest. The wines obtained had favorable quantities of ascorbic acid, while their antioxidant activity and polyphenol content were similar to red and white wines.

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Physicochemical Parameters, Macro And Micro Mineral Components Evolution In The Semi-Matured Cheese Of The Region Of Murcia Spain During Its Maturation

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The goat cheese in Murcia region has traditionally been made from goat's milk from the race Murciano-Granadina, whose main characteristic is its great capability to produce milk in big quantity and quality, in addition to having magnificent nutritional qualities, such as a great concentration of total dry extract, protein, and fat, being this the reason to develop this work with the main objective of determining during the maturation the main physicochemical parameters macro and micro mineral components of semi-matured cheese from pure goat's milk. The samples were obtained from the Central Cheese Montesinos in the region from Jumilla, Murcia. All the analysis were carried out in the laboratories of the Department of Food Technology, Nutrition and Bromatology of University of Murcia. The pH value, the percentages of protein, sodium chloride, ash (Casado, 1991), fat (FIL-IDF 5B-1986), water activity (hygrometer), dry extract (IDF 4a-1982), macro-minerals and micro minerals (sodium, potassium, calcium, magnesium, iron, copper, zinc and manganese) were analyzed by atomic absorption spectrophotometry with flame and phosphorus spectrophotometric method (FIL-IDF- 42B: 1990). All statistical determinations were performed by analysis of variance of one way with the software Statgraphics 2010. The results obtained show that the average value of pH and aw decline during the maturation, however, the dry extract, fat, ash and sodium chloride increase significantly during the maturation, obtaining results at the end of the maturation period of: 69.61%, 40.25 %, 3.92% and 1.87% respectively. The results obtained from the majority mineral elements (Ca, Na, K, P and Mg) generally increase as consequence of variation in the total solids content. With regard to the trace elements, it can be observed that during maturation there is a statistically significant increase, being the zinc which had a higher content at the end of maturation with 24.2 mg /kg, followed by iron (3,562 mg / kg), cooper (0381 mg / kg) and manganese (0.342 mg / kg). The variability in the results obtained of the mineral elements is related to the differences in the physicochemical characteristics of the starting milk and the type of goats feeding.

(Keywords: Cheese, maturation, macro and micro minerals)

Introduction

The cheese is a universal food that is produced in almost all regions of the world; it is made with milk of different species of mammals. The FAO has drafted a code of principles in which the following definition is given: "Cheese is the fresh or matured product obtained by drainage after coagulation of milk, cream, wholly or partly skimmed milk, milk fat or a combination of these components"

It has a high nutritional value and is one of the best foods available to man, it contains chemicals components such as: fat, protein, minerals (macro and micro minerals) also vitamins such as A, B_1, B_{12}. During maturation, these vitamins are used and synthesized by the microflora in the cheese (7).

The goat cheeses have desirable characteristics and are very typical products of the Mediterranean countries (14). Variations in traditional production processes of goat cheeses produced in different countries are a true reflection of national cheesemaking traditions as well as food habits.

The goat cheese in the Murcia region of Spain has traditionally been made from goat milk of Murciano-Grenadine race, whose main characteristic is its great capability to produce milk in big quantity and quality (8). On the orders of June 11, 1990 of the Ministry of Agriculture, Livestock and Fishery, the specific regulation of goat cheeses is approved referring them as "Quality Products of Murcia". Defining as "Goat Cheese of Murcia", the cheese produced from pasteurized whole milk of goat Murciano-Grenadine race and their crosses, and the cheese production area suitable for obtaining the distinctive throughout the Region of Murcia.

Based on the above, this research was conducted with the main objective of determining during the maturation the main physicochemical parameters macro and micro mineral components of semi-matured cheese from pure goat's milk of the Region of Murcia, in order to have data that allow us to know the nutritional contribution of this product.

Materials and Methods

Semi-matured Cheese Making

The cheeses were made with pasteurized goat milk from Murciano-Grenadine race, and it was manufactured as it is traditionally made in the cheese factory. "Central Quesera Montesinos" (Jumilla), located north of the Murcia Region, Spain.

Determination of physicochemical parameters of Cheese

Dry Extract. The dry extract was determined according to IDF Standard 4th (1982). 20 g of sea sand and a glass agitator were placed in an aluminum capsule. The capsule with the sand and the glass agitator was dried in the oven to constant weight. Later 3g of cheese prepared sample were placed and it was weighted again. The sand and the cheese were grounded together, at the end of this process the capsule was placed in the oven to constant weight.

Chloride content. The method is based on the destruction of organic matter of cheese with potassium permanganate and nitric acid and on the determination of the chloride content by Argentometric titration in a solution of nitric acid in the presence of ferric ammonium sulfate as an indicator (2).

Fat. The Van Gulik acid-butirometric method was used and a gravimetric method based on the principle of Schmid-Bondzynski-Ratzlaff (SBR) as a reference method that corresponds to the FIL-IDF Standard 5B (1986).

Ashes. This method is based on putting a porcelain capsule to constant weight, 10 g of cheese were added and dried in a drying oven at 102 ± 2°C for 24 hours. Subsequently it was introduced into the muffle at 650°C for 12 hours (2)

Proteins. They were determined with the nitrogen content expressed in percentage in weight and multiplied by the conversion factor, this was performed by the method of digestion boque (microkjeldahl) (2)

pH. In a glass beaker 50 g of grinded cheese were placed, 100 mL of distilled water were added and directly measured with a potentiometer (Thermo Orion, model 420) (2)

Aw. It was determined with a LabParther model Novasina hydrometer, and it was directly measured.

Determination of macro and micro minerals

Samples of cheese (10 g) were placed in a porcelain capsule, dried overnight (8 hours) at 102°C, burned in a burner and incinerated in a muffle at 460°C. After obtaining the ashes, they were dissolved in 1 mL of concentrated HNO_3 that was evaporated to dryness on a hot plate and placed back in the muffle at 460°C until they were completely white. The residue was dissolved on the hot plate with HNO_3 (1N), filtering the resulting solution and carrying up 10 mL with HNO_3 (1N). Subsequently the macro and micro minerals were identified by atomic absorption spectrophotometry with flame. We started with a commercial dilution series (Merck) of which the calibration curves were performed using standard solutions (9).

Phosphorus. In a porcelain capsule 1 g of cheese was weighted, dried in an oven at 105°C and calcined in a muffle (539°C) until white ashes were obtained, subsequently dissolved in 2 mL of hydrochloric acid (6

N) diluted with distilled water to 100 mL and filtered. 1 mL of the prepared dilution was taken and placed in a volumetric flask of 25 mL, 1 mL of ammonium molybdate solution was added (12.5 g ammonium molybdate heptahydrate in 69.5 mL of sulfuric acid and distilled water supplemented to 500 mL) and 0.5 mL of Elon solution (1 g of Elon and 3 g of sodium bisulfite, make up to 100 mL) 25 mL completed with distilled water and mixed. We waited 40 minutes and measured the optical density in a spectrophotometer at 640 nm, the equipment used was The Bausch & Lomb Spectronic 20. % Phosphorus = m/100M Where: m = mass of phosphorus expressed in micrograms, obtained from the calibration curve. M = mass of cheese expressed in grams, used for mineralization (9).

Results and Discussion

The table 1 shows the values obtained for the variables of pH, dry extract, moisture, protein, fat, ash, sodium chloride and water activity in the cheese samples at 7, 14, 21, 28, 35 , 42 and 49 days of maturation. The pH decreased during the period of maturation due mainly to lactic acid production by lactic acid bacteria present in the starter culture used for cheese elaboration. As expected, at all times the humidity values were decreasing, because when dry extract increases, humidity decreases proportionately, reaching average values of 30.39% at the end of maturation, these data are comparable with those obtained by various authors for Tenerife cheese made from goat milk (1).

The predominant protein type in cheese are the caseins, the variability in their composition influences the production of cheese as it affects the firmness of curd and the coagulation time (5). The protein values in the cheeses elaborated present significant differences during maturation time, these increases were due to the production of peptides and free amino acids of different size resulting from the degradation of proteins, since generally the hydrosoluble nitrogen and peptides content advance proportionally with the maturation (5). The results obtained were slightly higher than the cheeses of Càdiz, Málaga, Ibores, Badaia and Majorero industrial (7), as well as semi-matured cheese of Tenerife (12).

Table 1. Mean values of pH, dry extract, moisture, protein, fat, ash, sodium chloride and water activity during maturation

	pH	Dry extract %	Moisture %	Protein %	Fat %	Ash %	NaCl %	a_w
7	5.33^b	49.97^b	52.04^b	16.63^a	28.25^a	3.19^a	1.33^a	0.978^a
14	5.15^b	57.62^a	42.38^a	19.70^b	29.75^b	3.59^b	1.34^a	0.976^a
21	5.17^b	57.64^a	42.36^a	22.07^b	32.25^c	3.67^b	1.45^a	0.974^a
28	4.86^a	59.23^a	40.77^a	20.77^b	35.00^d	3.68^b	1.53^a	0.969^a
35	4.97^a	60.77^a	39.23^a	20.45^b	37.25^e	3.69^b	1.56^a	0.956^a
42	4.78^a	61.10^a	38.90^a	23.06^c	38.75^f	3.78^b	1.61^a	0.955^a
49	4.83^a	69.61^c	30.39^c	25.24^c	40.25^g	3.92^c	1.87^b	0.950^a

a, b, c, significant differences $p < 0.05$ between treatments by Tukey technique.

The fat is an essential component due to its participation in the formation of cheese aroma and flavor (13), The results obtained show a significant advance during the maturation. This behavior is closely related to the chemical composition of the departing milk, since fat is degraded by various lipases (esterases) non-fatty acids, which can subsequently be degraded by several enzymes in aromatic components.

With respect to the percentages of ash and sodium chloride, they significantly increase during maturation, getting to similar values reported for Tenerife cheese made from goat milk (3). Under normal conditions the water activity presents a decreasing evolution along the maturation phenomena due to the drying and hydrolysis that occur (15) as the cheeses studied behaved which values decreased during the whole maturation period.

Table 2. Mean values of the macro and micro minerals during maturation

	Macrominerals					Microminerals			
	Potassium (%)	**Sodium (%)**	**Magnesium (%)**	**Calcium (%)**	**Phosphorus (%)**	**Iron mg/kg**	**Copper mg/kg**	**Zinc mg/kg**	**Manganeseo mg/kg**
7	0.054[a]	0.425[a]	0.021[a]	0.786[a]	0.398[b]	1.006[a]	0.063[a]	18.4[a]	0.092[a]
14	0.056[a]	0.325[b]	0.022[a]	0.789[a]	0.400[b]	1.343[b]	0.065[a]	18.5[a]	0.083[a]
21	0.063[a]	0.513[c]	0.022[a]	0.790[a]	0.470[a]	1.653[c]	0.073[a]	18.7[a]	0.093[a]
28	0.074[a]	0.569[c]	0.026[a]	0.794[a]	0.489[a]	2.378[d]	0.086[a]	19.4[a]	0.895[b]
35	0.136[b]	1.018[d]	0.030[a]	0.814[a]	0.486[a]	2.857[e]	0.208[a]	20.7[a]	0.307[b]
42	0.137[b]	1.200[e]	0.033[a]	0.849[a]	0.490[a]	3.087[e]	0.220[a]	23.4[a]	0.319[b]
49	0.146[b]	1.258[e]	0.035[a]	0.851[a]	0.501[ab]	3.562[f]	0.381[ab]	24.2[a]	0.342[b]

a, b, c, significant differences $p < 0.05$ between treatments by Tukey technique.

We have studied the distribution of minerals until 49 days of maturation, with the goal of meeting the retention percentage of them in the cheese (Table 2). The values of calcium and phosphorus during the maturation gradually increase obtaining values at the end of the maturation of 0.851% and 0.501% respectively; these results can be compared with those obtained for the artisanal and industrial Majorero cheese made from goat's milk (7). The data obtained for magnesium and potassium are comparable to those found for Calahorra cheese made from milk from sheep and goats mixture (6).

The magnesium is retained in the mature cheese at a much lower rate than the calcium and phosphorus, because these elements are mainly found as a part of the complex of calcium phosphocaseinate, although a part of the magnesium is linked to casein and another important ratio of 70% is in solution. With respect to the values obtained for the trace elements, they show little variability during the maturation process, this is related to small differences in the physicochemical characteristics of the starting milk and the type of goats feeding (4). It can be concluded that the values obtained from the minerals generally increase along maturation as a result of the variation in the total solids content. The mineral composition of the cheese depends on the final ratio of the milk constituents and on the clotting, draining and salting methods.

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Water Diffusivity Determination During Osmotic Dehydration Of Kiwi Fruit And Guava Chips

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The present work studies mass transfer during osmotic dehydration of kiwi fruit and guava chips in sucrose solution. Both fruits have gained interest because kiwi fruit and guava consumption contribute to an adequate antioxidant activity and to reduce the probability of contracting cardiovascular diseases and cancer. The osmotic dehydration of fruits is a relatively inexpensive preservation process that reduces product discoloration and volatile and pigments compound loss. The industrial application of this technology is still restricted because the simultaneous mass transfer phenomenon between water and sugar (or salt) have not been elucidated completely. The weight and water loss of 6 mm thick chips were analyzed at different temperatures (25 and 50 °C) and sucrose concentrations (40 and 60 °Brix), in a 2^2 experimental design with replica. The fruits maturity index (°Brix/Acidity) was measured and controlled, as well as the solutions pH (3). The analytical solution of the mass transfer model was used for describing the mass transfer in osmotic dehydration of kiwi fruit and guava chips to obtain the diffusion coefficients (sample shrinkage was considered). Diffusion model fitting gave apparent mean diffusivity values of $5.1X10^{-6}$ cm^2 s^{-1} for kiwi fruit and to $5.9X10^{-6}$ cm^2 s^{-1} for guava. An Arrhenius type expression for Diffusivity was obtained. Parameter validation was calculated with the joint interval confidence analysis for non-linear regression model. Only the interaction terms were statistically significant ($P<0.05$).

(Keywords: Diffusion, Mass transfer, Osmotic dehydration, Modeling)

Introduction

Different researches have taken place to obtain osmotic characteristic of different fruits. Kiwi fruit and guava have gained interest because kiwi fruit and guava consumption contribute to an adequate antioxidant activity and to reduce the probability of contracting cardiovascular diseases and cancer. The osmotic dehydration of fruits is a relatively inexpensive preservation process that reduces product discoloration and volatile and pigments compound loss.in sucrose solution whit 30, 40 and 50% concentration. The osmotic dehydration or osmotic drying is a solid–liquid separation process performed by removing water from a cell through the membrane and lowering the water activity on the other side of the membrane (Waliszewski et al. 2002). Compared with other drying technologies (convective drying and/or freeze drying) osmotic drying presents the advantage of preserving foods flavor, color and nutritional value and prevent cell damage, consuming less energy and with minimum initial investment and lower operating cost.

In the present study osmotic drying of *kiwi fruit and guava* is presented as an alternative to process this fruit with low energy and operation costs and that can be commercialized to remote areas and extending its shelf life.

Materials and methods

Model Building

Numerous studies performed on osmotic dehydration (Azuara et al. 1992; Mauro and Menegalli, 1995; Yao and Le Maguer, 1996; Waliszewski et al. 2002; Panagiotou et al. 1998; Salvatori et al. 1998;

Salvatori and Alzamora, 2000). Mass transfer properties of water and solute diffusivity during osmotic dehydration of food products have been modeled by some authors solving the following mass transfer differential equation for a generalized geometry (Fick's Law):

$$\frac{\partial(\rho_{d\beta} X_{\beta})}{\partial t} = \nabla.\left[D_{\beta}\nabla\left(\rho_{d\beta} X_{\beta}\right)\right] \quad \text{in } V_{\beta} \tag{1}$$

Waliszewski et al. (1997) developed a solution to equation (1) based on a non-stationary diffusion in a flat slab of well-agitated and when ratio of solute/fruit volume divided into the partition constant is superior to 4. The mathematical expression for a 1D rectangular coordinate system is expressed as:

$$\psi_1 = 1 - \frac{8}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{(2n-1)}\exp\left[-\frac{(2n-1)^2\pi^2 D_{w\beta} t}{4L^2}\right] \tag{2}$$

Moisture Diffusivity can be calculated using non-linear regression to fit experimental results of osmotic dehydration of fruits to the above non-steady state analytical solution (Eq.2).

Once the water effective diffusivity is evaluated, nonlinear multivariate regression is used to model the behavior of response variables ($D_{w\beta}$) to process variables modification (T and Solute Concentration), generating predictive equations which correlate this response with variables studied in the process. The generalized polynomial model proposed for predicting the response variables as a function of independent variables is given as

$$Y = \alpha_0 + \sum_{i=1}^{k}\alpha_i x_i + \sum_{i=1}^{k}\alpha_{ii} x_i^2 + \sum_{i=1}^{k-1}\sum_{j=i+1}^{k}\alpha_{11} x_i x_j \tag{3}$$

These predictive equation (model) can be used to estimate the expected response to combinations o factors not directly tested. For each treatment, the dependent variable was evaluated and the data submitted to a multivariate regression analysis, whose model contained linear, quadratic and interacting terms for the two independent variables. To prepare the adjusted model only the statiscally significant parameters at $P<0.05$ were considered. The validity of the model was evaluated as a function of their respective coefficients of determination as well as by an analysis of the lack of fitness. The coefficients were determined by means of the multiple regression tool of the Microsoft Office Excel software. In this case the equation (3) es rewritten as follows:

$$\ln\left(D_{w\beta}\right) = \alpha_0 + \frac{\alpha_1}{T} + \alpha_2\frac{\text{Brix}}{T} + \alpha_3\frac{\text{Brix}^2}{T} \tag{4}$$

Raw Materials

The material used was kiwi fruit and guava adquired at the local market. The fruits were washed and cut into slices of 6mm thick. All the fruits had the same maturation state, which was monitored measuring the fruits Total acidity and °Brix and maturity index. To prevent the fruits darkening a pretreatment with 0.1% w/w citric acid solution was performed. Two class commercial grade sucrose concentrations were used as hypertonic solution (40 and 50 ° Brix) and three different temperatures (25 and 50°C) the solutions pH was adjusted to 3.

Equipment

The equipment used for drying kinetics was an analytical balance Scientech SA80 (d=0.0001 g, max. 80 g), stainless steel cutter, a plastic strainer, polystyrene vessels with capacity 7 oz, spectrophotometer Spectronic 21D Milton Roy, incubator with agitation Lab Line SHAK-R-BATH mod 3580, potentiometer Hanna Instruments pH 210 Microprocessor pH Meter, a thermometer and a vernier caliper.

Experimental Design

To determine the temperature and sucrose concentration effect on the osmotic drying, a 2^2 factorial experimental design was proposed (Table 1). Sucrose concentration and temperature were the factors and each of them had three levels, which gives us 9 treatments, all of them with a replica.

Table 1. Experimental Design.

Treatment /Variable	**Temperature (°C)**	**Concentration (°Brix)**
1	25	30
2	25	40
3	50	50
4	50	30

Drying Kinetics Methodology

After selection, kiwi fruits and guavas were washed with soap and water to remove excess of gum or dirt. The fruits were peeled and cut into slices of 6mm thick and weighed on an analytic balance. Obtained slices were submerged for 1 min in an aqueous solution of 0.1% citric acid to prevent darkening. Subsequently were removed, drained and placed into the respective osmotic dryer with the appropriate conditions of temperature and concentration. The volume ration between the solid and the solution was 1:4. Subsequently all the vessels were placed with the sample in an incubator at the desired temperature with slow agitation. Slabs were removed in sequential order of every hour for 10 h of drying. Slabs were gently washed with distilled water to eliminate syrup from the surface and water residue was removed with tissue paper before weighing. Moisture content was determined by weight loss. The dimensions of each slice were monitored with a vernier caliper. Sucrose concentration of the solution was monitored using a portative brixometer.

Results and Discussion

Results of equilibrium water loss were modeled with respect to process variables using a second order polynomial equation. Parameter validation was calculated with the joint interval confidence analysis for non-linear regression model.

The estimated regression coefficients of the two independent variables, along with the corresponding R^2 value were displayed in Table 2.

Table 2. Regression coefficients of the model for kiwi fruit and guava.

	α_0	x_1	x_1x_2	$x_1x_2^2$	***Fruit***
Coefficients	-25.4859	-1557.51*	23.66	-*	Kiwi fruit
p	0.00068	0.47	0.0077		
R^2	0.9947				
Coefficients	-26.2578	0*	68.627	-0.7416	Guava
p	$5.5X10^{-31}$	-	$3.31X10^{-5}$	$1.16X10^{-5}$	
R^2	0.97				

A high R^2 (0.9947 and 0.97) indicates that the data fitted satisfactorily to the second-order polynomial model. By analysis of variance, the R^2 value of this model was determined to be over 0.9, which showed that the regression model represented adequately the behavior of the system.

Figures 1 to 2 show that the proposed models adequately represent the experimental conduct. Table 2 show that only the interaction terms of the variables (1/T) and (°Brix) are significant for a $p<0.05$ for kiwi fruit. The temperature term by itself has no effect on the response variable.

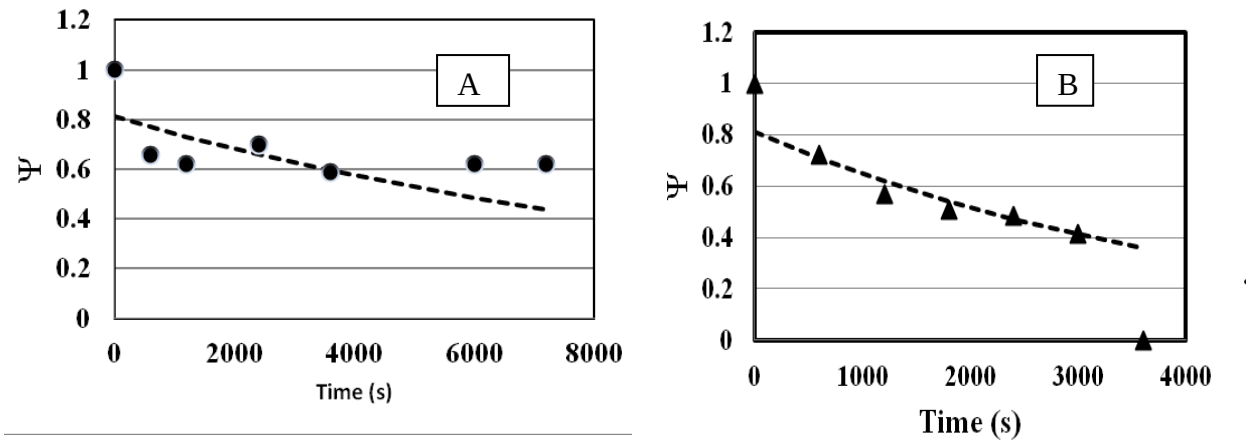

Figure 1. Experimental (♦) and predicted kinetics (- - -)of water loss during osmotic drying of kiwi fruit slabs at 60°Brix of sucrose concentration and 50°C (A) and 25°C (B).

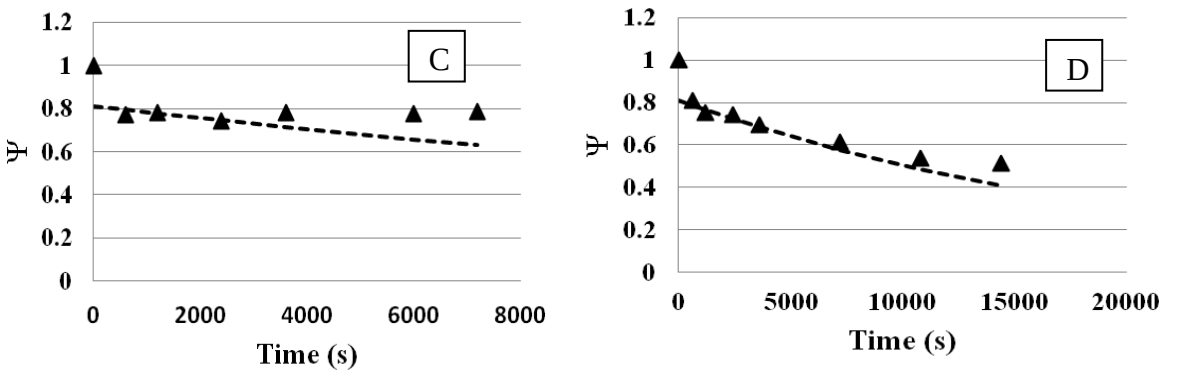

Figure 2. Experimental (♦) and predicted kinetics (- - -)of water loss during osmotic drying of kiwi fruit slabs at 25°C and 40°Brix (C) and 50°C (D).

For guava only the temperature term by itself of eq. (3) parameters wasn´t statistically significant (P<0.05). The interaction of the variables (sucrose concentration and temperature) have an effect on water diffusivity results.

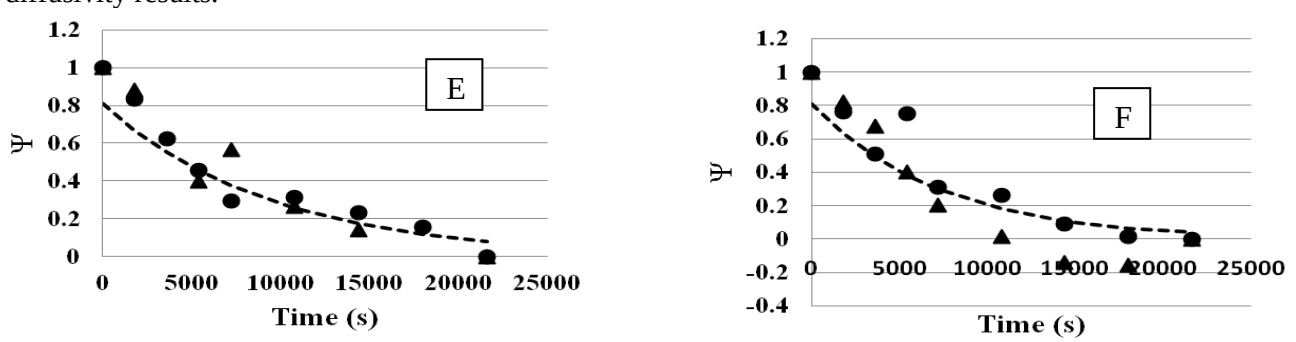

Figure 3. Experimental (♦) and predicted kinetics (- - -)of water loss during osmotic drying of guava slabs at 40°Brix of sucrose concentration and 50°C (E) and 25°C (F).

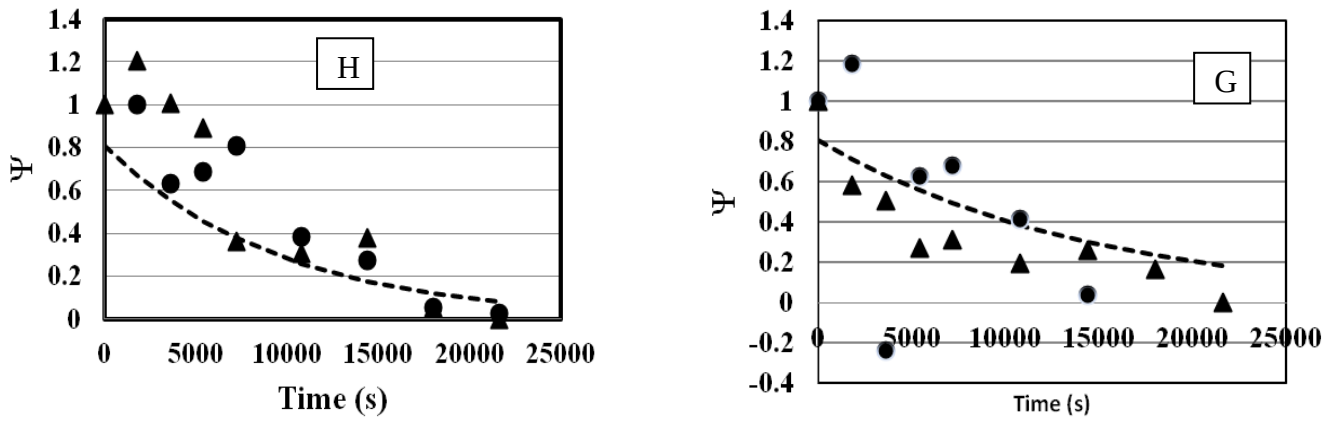

Figure 4. Experimental (♦) and predicted kinetics (- - -)of water loss during osmotic drying of guava slabs at 25°C and 60°Brix (G) and 50°C (H).

In Figure 4 the effect of temperature is evident for the same drying time a greater reduction of water content is achieved for the highest temperature. The mean value for guava and kiwi fruits water effective diffusivity are $5.1X10^{-6}$ cm^2/s and $5.9X10^{-6}$ cm^2/s respectively, this value is similar to other reported fruits like pineapple reported by Waliszewski et al. (2002) $1.717X10^{-5}$ cm^2/s and Beristain et al. (1990) $6X10^{-6}$ cm^2/s for pineapple rings.

Conclusions

In this work, it was shown that the analytical solution of mass transfer equation reproduces the moisture experimental evolution adequately. The obtained water diffusivity equations (3) shows that the osmotic drying of the kiwi fruit slices is affected by temperature and syrup concentration interaction. The obtained expressions might be used to design a fixed bed osmotic dryer or for osmotic drying optimization.

Nomenclature

A	specific surface area between phases (m^2m^{-3})
°Brix	Sucrose concentration in °Brix.
D	effective diffusivity (m^2 s^{-1})
L	Diffusion path, thickness of the chip (m)
T	temperature (°C)
T	time (s)
V	volume (m^3)
X	Independent variables
X	water content (kg water (kg dry matter)$^{-1}$)
Y	Predictive response

Greek Symbols

α	Regression coefficients
β	indicate solid phase

ρ density (kg m^{-3})

Subscripts

w indicate water

0 indicate reference state

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Changes In Pericarp During Nixtamalization Process By Using Modulated Temperature Differential Scanning Calorimetry And Differential Photoacoustic Cell Study In Situ

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Introduction

In the last decades an increasing interesting to study the physicochemical changes into the organic materials, like biopolymers and food systems; in special the study kinetics of the transformation process. The corn is the main cereal for human consumption in Latin America and the south part of United States. In this region the corn kernels is consumed mainly as nixtamalized product such as tortilla, tamales and increasing volume of snacks. During the nixtamalization process, the water diffusion and calcium into the corn kernel is one of the most important process; producing important physicochemical changes in pericarp, endosperm and germen, which are reflected in nutrimental and sensorial properties (4). During the nixtamalization process, the corn is cooked in presence of water and lime, and during the cooking there exist simultaneous calcium and water diffusion into the structures of the corn. The first barrier to this diffusion process is the pericarp and studied the calcium ion diffusion in the maize as a temperature dependent for the nixtamalization processs, and concluded that the diffusion is governed by physicochemical changes in the pericarp (4). Rojas-Molina et, al, 2009 (7) studied a photoacoustic (PA) technique that could help to the study in-situ, physicochemical changes in the corn kernels during the thermo-alkaline process. PA is a modulated technique that provides a signal that is related to the optical and thermal changes that take place in the sample as a result of specific process. Recently, (5) report the microstructural changes in the maize pericarp during the critical lime-cooking step of a traditional nixtamalization process. In order to avoid the noise and to obtain at the same time the reference and the signals from the samples, it is necessary to use a Differential Photoacoustic Cell (DPC).The objective of this investigation was to use Modulated Temperature Differential Scanning Calorimetry and the Differential Photoacosutic Cell to study in-situ the physicochemical changes that take place during the lime process mainly in pericarp of corn kernels at different temperatures for the first step of the nixtamalization process (cooking), in corn kernels with and without pericarp.

Materials and Methods

1.1. Physical properties of maize

Quality Protein Maize (H-368C) is a White dent maize hybrid, which was provide by Instituto Nacional de Investigaciones Agrícolas y Pecuarias (INIFAP) and Centro de Investigación para el Mejoramiento del Maíz y Trigo (CIMMYT). Crop management was done according to INIFAP recomendations. The ears were hand harvested on October 2011 and the grain was stored at 4ºC until use. Maize samples were cleaned by removing foreign material, impurities and broken kernels before evaluations. Clean grains

were characterized for hectoliter weight by the 84-10 method (1). The 1000-kernel weight was measured according to the methodology reported by (8).

1.2. Cooking time of the samples in a Differential photoacoustic cell process (DPC)

The cooking stage of the corn kernels was conducted according to the methodology described by (5) In the lower chamber 40g of corn were placed, 80 mL of water and 4 g of $Ca(OH)_2$ to flood the grains , including the point of the upper chamber. The cell was installed to differential photoacoustic system. The mirrors to direct the light beam aligned photoacoustic cell. When starting the experimental run the oven was heated by a temperature controller Fashion Watlow-96i/116D/N. Temperatures were monitored by two thermocouples (Type 6,302,986). At two saucers superior quartz windows make them reach the excitation source of 532 nm from a laser COHERENT Compass 415M model. The radiation of the laser was modulated at 5kHz through an opto-acoustic modulator (ISOMET model 232A-2). The laser beam is split into two beams: 60 and 40% by a filter and only the pericarp was issued. The output signals depended on the amplitude and phase signals were amplified in a lock-in (OPAM-TL084) and connected to an interface SRS 830 (6). The samples were run independently at 92, 82 and 72 °C. The cell was asylee with a jacket to prevent heat transfer with the environment. The heating rate started at 22 °C with a heating rate of 2.5 °C / min, up to 92 °C. The second ramp maintenance is an isotherm was maintained for 25 minutes. Subsequently, different samples were run at 80 and 70 °C isotherm times 40 and 65 minutes respectively.

2.3 Simulation of the cooking step in differential scanning calorimeter (MDSC*)*

Pericarp samples were removed from native corn grain previously hydrated. Subsequently, dried, cut flakes or plates of 4 mm diameter with a stainless steel punch. The pericarp foil was placed on the tray. Were added 3.4 ml of alkaline solution of $Ca(OH)_2$ prepared at 0.3% based on the amount of calcium that the pericarp fixed during firing (4). The sample was sealed and placed in the differential scanning calorimeter (DSC-Q100) TA. Instruments with a mechanical cooling system (RCS), and using nitrogen as carrier gas at 80 mL / min in the cell and 25 mL / min in the RCS. The equipment was calibrated with the methodology proposed by (2). Indium and mercury was used to -38 °C and 158.4 for cell constant and temperature. For a constant Cp was calibrated with sapphire. The corresponding values were: cell constant = 1.0692, constant slope Cp = 1.1711 and 0.0016 baseline. Standardized the team, the run began. The method applied to each sample to the same fuel DPC.

2.4 Characterization by low vacuum scanning electron microscopy (LV-SEM)

For each sample was analyzed in an LV-SEM, JSM 5600LV, with a resolution of 5 nm in low vacuum and fitted with an energy dispersive X-ray spectrometer (Noran instrument, model Voyager 4.2.3). Prior to the analysis, the maize samples were fixed on an aluminum specimen holder with carbon tape. The analysis was performed employing a 20 kV electron acceleration voltage and a pressure of 12–20 Pa in the specimen chamber, thus obtaining images on the fracture surfaces with the backscattering electron signal. The micro-compositional analysis of the pericarp was carried out, using an Energy Dispersive Spectrometer. The pericarp thickness of 10 samples was measured in 25 different regions. Each sample was turned to move the focus position of the microscope. Further, surface views of isolated pericarps were taken to obtain the micrographs.

2.5 Statistical analysis

In order to assure reproducibility of samples, it was necessary to calculate the relative coefficient of variation by the ratio of the coefficient of variation for temperature values observed in an experiment and the coefficient of variation of temperature values observed in the reference sample. The reference sample showed the lowest value of the relative coefficient of variation.

Results and Discussion

3.1 Physical properties of maize samples

QPM H-368C samples showed a weight in hectoliters of 74.3 kg/ hl and a 1000-kernel weight of 289 g. These values are similar to those reported by (8). for a commercial yellow food-grade corn with

outstanding alkaline-cooking properties, a yellow quality protein maize and a white quality protein maize. These authors mention that QPM varieties with these physical properties can be processed into good table tortillas by altering the cooking conditions, since QPM grains do not meet all the optimum properties that are required for alkaline processing of maize; for example: a test weight of at least 77.3 kg/hl and a 1000-kernel weight of at least 300 g. The ratio of hard to soft endosperm was 4:1 indicating that QPM H-368C contains a much larger portion of hard endosperm. (9) reported that QPM varieties require a longer cooking time, since they have a greater amount of corneous endosperm than that of the normal maize. Nevertheless, according to (8) the reduced kernel size of QPM, as in the case of QPM H-368C, shortens its optimum cooking time

3.2 In situ photoacoustic studies in pericarp

Figure 1 shows the photoacoustic signal from the differential photoacoustic cell directly related to the changes that occur in the pericarp during the cooking step to temperatures of 72, 82 and 92 °C respectively. This graph shows the photoacoustic signal as Log-Log to have a better view of the process.

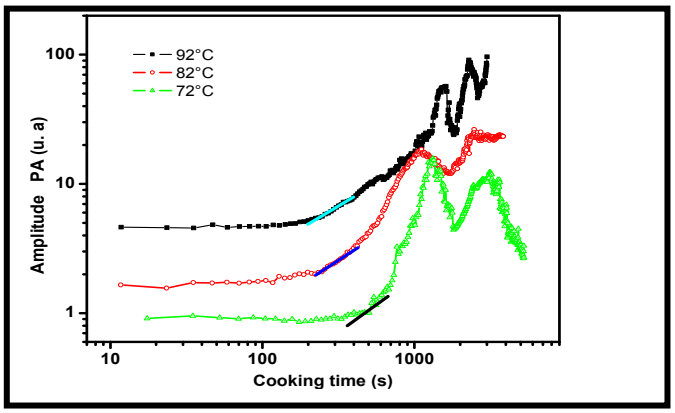

Figure 1. Pericarps photoacoustic signals during cooking step

For short cooking time, we did not detect any significant change in the behavior of the signal, indicating that there were no changes in the corn kernels. This part of the figure shows directly that the corn is impervious to the alkaline solution for short times at lower cooking temperatures. The figure 1 shows two peaks that are detected with greater intensity at 92 °C. The speed at which the second peak appears also was most notable at this temperature.

These peaks represent a set of changes that could be kinetic or structural type. This can be supplemented with the results of differential scanning calorimetry. The first change in the signal is because the water diffusion begins and calcium removes the waxes or fats in the epidermis, which is in the outermost layer and that made impermeable. This change required about ten minutes; subsequently a different peak appears, probably due to a set of transformations, calcium compound formation, pectin dissolution present in the cell wall of the pericarp. In the middle lamella and the inside wall the presence of alkali form thermo-reversible gels with Ca_2 ions. The alkali promotes hydrolysis of esters such as the glycosidic bonds, according to that reported by (3). The second peak represents another set of changes, uronic acid neutralize the hemicellulose and break the diferulics bridges and, detachment of lignin and hemicellulose from the pericarp. These modifications in the pericarp, cause changes in its morphology, its degradation is more noticeable at higher temperature, which is evident in the micrographs obtained by scanning electron microscopy.

3.4 Simulation of the cooking step in the differential scanning calorimeter (MDSC)

Figure 2a shows the thermogram of heat flow versus time obtained in situ changes, by MDSC. These studies were performed with respect to time for comparison with those made in photoacoustic cell.

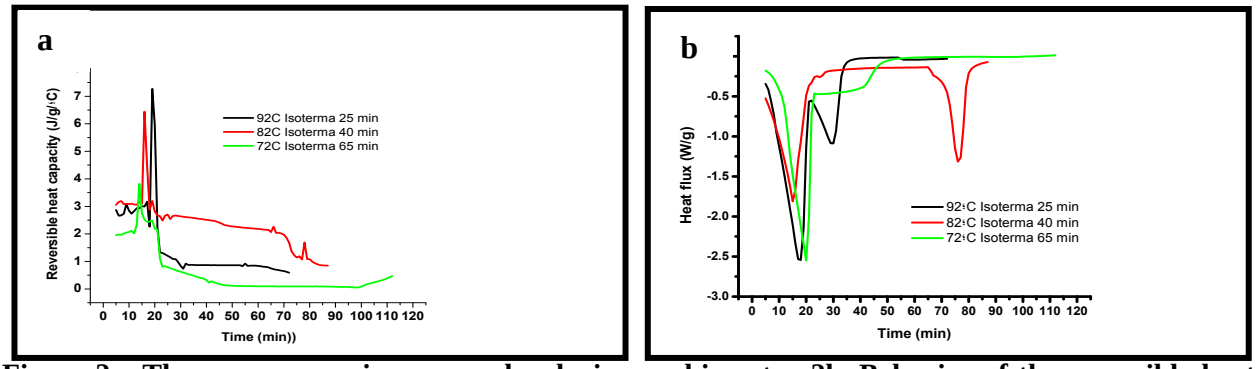

Figure 2a. Thermogram pericarp samples during cooking step 2b. Behavior of the reversible heat capacity isothermal zone

In the first stage, can be seen in the thermogram a period between ten and twenty minutes of warming, is a fall where an endothermic transition occurs. This is for the three temperatures 72, 82, 92 °C. The transition temperature range occur from 25 ºC to 60 ºC, only pericarp components can be affected at those temperatures that was pectic substances and fats in the outer layers of corn pericarp. The peak time succeeded 17.49, 15.33 and 20.16 minutes, corresponding to the three temperatures 72, 82, and 92 °C. In the second ramp or isotherm is also seen another endothermic transition at 82 °C, the transition at this stage is more pronounced, probably because the isotherm has a duration of 40 minutes, giving you time to calcium hydroxide to react with the pericarp components, while the isotherm is short at 92 °C in 25 minutes. In addition the isotherm duration to the 72 °C is 65 minutes. Therefore, the temperature is not sufficient to produce changes in the pericarp components, for that reason can be hardly perceive a very smooth transition. The changes in this transition had to do with the neutralization of uronic acids and phenolic acids. Modifications such as breaking the diferulics bridges, hemicellulose unions can be cause rupture of the fibrous matrix and consequently the defoliation of the pericarp, which is shown in the micrographs in figure 3. The MDSC, for isothermal experiments, show as zero in the reversible heat flow, since the heating rate is zero, this means that in the cooking step in the isothermal zone no changes in Cp therefore no changes in the structural components of the pericarp. Figure 2b shows the behavior for reversible heat capacity, wherein the maximum time shift values are 8 J / g / °C initial value of 2 to 3.5 J / g /ºC. This shows structural changes on the reaction product of the alkaline hydrolysis and thermal components in pericarp. In all cases for temperatures of 72, 82, and 92 °C, the structural changes occurring during the heating ramp, before reaching the isothermal temperature maintenance. This is important because during nixtamalization process, corn must be added together with water and alkali, so that in combination with the time and temperature to achieve the action of thermal and alkali hydrolysis, acting particularly on the pectin content, starch pericarp, waxes and the uronic acid neutralization.

3.3 SEM microstructural changes as a function of the thermoalkaline treatment

Figure 3 shows micrographs for native pericarp and obtained at the end of the cooking stage. 3 a) pericarp native 3b) Pericarp treated at 72 °C, 3c) Pericarp treated at 82 °C, 3d) Pericarp treated at 92 °C.

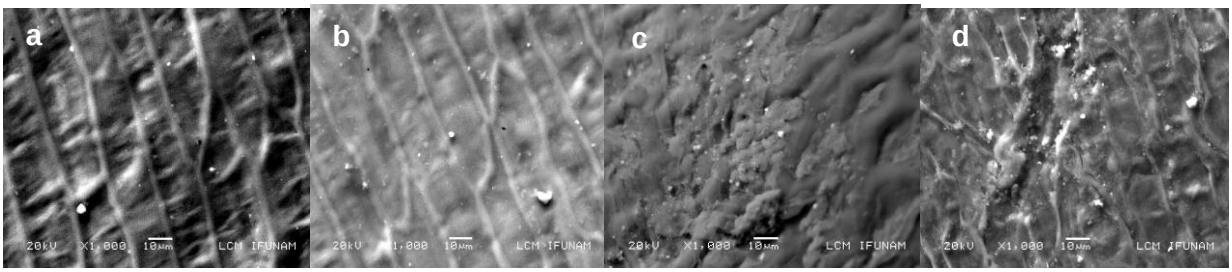

Figure 3. Micrographs corn pericarp, 4 a) native pericarp, 4 b) treatment pericarp 72ºC, 4c) treatment pericarp 82ºC, 4d) treatment pericarp a 92ºC

In Figure 3a, pericarp native native sample integrity. Figure 3b shows the micrograph taken at the end of the cooking step conducted at 72 °C, and the gloss is not observed characteristic of the outer layer of the pericarp. The panels routed fibers of cellulose and hemicellulose are less pronounced. Figure 3c processed pericarp temperature of 82 °C can be observed that the corn pericarp undergoes changes in morphology, lost the brightness in the structure; temperature obviously affects the waxy layer of the pericarp. Furthermore, the cellulose fibers and hemicellulose fibers are completely swollen, have increased considerably in size, this is due to water absorption the calcium, which soften fabrics and allowed to expand the fibrous matrix that constitutes the pericarp but no tissue breakdown. When compared with the micrograph of Figure 3d pericarp obtained corresponding to the end of the cooking step at 92 °C. In this figure we can say that the cellulose and hemicellulose fibers are not as swollen, but the pericarp tissues are torn. This is because the calcium reacts with components present and caused the degradation of the structure.

Conclusions

The pericarp is not a homogeneous structure. Consequently, water and calcium diffusion might have different diffusion mechanisms. The lime treatment promotes waxy thin layer removal, which allows the simultaneous entry of calcium and water into the pericarp at the beginning of the nixtamalization process. The interaction between the corn, water and temperature promotes the hardness pericarp reduction and not structure transformations. The lime addition, promotes structural changes into the pericarp and the calcium diffusion onto the pericarp and, as a consequence the starch change. The DPC technique, show that the structural changes in the pericarp is possible to be measure in situ and, the changes in signals (amplitude and phase) was only attributed to the pericarp changes. The evaluation of corn kernel without pericarp does not have the same response as the corn kernel with pericarp, because the starch is transparent to the laser radiation, so the PA signals were due to structural changes in the pericarp. The analysis showed that higher MDSC structural changes occurred at 92 °C, which was evidenced by the micrographs.

Acknowledgements

This work was partially supported by projects PAPIME (No. PE203711) Y PAPIIT No. (IT231511) UNAM, México. We thank Posidia Pineda Gómez Facultad de Ciencias Exactas y Naturales, Universidad de Caldas, Manizales, Caldas, A.A.275, for giving us the facilities to use the laboratories installations and equipment MDSC.

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Effect Of Temperature And Maltodextrin Addition On The Anthocyanins Stability During Storage Of Capulin Juice

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Abstract

The purpose of this work was to improve the preservation of anthocyanins adding maltodextrin 10 DE to the capulin juice. Fresh capulin juice (CJ0%MD) and capulin juice with 8%(CJ8%MD), 10%(CJ10%MD) and 12%(CJ12%MD) of MD10 were processed by sonication during 30 min (pulses of 5 s). After this treatment, all capulin juices were stored at 8, 15 and 25ªC, taking samples during during 21 d to determine color, anthocyanins content and transversal relaxation times T2. Color degradation was lower for CJ10%MD than for CJ0%MD. Results indicated that the degradation of anthocyanins followed first-order reaction kinetics. Highest values of anthocyanins retention and the lowest values of T2 were found in the CJ8%MD, CJ10%MD and CJ12%MD samples. After 21 d of storage at 25°C the retention of anthocyanins was 83 and 58% for CJ10%MD and CJ0%MD, respectively. Higher temperatures increased the anthocyanins degradation. Maltodextrin represents a promising biopolymer that could improve the preservation of anthocyanins during storage of juices.

(Keywords: capulin juice, anthocyanins, maltodextrin, storage)

Introduction

The elaboration and processing of liquid food like fruit juices have to be carried out under hygienic conditions that ensure final product safety. Treatments on food products are carried out to extend its shelf life, but above all to ensure its safety for consumers. Thermal technologies are the most widely applied to achieve these purposes, but these treatments have a negative effect on certain components of the food itself, reducing its vitamin content and other nutrients, as well as sensory features that make them less attractive in terms of color and textural properties. Non-thermal technologies are an alternative to thermal treatment that is being studied and developed in order to obtain a better final product sensory quality, but without neglecting microbial safety. In this way, these alternatives to thermal technologies can produce food products without enzymes and harmful microorganisms, while maintaining nutritional characteristics and minimizing the loss of quality in terms of flavor, color and nutritional value (1). The application of ultrasonic waves generating cavitation in suspension which contain microorganisms and enzymes often has a lethal result and deactivating action (2). Ultrasound amplitude at ≥40% (≥80 W) combined with moderate temperature (50 °C) resulted in up to 6 log inactivation of yeast in orange juice after 15 min treatment (3).

The Capulin is an endemic fruit in Los Tuxtlas and its latin name is *ardisia compressa*. Capulin juice is a good source of health related compounds such as anthocyanins, thus having high antioxidant activities. Anthocyanins is a widespread group of plant phenolic compounds which have been regarded as a natural alternative to replace synthetic food colorants. Recently, increased attention has been given to their potential health benefits in preventing heart diseases and cancers due to their powerful antioxidant properties (4). It has been demonstrated that heat degrades anthocyanins, diminishing the nutritional quality and the antioxidant properties of juices (5). Processed fruit juices have lower nutritional value than their respective fresh commodities due to the loss of antioxidant compounds during processing and storage.

The purpose of this work was to obtain a healthy and safety capulin juice using ultrasound and to improve the anthocyanins retention during storage by adding different concentrations of maltodextrin.

Materials and Methods

Materials.
Fresh capulin fruit with no apparent physical or microbial damage were purchased in the city of San Andrés Tuxtla, Veracruz, Mexico. Maltodextrin (with 10 DE) was purchased from Productos Zave, S.A de C.V. (Xalapa, Ver., Mexico).

Preparation of the capulin juice.
The fruit pulp was separated from the seed and squeezed in a juicer. Next capulin juice was clarified in a centrifuge. Finally, the juice was diluted adding water to obtain a product with 10°Brix. This sample of clarified capulin juice (CJ) was divided in four batches with different concentration of maltodextrin (MD): 0% MD (CJ0%MD), 8% MD (CJ8%MD), 10% MD (CJ10%MD) and 12% MD (CJ12%MD). All batches were treated with ultrasound (Branson Ultrasonic Corporation, model 250D) using an amplitude of 70% (1500 W) with moderate temperature (55°C) during 30 min (pulses of 5s). After this treatment, all batches were stored at 8, 15 and 25°C, taking samples during 21 d to determine color, anthocyanins and transversal relaxation times T2.

Color changes.
Color Flex CX1115 HunterLab colorimeter was used to determine the changes with storage time of the L*, a* and b* color parameters in the CIELab scale. The total color difference (ΔE) with respect to the fresh capulin juice was calculated by using the following equation:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2} \qquad (1)$$

Anthocyanins.
The anthocyanins content of capulín juice was determined with the pH differential method described by Wrolstad et al. (6), using two buffer systems: potassium chloride 0.025 M at pH 1.0, and sodium acetate 0.4 M at pH 4.5. The absorbance of the mixtures at pH 1.0 and 4.5 was then measured with a spectrophotometer at 517 and 700 nm against distilled water blank. The anthocyanins content was calculated according to Equation 2:

$$TA = \frac{[(A_{517} - A_{700})_{pH1.0} - (A_{517} - A_{700})_{pH4.5}] \times MW \times DF \times 1000}{\varepsilon \times L} \qquad (2)$$

where: TA is the total content of anthocyanins, A is absorbance, MW is the molecular weight of cianidin-3-glucoside (49.2 g/mol), DF is the dilution factor, ε is the molar extinction coefficient for cianidin-3-glucoside (26900 L/mol.cm), and L is the pathlength in cm.

Simple first-order model.
Simple first-order kinetics have been commonly used to fit thermal degradation of anthocyanins in juices and nectars as a function of treatment time or storage time (7,8). In this way, the degradation of anthocyanins determined in all batches of capulin juice was described with a first-order model, as follows:

$$\ln (TAt/TAo) = - k_1 t \qquad (3)$$

where: TAt is the total content of anthocyanins at time t, TAo is the initial total content of anthocyanins, k_1 is the first-order kinetic constant and t is the storage time.

Relaxation times T2.

Samples of capulin juice were placed in 10-mm-diameter NMR tubes. Sample height within the tubes was maintained at 4 cm and the equipment temperature was let to equilibrate for 5 min before proceeding with the measurements. Relaxation times T2 (spin-spin) and 1H (spin-echo and inversion recovery) measurements were done with NMR Bruker minispec (mq20, Bruker Optik Gmbtt, Rheinstetten, Germany) equipment. The pulse sequence method of Carr-Purcell-Meiboom-Gill (CPMG) is the most commonly used for determining T2. Each measurement was performed by establishing 100 points, pulse separation of 2.6 ms, silent echo 4, scans 4, and temperature of 25°C. The gain was adjusted in order to optimize the signal/noise ratio. T2 values were calculated using the NMR software. Each sample was measured three or four times.

Results and Discussion

Figure 1 shows the degradation kinetics of anthocyanins at 8, 15 and 25°C for the capulin juice stored without maltodextrin (CJ0%MD). As can be observed, an increase of storage temperature produces higher anthocyanins degradation rates. In the juice CJ0%MD, the first-order kinetic constans were 0.0044 d^{-1}, 0.0141 d^{-1} and 0.0307 d^{-1} for 8, 15 and 25°C, respectively. The anthocyanins retention in the capulin juice without maltodextrin was 94% at 8°C, 81% at 15°C and 58% at 25°C, after 21 d of storage.
Anthocyanins retention at 25°C for capulin juice mixed with different concentrations of maltodextrin, can be seen in the Figure 2. Maltodextrin produced a non-linear protective effect on anthocyanins, where 10% was the optimum concentration. This Figure 2 also shows a lower anthocyanins degradation in all capulin juices with maltodextrin, producing a 65% of retention in CJ8%MD, 83% in CJ10%MD and 63% in CJ12%MD, for a storage temperature of 25°C.

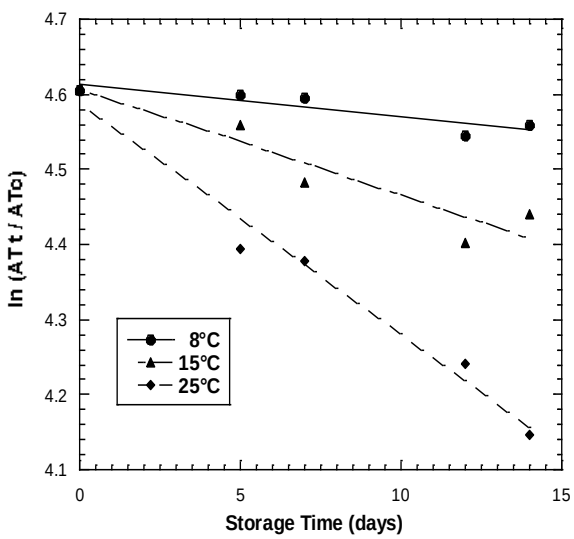


Figure 1.Degradation kinetics of anthocyanins for capulin juice without maltodextrina

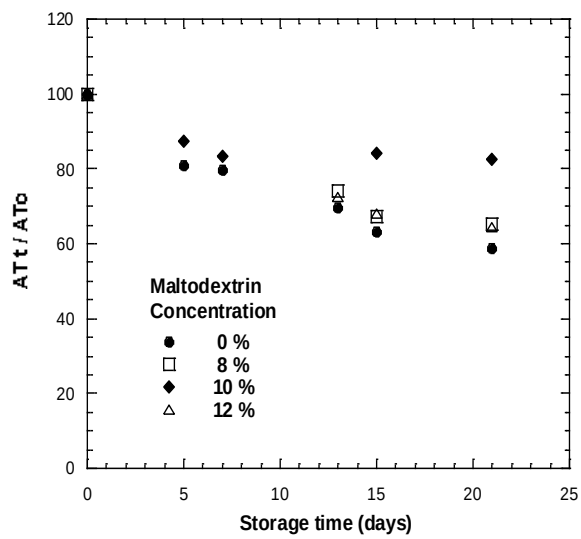
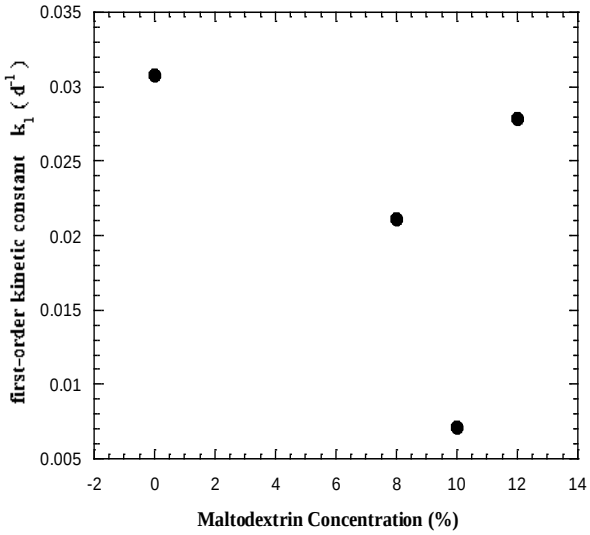


Figure 2.Anthocyanins retention in capulin juice as a function of maltodextrin concentration.



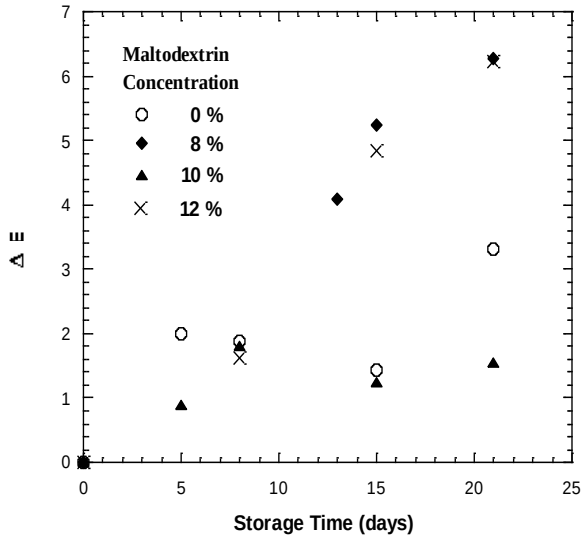


Figure 3.Kinetic constants at 25°C as a function maltodextrin concentration.difference for

Figure 4.Changes of total color of capulin juice with different maltodex-trin concentration.

All experiments of anthocyanins degradation during storage were described with a first-order kinetic model. Figure 3 depicts the values of the first-order kinetic constans as a function of the maltodextrin concentration, at 25°C. Increasing the maltodextrin concentration the first-order kinetic constant decreased until reach a concentration of 10%, point of maximum protective effect, where the tendency changed to an opposite way. The existence of an optimum value for maltodextrin concentration, suggests a protective mechanism in which at first maltodextrin molecules interact with water molecules in the juice to protect the anthocyanins against degradation reactions. However, for concentrations higher than 10%, the increasing viscosity of the juice favors a new interaction among maltodextrin molecules, it which generates steric effects that diminish the protective role on anthocyanins from this biopolymer.

Another quality parameter for the juice is its color, where some changes during storage can be related to the enzymatic activity. In Figure 4, the changes of total color difference (ΔE) for all batches of capulin juice are plotted as a function of storage time, at 25°C. As can be observed, capulin juice color was protected by maltodextrin addition, showing the lowest values of ΔE for the concentration of 10%.

In order to acquire more information to explain the protective mechanism induced by maltodextrin, transversal relaxation times T2 (spin-spin) were measured during 21 d of storage. The addition of maltodextrin to the capulin juice decreased the transversal relaxation times T2 of water molecules (Figure 5). Besides, T2 relaxation times for water molecules in the juice CJ10%MD were almost constans during 21 d of storage, suggesting a higher stability for this liquid system.

Conclusions

The addition of maltodextrin 10DE to capulin juice improved the anthocyanins retention in this product during storage. Probably the protective mechanism is a consequence of the lower mobility of water molecules produced by the interaction among biopolymer-water-anthocyanins.

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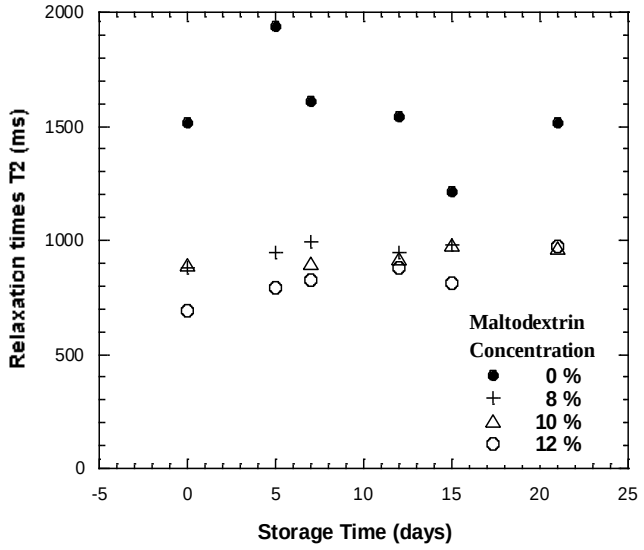

Figure 5. Variation of relaxation times T2 in capulin juice with different maltodextrin concentrations.

Improving Physical Stability Of Sweet Potato Starch Films By Adding Nanostructured Alginate

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Abstract

The effort to extend the shelflife of food and the search for solutions to reduce the environmental pollution has directed the research to the exploration of new materials such as edible film to replace the synthetic polymer packings.In this work two types of films were produced: one from sweet potato starch (SPSF) and other from nanostructured alginate 33.33% / sweet potato starch 66.67% (NASPSF). Adsorption isotherms of SPSF and NASPSF were determined at 15, 25 and 35 °C in a water activity range of 0.1 to 0.9 using the static gravimetric method. The moisture content corresponding to the micropore volume (pore diameter< 2 nm) was obtained using the Dubinin-Radushkevich equation. Tensile tests were performed with a texture analyzer TA-XT2 for each water activity studied. Longitudinal relaxation times (T1) were quantified using a low-field NMR Bruker Minispec.The differential and integral thermodynamic properties were calculated with the method of Othmer-Hill. Minimum integral entropy of water molecules occurred at moisture content of 6.8 % d.b. (a_w= 0.33) and 17.3 % d.b.(a_w= 0.63) for SPSF and NASPSF, respectively. Moisture content (MC) corresponding to the micropore volume (7.2 % d.b. for SPSF and 16.9 % d.b. for NASPSF) and MC for the minimum integral entropy were very similar. In both films, the maximum resistance to the fracture and the minimum longitudinalrelaxation time were observed at the minimum integral entropy. The NASPSF films showed lower water vapor permeability and water solubility than SPSF films. The results of this work suggest that the integral entropy is an useful parameter for predicting physical stability of edible films.
(Keywords: integral entropy, nanotechnology, edible film)

Introduction

Currently, the world produces 200 million tons of non biodegradable garbage. Of the total production of plastics, 12 million are earmarked for packaging(1).Plastics are made from synthetic polymers, which are not biodegradable. For this reason it is necessaryto bring to market edible films in order to reduce the environmental pollution.

Starch edible films are a great alternative to preserve food since starch is a biodegradable polymer, cheap, abundant in nature and easy to obtain (2). They have good mechanical properties and good barrier properties to gases(3,4).However the use of starch edible films is limited because the relative humidity affect their mechanical properties producing very brittle films in dry environments, and films with low elasticity and high permeability in wet environments (5) (6).

It is possible to preserve the foods with high moisture contents by increasing the number of micropores (7). So a solution to this problem is increase the number of micropores in the edible films to control the adsorption process. Thus, it is possible produce biodegradable films with higher moisture content without destroy their structure, because the stability of a food depends of its moisture sorption characteristics (8).

The objective of this work was to produce nanostructured starch edible films by increasing the number of micropores in order to increase their stability and to improve their mechanical properties and permeability, during storage in wet environments.

Materials and Methods

Materials. Starch was obtained from sweet potatoes purchased in Xalapa, Ver. Calciumchloride, sodium alginate, andglycerol were obtained from Sigma Aldrich (Mexico).

Preparation of SPSF

3g of sweet potato starch were dispersed in 100ml of distilled water, stirred for 5min at room temperature and then heated to 85°C for 20min, was added glycerol6% (w/w starch dry base) and stirred for 10min. 10ml of this suspension were placed on plastic dishes and dried at 45°C.

Preparation of NASPSF

Sodium alginatesolution 2% (w/v)was spraying inasolutionof calcium chloride 2% (w/v)at a pressure of35psi to obtain calcium alginate particles. Excessof calciumchloride was separated bycentrifugation at 1500rpm in a time of5min.The obtained particleswere spray-dried withinlet temperature of200°Candoutlet temperature of 110°C. After obtainingthis powder, PANACfilmswere madein a same way asPACfilms,usinga ratio ofalginate-starch potato1:3.

Adsorption isotherms

Samples of the SPSF and NASPSF were placed in desiccators containing P_2O_5 for 3 weeks at room temperature.Samples were placed in separatedesiccators containing saturated salt slurries in therange of water activity from 0.11 to 0.85 using the $a_w$reportedby Labuza (9). The samples were held at 15, 25, and35 °C until equilibrium was reached.Equilibrium was assumed when the difference between 2consecutive weightings was less than 1 mg/g of solids. Thetime to reach the equilibrium varied from 8 to 14 days. The Guggenheim-Anderson-De Boer (GAB) equation wasused in modeling water sorption(10).

$$M = \frac{M_o C K a_w}{(1 - K a_w)(1 - K a_w + C K a_w)}$$

where a_w is water activity; *M* is water content of the sampleon dry basis; *Mo* is the monolayer water content; *C* is theGuggenheim constant = *C'exp (hm-hn)/RT*; *hm* is the heat ofsorption of the first layer, *hn* is the heat of sorption of themultilayer, *K* is the constant correcting properties of multilayermolecules with respect to bulk liquid = *k' exp (h1-hn)/RT*; *h1* is the pure water heat condensation; *T* is the absolute temperature; and *R* is the gas constant. The parameter valuesof GAB equation (*Mo,C,and K)* were obtained by nonlinearregression analysis. The integral entropy was calculated.

Mechanical properties

Tensile tests were performed in SPSF and NASPSF films with a texture analyzer TA-XT2 for each water activity studied at 25°C and the maximum resistance to the fracture was obtained.

Micropores volume

The moisture content corresponding to the micropore volume (pore diameter< 2 nm) was obtained using the Dubinin-Radushkevich equation (11).

Water vapor permeability (WVP)

Water vapor permeability was determined by the method ASTME96-90 reported by Westling (12).

Solubility in water

Solubility in water was determined by the method Bertuzzi (13) with some modifications.

RMN

Longitudinal relaxation times (T1) were quantified using a low-field NMR Bruker Minispec.

Results

Fig.1 and Fig. 2 show the results of integral entropy of the sweet potato starch films (SPSF) and the nanostructured sweet potato starch films (NASPSF) in a water activity range of 0.1 to 0.9. Maximum resistance to the fracture of SPSF and NASPSF is showed in Fig. 1 and Fig. 2, too. Minimum integral entropy of water molecules occurred at moisture content of 6.8 % d.b. (a_w= 0.33) and 17.3 % d.b.(a_w= 0.63) for SPSF and NASPSF, respectively, so NASPSF can be used in products with higher moisture contents than SPSF and NASPSF can be storage in relative humidities from 50% at 70%; however SPSF can be storage in relative himidities 33%.The maximum resistance to the fracture was observed at the minimum integral entropy in both films.

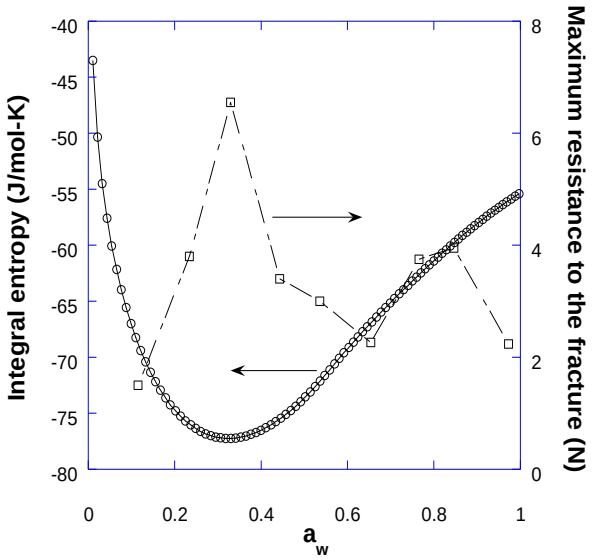

Fig. 1 Integral entropy and maximum resistance to the fracture of SPSF

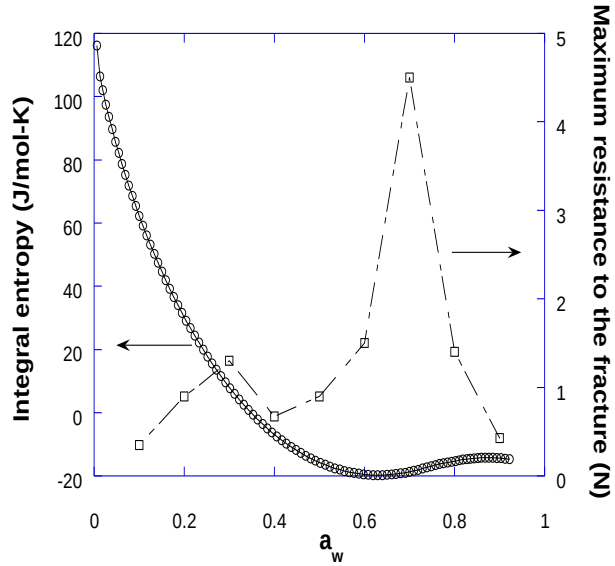

Fig. 2 Integral entropy and maximum resistance to the fracture of NASPSF

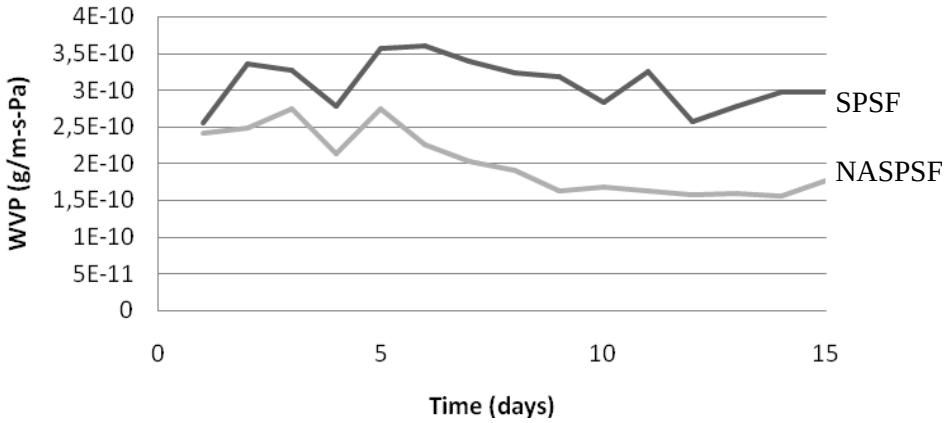

Fig. 3 Water vapor permeability (WVP) of SPSF and NASPSF equilibrated at relative

Fig. 3 in the previous section, showthe water vapor permeability of SPSF and NASPSFequilibrated at relative humidity of minimum integral entropy and 25 °C. These results show that NASPSF had low water vapor permeability than SPSF

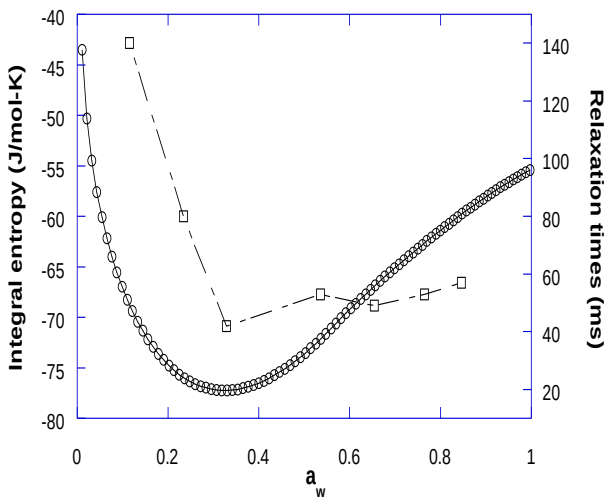

Fig. 4 Integral entropy and longitudinal relaxation time (T1) ofNASPSF

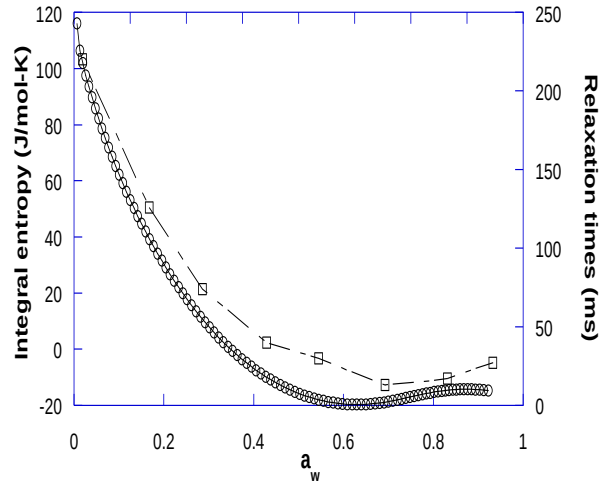

Fig. 5 Integral entropy and longitudinal relaxation time (T1) of SPSF

Figures 4 and 5 show the longitudinal relaxation times and the integral entropy for SPSFand NASPSF, the relaxation times were observed in the minimum integral entropy. This indicates that the better arrangement of water molecules is in the minimum integral entropy because the water molecules are occupying the available micropores volume and there is less molecular mobility.

Table 1 shows the moisture content corresponding to the minimum integral entropy and moisture content for micropores volume (7.2 % d.b. for SPSF and 16.9 % d.b. for NASPSF). These results were very similar. Increasing of the micropores number increased the adsorption of water in low water activities, so the films were more stable at higher relative humidities.

Table 1 Moisture content in the micropore

Edible films	Temperature	Minimum integral entropy (g water/100g s.s.)	Volumeof micropores (g water/100g s.s.)
SPSF	15°C	7	7.4
	25°C	6.8	7.2
	35°C	7	7.49
NASPSF	15°C	19.7	19.45
	25°C	17.3	16.9
	35°C	19.8	17.8

Conclusions

Nanostructured films of sweet potato starch were stable in higher humidity environments than the non nanostructured films.

NASPSF showed less water vapor permeability and solubility in water than SPSF.

The thermodynamic method is a good tool to determine the physical stability of edible films because the longitudinal relaxation times and the maximum resistance to the fracture were performed in the water activities where occurred the minimum integral entropy.

The lower molecular mobility was presented in the minimum integral entropy, where the water molecules have the better arrangement and this happened when the available micropores are filled.

Thephysical stability of edible films was improved because these were more stables in a wider range of relative humities (50 % – 75%) due to increased nanocavities.

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Protective Role of Calcium Alginate Nanostructured Matrices on Ascorbic Acid Preservation

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The objective of this work was to study the stability of the ascorbic acid (AA) encapsulated by coacervation into a matrix of calcium alginate, modifying the microstructure, but conserving the same chemical composition of the food matrix. Three powders were obtained: a) AA encapsulated into non-nanostructured matrix of calcium alginate and then freeze dried (NNAA) b) AA encapsulated into a nanostructured matrix of calcium alginate and then freeze dried (NAAFD), and c) AA encapsulated into a nanostructured matrix of calcium alginate and then spray dried (NAASD). Adsorption isotherms were determined at 15, 25 and 45 ° C in a range of water activities (a_w) 0.103-0.892, using the gravimetric method with saturated salt. The integral thermodynamic properties were calculated with the Othmer-Hill method. The longitudinal relaxation times (t_1) were quantified using a low-field NMR Bruker Minispec. The mechanical properties of the powder were measured by compression tests using equipment TA-XT Plus. The retention of ascorbic acid was evaluated after 30 and 60 days of storage with a second derivative spectrophotometric method. The minimum integral entropy of water molecules occurred at a_w = 0.230 in the non-nanostructured powder, a_w = 0.706 in the nanostructured powder and then freeze dried, and a_w = 0.580 in the nanostructured powder and then spray dried. For the three powders, the highest values of retention of ascorbic acid, the lowest values of t_1 and the highest values of Fmax, were found in the a_w corresponding to the minimum integral entropy. The results showed that the nanostructuration of the food matrix could be a good option to conserve the AA during storage.
Keywords: (ascorbic acid, minimum integral entropy and nanostructured matrix).

Introduction

The great progress made in the application of nanotechnology in the food area, increase the possibility of using its principles to the production of food materials that enable controlled interaction between water molecules and microstructure. Water is the most important constituent of the food, affect food safety, stability, quality and physical properties. Nanoscale studies have shown that the behavior of water in nanospaces is different than at the macroscopic level, which favors the stability of foods during storage (1). Also recent researches has shown that minimum integral entropy can be considered as the point in which food has its maximum stability and sometimes does not correspond to the value of monolayer or glass transition temperature, proposed by many authors as the point of maximum stability (2). This thermodynamiccriterion has been confirmed experimentally, showing that the best humidity conditions for preservation of food, is the minimum integralentropy of water (1, 2, 3). Azuara and Beristain (3) also showed that the moisture content corresponding with the minimum integral entropycoincidesto the micropore volumen calculated with the Dubinin-Radushkevich model, where the water adsorption process is controlled by entropy when the molecules are adsorbed in the micropores. The objective of this work was to study the effect of the food microstructure on the chemical stability of ascorbic acid and the texture changes when the product was stored at different relative humidities.

Methods and Materials

Materials: Sodiumalginate, calcium chlorideand ascorbic acidwere obtainedinpowder form from Sigma-Aldrich(Mexico).

Preparation of ascorbic acid encapsulated in a non-nanostructured matrix of calcium alginate using the dripping method (NNAA):An initial solution was prepared mixing equal volumes of solutions with 2 % sodium alginate and 1% ascorbic acid. This solution was coacervated by dripping on a solution of 2% calcium chloride at room temperature. The capsules formed were separated from the calcium chloride by filtration and were dried by lyophilization (Labconco, Lyph Look 4.5, USA).

Preparation of ascorbic acid encapsulated in a nanostructured matrix of calcium alginate using freeze-drying (NAAFD) and spray-drying (NAASD):An initial solution was prepared mixing equal volumes of solutions with 2 % sodium alginate and 1% ascorbic acid. The nanostructuring process, was made feeding this initial solution witha peristaltic pump (Thermo Scientific), which allowed maintaining a constant feed flow (0542 l/h). The feed passed through a hose connected to a nozzle of stainless steel of two-fluid (Spraying Systems Co., 1/8JJ, USA) and was atomized(air pressure of 35 psi) from a height of 10.0 cm on a solution of 2% calcium chloride at room temperature. Afterward, the excess of calcium chloride was removed by filtration. Next, the capsules obtained with the coacervation process were divided in two batches. One batch called NAAFD was freeze-dried (Labconco, Lyph Look 4.5, USA), and the other one called NAASD was spray-dried in a Buchi equipment model 190(inlet temperature of 200°C and outlet temperature of 110°C).

Adsorptionisotherms: Equilibrium moisture properties of nanostructured and non-nanostructured samples were determined using the gravimetric method with saturated salts(4) at 15, 25, and 45°C. From 1-2 g of samples were placed in desiccators containing (P_2O_5) for 2 weeks. Nine saturated salt solutions were prepared corresponding to the water activity range of 0.103-0.892. Water activity data at the 3 temperatures were obtained from Labuza and others(5).The GAB equation was used in modeling water sorption:

$$M = \frac{M_o C K a_w}{(1 - K a_w)(1 - K a_w + C K a_w)}$$

WhereM is water content of the sample on dry basis, a_w is the water activity, M_0 is the moisture content (dry basis) corresponding to monolayer (BET) and C and K are constants related to the temperature effect.

Determination of thermodynamic parameters: The integral thermodynamic properties were obtained with the method Othmer-Hill (6) using the equations:

$$\ln P_v = \left[\frac{H_V(T)}{H_V^0(T)}\right]_\phi \ln P^\circ_v + A \quad \phi = \mu_{ap} - \mu_a = RT \frac{W_{ap}}{W_V} \int_0^{a_w} M d \ln a_w$$

Where$P_v$isthe vapor pressureofwaterin the food,P°_v isthe vapor pressureof pure water atthe same temperature,$H_v(T)$ is the integral molarheatof wateradsorbed$H^\circ_v(T)$ is the heatofcondensationof pure water,A is a constantadsorption, ϕ is diffusion pressure orsurface potentialof the food,$\mu_a$is chemical potentialof the adsorbentparticipatinginthe condensed phase, μapis the chemical potentialof thepureadsorbent, $W_{ap}$is themolecularweightof the adsorbent,and$W_v$is themolecularweightof the water.Integralmolarenthalpy$(\Delta H\text{int})_T$wascalculatedwiththe equation: $(\Delta H_{\text{int}})_T = \left[\frac{H_V(T)}{H_V^0(T)} - 1\right]_\phi H_V^0(T)$

When values for $(\Delta H\text{int})_T$are obtained, changes in integral entropy can be calculated using equation: $(\Delta S_{\text{int}})_T = \frac{-(\Delta H_{\text{int}})_T - \Delta G}{T}$

Calculation of moisture content corresponding to the micropore volume: The moisture content corresponding to the micropore volumen (n_0) was obtained using the Dubinin–Radushkevich equation (3).

Relaxationtimes (t_1): Nanostructured and non-nanostructured samples were placed in 10-mm-diameter NMR tubes. Sample height within the tubes was maintained at 4 cm and the equipment temperature was let to equilibrate for 5 min before proceeding with the measurements. Relaxation times t_1 (spin-lattice) were done with NMR Bruker minispec (mq20, Bruker Optik Gmbtt, Rheinstetten, Germany) equipment. Each measurement was performed by establishing 50 points, pulse separation of 2.6 ms, silent echo 4, scans 4, and temperature of 25°C. The gain was adjusted in order to optimize the signal/noise ratio. t_1 values were calculated using the NMR software. Each sample was measured three or four times.

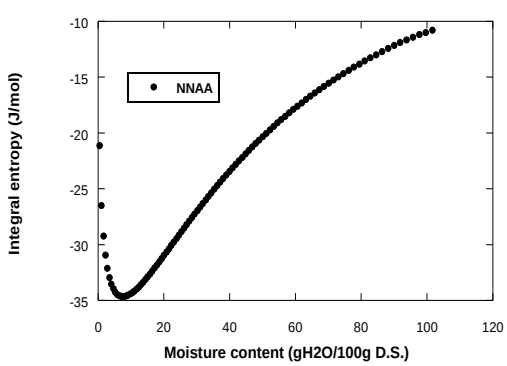
Compression tests: Mechanical compression tests were done using a texture analyzer TA-XT Plus (Stable Micro Systems)according to methodology proposal by Teliz and Martinez- Navarrete (7).

Determinationofascorbicacid during storage time(AA): The determination of ascorbic acid was evaluated usingsecond derivative spectrophotometric method (8).

Results and discussion

Integral entropy (ΔS)

In Figure 1 is observed that in non-nanostructured sample (NNAA), the minimum integral entropy is present in low values of moisture content (9.18 gH2O/100g D.S.). In Figure 2 is observed that in nanostructured samples the integral entropy decreases to a minimum, that occurs at moisture content of 38.01 (gH2O/100g D.S.) for NAAFD samples and 30.78 (gH2O/100g D.S.) for NAASD samples. In the minimum integral entropy the water moleculas are best organized and less available for taking part in spoilage reactions (2). Viganó and others (9) reported that an increase in the moisture content corresponding to minimum integral entropy zone in pineapple pulp powders obtained by different drying methods, can be related with differences in the micropore volume. These results indicate that differences in the minimum integral entropy in NAAFD and NAASD samples compared with NNAA sample are a direct consequence of the changes in the microstructure, allowing the displacement of the minimum integral entropy at higher moisture content causing a positive effect on the stability of AA during storage.



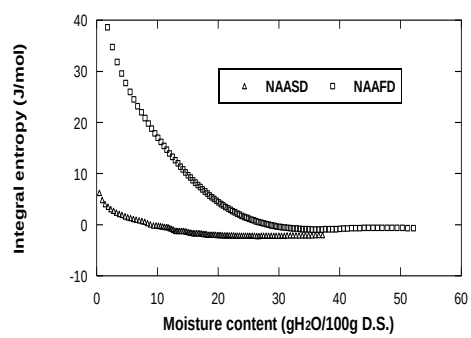


Fig. 1.

Integral entropy changes of adsorbed water on NNAA Fig. 2. Integral entropy changes of adsorbed water on as a function of moisture content at 25°CNAAFD and NAASD as a function of moisture content at 25°C

Micropore volume

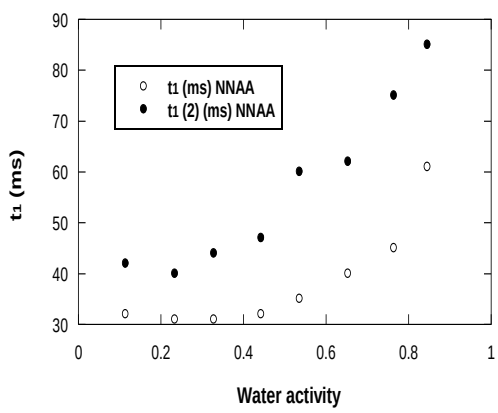
The adsorbed moisture contents corresponding to the micropore volume (n_o) and the moisture contents at the minimum integral entropy are given in Table 1, is observed that the moisture content adsorbed corresponding to the micropore volume is greater for NAAFD and NAASD samples that for NNAA sample. Also is observed in the Table 1 that the moisture contents corresponding to the micropore volume and the minimum integral entropy are very similar.

Table 1. Dubinin-Radushkevich parameters for sorption isotherms of NAAFD, NAASD and NNAA products and moisture content at the minimum integral entropy at 25°C

Product	n_0(gH$_2$O/100g D.S.)	r^2	Moisture at the minimum $(\Delta s_{int})_T$(gH$_2$O/100g D.S.)
FDAA	38.13	0.944	38.01
SDAA	30.90	0.980	30.78
NNAA	13.808	0.998	9.18

Relaxation time (t_1)

Figures 3 and 4 show that the more ordered configuration of water molecules during adsorption process through of the micropore, occurs when these molecules have minimum molecular mobility (t_1), for all samples the minimum value of t_1 is present in the values corresponding to the minimum integral entropy. Also is observed that the non-nanostructured samples (Figure 3) show two values for t_1 indicating the interaction of water molecules with the active sites and the interaction the water molecules among themselves.Yoshioka and Aso (10) studied the molecular mobility of water determined by t_1 on the hydrolysis rate of cephalothin in freeze dried formulations. Where observed that the water molecules exhibit minimum value spin-lattice (t_1) in the minimum hydrolysis rate of cephalothin.



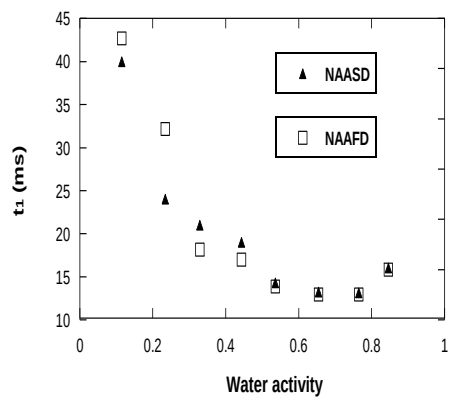


Fig. 3.Values of $t_1$in NNAA products at 25°C Fig.4.Values of $t_1$in NAAFD and NAASD products at 25°C

Stability of ascorbic acid (AA) during storage

In Table 2 is observed that in the NAASD samples the maximum retaining percentage of AA is 91.29% at 30 days storage at water activities of 0.536 (aw near the minimum integral entropy) and 78.65 at the 60 days, for NAAFD samples the maximum retaining percentage of AA is 98.20% at 30 days storage at water activities of 0.765 (aw near minimum integral entropy) and 81.95 % at 60 days. By contrast, non-nanostructured samples (NNAA) at low water activities (0.23), has a retention of 45% of AA at 30 days of storage and 4% at 60 days, in high values of water activity, the AA is completely lost since the earliest days.

Table 2. Ascorbic acid retention during storage time at 25°C

	NAASD		NAAFD		NNAA	
a_w	30 day	60 day	30 day	60 day	30 day	60 day
0.234	82.310	71.332	83.47	73.13	45	4
0.443	73.162	64.622	74.25	66.07	17.64	0.00
0.536	91.290	78.652	73.162	63.622	14.26	0.00
0.765	79.261	70.112	98.20	81.95	0.00	0.00

CompressionTesting

Figure 5 shows the the maximum force points (Fmax) obtained for all samples at different water activities. In the samples non- nanostructured (NNAA) with increased the water activity, the maximum force was

declining and the water acted as a plasticizer, in the nanostructured samples (NAAFD and NAASD) the Fmax was increasing at highest values of aw showing an Anti-plasticization effect. Also is observed that the highest Fmax in all samples corresponded to the moisture content given by the minimum integral entropy.

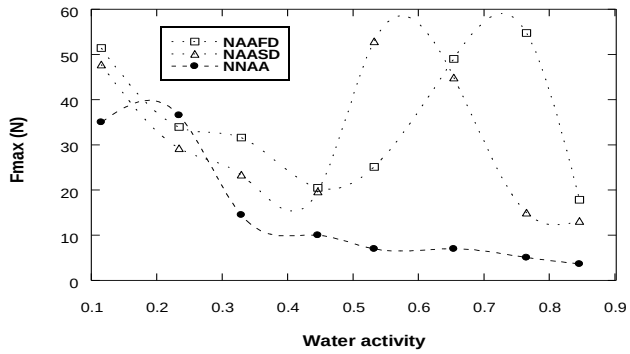


Fig. 5. Effect of water activity on the maximum force (F_{max}) of NAAFD, NAASD and NNAA products at 25°C

Conclusions

With the modification of the microstructure is possible to change the properties of adsorption water and micropore volume, in materials with same chemical composition, displacing the minimum integral entropy at higher moisture content and water activity. Maximum values of Fmax as well as minimum values of the relaxation time (t_1) occurred when all samples studied were equilibrated at water activities corresponding at the minimum integral entropy. Ascorbic acid is preserved at high water activities, when it was encapsulated in a nanostructured matrix of calcium alginate. Thus demonstrating that nanotechnology is a good choice to improve the stability of ascorbic acid during storage.

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Extraction and Characterization of Faba Starch(*Viciafaba l.*) As a Source of Resistant Starch

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Introduction

Starch is the only food polysaccharide occurring naturally which can be digested by the intrinsic enzymes of the human gastrointestinal tract(GIT). This starch is termed resistant starch (RS) and is defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals (Asp, 1992).The RS exists in foods for a variety of reasons (Topping &Clifton, 2001). Raw starches (e.g. in unripe banana) are digested poorly and their digestibility is enhanced through cooking, especially in the presence of water, which gelatinize the starch giving greater access to amylases. Disruption of food structure by milling and other processes (e.g. chewing) also enhances digestion by permitting access to the matrix for the digestive enzymes. While cooking increases starch digestibility, subsequent cooling leads to the formation of crystallites that are resistant to digestion. This process is termed retrogradation and the RS content of foods appears to increase when they are subject to increasing numbers of heating and cooling cycles. Chemical structure is an important factor in starch digestibility, especially amylose:amylopectin. Most food starches are predominantly (about 70 %) amylopectin. The remainder is amylose which occurs within the amylopectin matrix. The greater the content of amylose, the more difficult the starch is to gelatinize and the more susceptible to retrogradation (Colonna & Mercier, 1985). Ungelatinised high-amylose starches (60–70 % total starch) are resistant to amylolysis and are used commercially as an ingredient to increase the RS content of processed foods (Brown et al. 2000).

There are a number of advantages to using commercially manufactured RS in food products. Unlike natural sources of RS (that is, legumes, potatoes, bananas), commercially manufactured RS are not affected by processing and storage conditions. The RS shows improved crispness and expansion in certain products and better mouthfeel, color, and flavor over products produced with some traditional, insoluble fibers. The industrial applications of RS are mainly in processed cereals, snacks, pasta/ noodles, baked goods, and fried foods and permits for easy labeling as simply starch, conferring additional nutraceutical benefits. Compared with conventional fibers, it is white and has a bland flavor and a fine particle size between 10 and 15 µm. It also has a reduced caloric content and may be used as a bulking agent to complement reduced sugar or reduced-fat formulations. Most importantly, the commercial RS has a much lower water-holding capacity than do various traditional fibers. Because it absorbs less water, adjustments in product formulations and processing are substantially minimized. It is classified as a fiber component with partial or low fermentation. Technically, it is possible to increase the RS content in foods by modifying the processing conditions such as pH, heating temperature and time, number of heating and cooling cycles, freezing, and drying. A number of commercially available RSpreparations would make it possible for a wide range of applications with nutraceutical implications (Thompson, 2000)

Objetive

The aim of this study was to characterize faba starch (*Viciafaba*L.) as a source of RS and its application as an ingredient in food.

Material and methods

Samples

Beanseeds(*ViciafabaL*) werepurchased from thesupply center inMexico Cityand storedin a cold roomuntil processing.

Extraction of Faba bean starch

One kilogram of fava beans (*Viciafaba*L.) was milled in a Pyrex mill, soaked in 3 L of 0.05 *N* NaOH overnight at 5°C, and then subjected to wet milling. The suspension was filtered through 30-, 100-, and 200-mesh sieves (US standard), and the residues retained on each screen were washed with water to recover the starch. The starch extract was allowed to stand overnight at 5°C to drain excess water and was then dried at 40°C and stored in plastic bags at room temperature (24°C).

Chemical characterization of starch

The amount of resistant starch in the starch extract was measured using the enzymatic method of Goñi et al. (1996). Analysis of moisture, protein content, lipid content, and ash of the starch extract were performed by using official procedures of AOAC (2000).

Pasting properties of starch

The pasting properties of starches were determined using a Rapid ViscoAnalyzer (Model RVA Series 4, Newport Scientific Pvt. Ltd., Warriewood, Australia). About 2.5 g of starch (corrected to 14% moisture basis) and 28mL of distilled water were combined and stirred in the aluminium RVA sample canister. Temperature was held at 50 ◦C for 1min, raised to 92°C at 5.5°C/min, maintaining the temperature by 5 min, cooled to 5°C and maintaining for 2 min. The units of viscosity are expressed as centipoises (cP's).

Differential scanning calorimetry (DSC)

Starch samples (3 mg) with 7 mg distilled water were hermetically sealed in DSC C822 (Mettler-Toledo, Switzerland) Theheatingintervalwas from 30 to110°Cata heating rateof10°C/min.In the thermogramsobtainedwere determinedonset (To), peak(Tp)andend (Tc) temperatures and the transition enthalpy (ΔHg).

X-ray diffraction (XRD)

X-ray diffraction data were obtained using a Rigaku D/Max-B X-ray diffractometer (Rigaku Corporation, Tokyo,Japan). The samples were scanned from 5° to 55°in the diffractometer. Data were collected as counts per second (cps) versus Bragg angle (θ/2θ) with0.02s of integration time, at30KVand16 mAof operating withCoKα1of radiation andλ=1.7889Å. Percent relative crystallinitywas calculatedaccording to themethodofNaraandKomiya(1983). Peak fitting software Origin Version 6.0/SR-4 (Microcal Software Inc., Northhampton, MA, USA) was used to calculate absolute differences betweenXRD profiles.

Scanning electron microscopy (SEM)

Starch samples were mounted on metal stubs of 12.69 mm of diameter type PIN and previously prepared withdouble-sidedtapegraphite. Samples were then observed using a Philips XL30 scanning electron microscope at an acceleration potential of 15 kV. Microphotographs were registeredat different magnificationsthrough anelectrondetectorgas.

Results and discution

Chemical characterization of starch

The purityof starchwas describedby moisture(10.59% ±0.011), ash (0.06% ±0.056), fat(0.15% ±0.029)and protein(0.35% ±0.270) composition. Protein contentforbeanstarchwas abovethat these reported byother authors(HaaseandShi1991).These differences maybe due to theextraction processused. Thehighvalues ofproteinmay be importantdue tothepossibilityof forming complexesstarch-protein, that interactions has been to reduce RS contents(Sajilataetal., 2006). RS contentofrawstarch was 2.90% and

afterpasteurization(75°Cfor 15s) was 5.84%.Accordingto the classificationof RS contentproposed byGoñietal.(1996);rawbeanstarchcorrespondatintermediatelevelofAR(2.5 to5%) andafter pasteurizationat high level(5 to 15% AR). The increaseinRS has beenattributedto changes inprocessing conditionssuch as heatingandcooling(TharanathanandMahadevamma, 2003). Amyloseretrogradationwas identified asthe main mechanismfor the formation ofAR3.

Pasting properties of starch

The valuesof theviscosity profilebeanstarchare presentedin Table 1. Theviscosity profilebeanstarchischaracterized by alowV_p(1169cP) and a highV_f(1709cP).It has been reportedthatthesedifferencesare due tothestarchamylosestarchcontainingabout 30% amylose generally exhibita decreaseinthepeak$V_p$although$V_f$tends to increasewith increasingamylose content(Yamamori andQuynh, 2000).

Table11.Thermal propertiesbeanstarch

Starch	V_i (cP´s)	T_i (°C)	V_p (cP´s)	T_p (°C)	V_h (cP´s)	V_f (cP´s)	V_r(cP´s)
Haba(*Vicia Faba* L)	28	68.5	1169	89.46	1014	1709	695

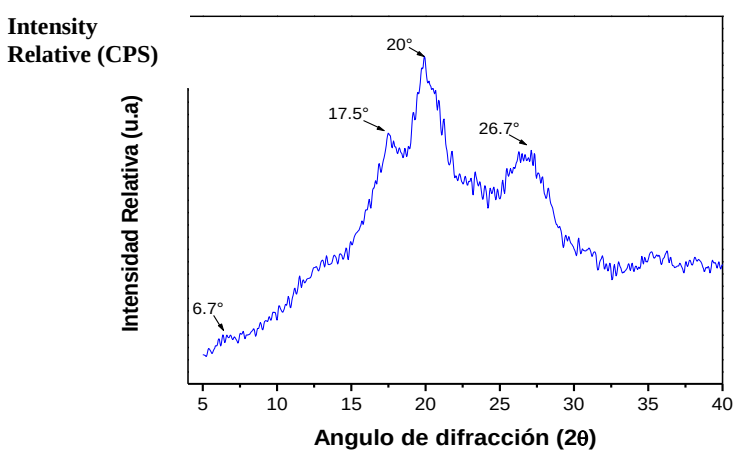
Means of three determinations.V_i = pasting viscosity, T_i = pasting temperature, V_p=viscosity peak, T_p= temperatureviscosity peak,V_h= hold viscosity, V_f= final viscosity, V_r= Retrogradation viscosity.

Differential scanning calorimetry(DSC)

The DSC parameters of faba starch were To= 55.88°C, T_P= 62.31°C,Tc= 68.21°C, (Tc-To) = 12.33 and ΔH_G= 10.28J/g.This starchcanbeusedin processeswhich do notrequirehigh operating temperaturesto gelatinizeandformAR3such aspasteurization(65°C or 75°C).

X-ray diffractionand relative crystallinity

The X-ray diffraction patternofbeanstarchis shownin Figure1.Thebeanstarchshoweda small peakat 6.7°characteristicof type B patternand another peakat 17.5°characteristicof type AandB pattern. Beanstarchshowed aC-type diffraction pattern, a combinationoftypeAand type B, which is typical oflegumestarches(Donald, 2004), which is generallymoreresistantto digestion.Regarding therelative crystallinity(Cr)the sample analyzedinthisstudyshowed a20.45% lowerthan reportedbySandhuandLimin starch of legumes(2008).The presence of branch points within amylopectin allows the short linear chains to pack together efficiently as parallel left-handed double helices, forming the basis of the semicrystallinestarch granule. The precise location of amylose within the granule is a subject of much debate; it is thought to be found predominantly in the amorphous, less-crystalline regions.



Microscopia Electrónica de Barrido (SEM)

Figure1. X-ray diffraction patterns of bean starch

Diffraction angle (2θ)

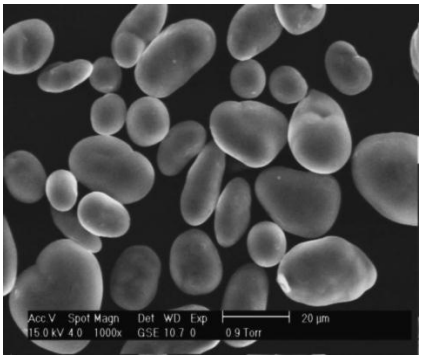


Figure 2. Scanning Electron Microscopy (SEM) of resistantbean starch. Increase to 1000x

Beanstarch granulesshowedformsofkidney, oval and spherical (Figure2).Ovalstarchgranulespresented an averagediameterof14.4µm and23.24µmlong. The spherical granulespresented a diameter about12.48µm.Thegranulestarchsizeis related, according tothe relationship betweensurface areaand volume of thestarchandthusthe contactbetween the substrateandthe enzymedecreases as thesizeof the granule(Svihusetal., 2005).

Conclusions

Beanstarchshowed an increaseinthecontentofRSafter thepasteurizationprocess, obtaining a highlevelofAR,attributingtoretrogradecapacityofthe sample, which was observedintheprofileofviscosity, which yieldeda curvesimilar tohigh amylosestarcheswhich tendto comprisea higher content ofAR3,so thatthisstarchwillmakefood pasteurizedwith increasedARduring processing. Therawbeanstarchshoweda pattern ofX-ray diffraction typeC, characteristicof legumes, a low degree ofcrystallinityandstarch granules inkidney shapes, ovaland sphericalgreater than 10µm. For these characteristicseven thoughtheliteratureindicate a highpercentageoflegumeAR, in this study thecontentsofRA wasintermediate(2.90%) beingnoclear relationshipbetweenresistantstarchandtheir physicochemical characteristics.

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Determination Of Thermal Properties And Heat Transfer Mechanisms During Canning Process Of Escamoles (*Liometopum apiculatum*)

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Abstract

The "escamoles" are the ant pupae (*Liometopum apiculatum*) and are considered as one of the most consumed insects in Mexico, due to their nutritive and sensorial characteristics, besides of being considered as the "Mexican caviar", due to the analogy made with the "esturion caviar". They are highly perishable, because of their chemical composition so that's why it was necessary to procure the canning process to extend their shelf life. So the objective of this work was to obtain the thermal properties (conductivity (k), calorific capacity (Cp) and density (ρ) of the product and the heat transfer mechanisms during the canning process of two escamoles products. Sixteen experiments (original and replicate) were realized according to a random factorial design, each one of three factors with two levels. The factor studied were: type of product: (escamoles in brine and escamoles in vinegar); process temperature: (115 and 121°C) and type of sterilization (steam treatment and immersion in water). Thermal properties were calculated in function of temperature and chemical composition using equations of (1), heat transfer mechanisms were calculated during temperature evolution in cold point of the can, using Computational fluid dynamic simulation (CFD) with Comsol Multiphysics software. For any kind of product and type of sterilization, was observed that when temperature arises the thermal conductivity was increased ($p< 0.05$) and the specific heat and density decrease. Products elaborated in brine showed heat transfer by conduction and those elaborated with vinegar presented convection heat transfer at first, and then conduction. In conclusion, in this work was possible to elucidate the heat transfer mechanisms and thermal properties during canning process of each product. These results are the base to develop the canning process of escamoles and to obtain a new alternative of conservation and marketing of these products.

Keywords: Escamoles, thermal properties, heat transfer, canning process

Introduction

Entomophagy is referred to consume of insects as food. Actually this habit has been significantly increased in the whole world due to the sensorial and nutritive properties of insects. These are characterized by their high content of proteins, lipids, minerals and vitamins (3). For this reason, the Food and Agriculture Organization (4) considered insects as a potential food that will help to fight malnutrition and problems related with food safety in underdeveloped countries.

At a global level, there are about 2000 species of eatable insects distrusted in 120 countries. From these species, 540 are localized in different states of Mexican republic, being the escamoles the most appreciated insects for Mexican people (5). Escamoles or pupas have a length or 7-10 mm and a diameter of 3-5 mm and have a white pearl color. Exist few studies about escamoles, and are focalized on biogeographical aspects, nutritive value analysis and commercialization. Based on these it is known that are localized in the states of Mexico, Hidalgo, Tlaxcala, Zacatecas and San Luis Potosi, and that their recollection period is from February to April.

Their commercialization is developed only in frozen state, which causes structural damage when they are defrosted. They are offered to recognized restaurants as a “gourmet plate” because of their special flavor and elevated cost. The presence of proteolitic enzymes and a high content of carbohydrates in their chemical composition promote a fast spoilage and at this date there are no studies about preservation process that would help to provide longer shelf life. For this reason, the objective of this work was to obtain the thermal properties and heat transfer mechanisms during the canning process of escamoles

Material and methods

Product elaboration

Escamoles were collected in Pocitos Charcas San Luis Potosí and were washed and frozen at -20°C until their utilization. Sixteen products (original and replica) were elaborated according to an experimental factorial design completely random of 3 factors with 2 levels each one. The studied factors were: Type of product (escamoles in brine and escamoles in vinegar); process temperature (115 and 121°C) and sterilization method (vapor and water immersion). Canned products were elaborated according to the formulation established en NOM-SCF-002.

Cans filling process was developed at 80°C. During sterilization process, cans were subject to a heating temperature of 115or 121°C (with direct vapor or water immersion) using a vertical stationary autoclave. Each can was arranged with cupper-constantan couples in radial direction in three different positions considering three different heights in the can (1/2, 1/3 and an intermediate between ½ and 1/3) and set at the geometrical center. The equipment used to obtain the temperature evolution was a digital CALplex assisted by the CALSoft Collect software, adjusted to collect the temperature values at times of 30 seconds in each thermocouple. In this way were obtained the temperature profiles depending on the time, including cooling stage until a temperature of 40°C. With these data was located the “cold point” and analyzed the heat transfer mechanism. “Cold point” was used as a reference to evaluate the temperature evolution and so obtaining thermal properties and heat transfer mechanisms.

Estimation of thermal properties by Choi Okos method

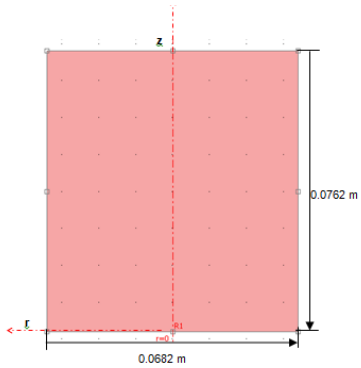
Thermal conductivity (k), thermal capacity (Cp) and density (ρ) were calculated by equations proposed by (1), in function of temperature and chemical composition (masic fraction and volumetric fraction) of each one of the constituents considering these equations

$Cp = \Sigma(Cp_i X_i^m)$ (1) $\rho = 1/\Sigma(X_i^m/\rho_i)$ (2) $k = \Sigma (k_i X_i^v)$ (3)

Where X_m is masic fraction, X_v is volumetric fraction, *i* represents constituents. With those equations, global heat transfer coefficient (h) could not be estimated, so it was supposed a really high value ($1x10^{10}$ W/m^2 °C) that implicates heat transfer resistance by negligible convection and determined by simulation.

Heat transfer simulation

Simulation was performed with COMSOL Multiphysics software version 3.5., supposing and homogeneous food mixture inside the can (ideal). A 2D geometry (radial and axial) with cylindrical coordinates (figure 1) and convection energy flow frontier conditions were used.



Ecuation 4 governs heat transfer behavior

$$\frac{\partial T}{\partial t} = \frac{k}{\rho C_p}\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial T}{\partial r}\right) + \frac{\partial^2 T}{\partial z^2}\right] \quad (4)$$

Where: T= temperature, t= time, r = radio y z= height. Model parameters were estimated from experimental data using equations 1 to 3 (1) and by no linear regression.

Statistical analysis

A linear model with interactions (Eq. 5) was used to analyze the effect of king of product (A), sterilization method (B) and temperature (T) on k, h, Cp and ρ.

$$Y = b_0 + b_1 A + b_2 B + b_3 T + b_4 A*B + b_5 A*T + b_6 B*T + e \qquad (5)$$

Where b_0 to b_6 are regression constants of the model and ε is the experimental error ANOVA was performed with a confidence interval of 95% with Modde versión 7.0. software.

Results and discussion

Evolution of temperature and heat transfer

Localization of cold point depends directly of food properties, such as density, viscosity temperature and sterilization method (6). Cold point is considered as the longest time to get the desired temperature in the process. In the case of brine product treated in water immersion, cold point was localized at ½, and for the product treated with direct vapor was between ½ and 1/3. With respect to product in vinegar in both treatments (immersion and steam) the cold point was localized at ½. Figures 2 a and b show results of temperature evolution determined at cold point. As an example results for brine/immersion method at 115 °C are shown (figure 2a) due that in all the experiments have similar behavior. In this figure can be observed that temperature evolution was slow, because time required to get 115 °C was 4000 s (66 min). Respect to figure 2b it can be observed a faster heating process, a time of just 2100 s (35 min) was required to get a temperature of 115 °C. A similar time for temperature evolution was reported by (6), when they develop studies about temperature evolution at cold point in liquid food with different viscosities.

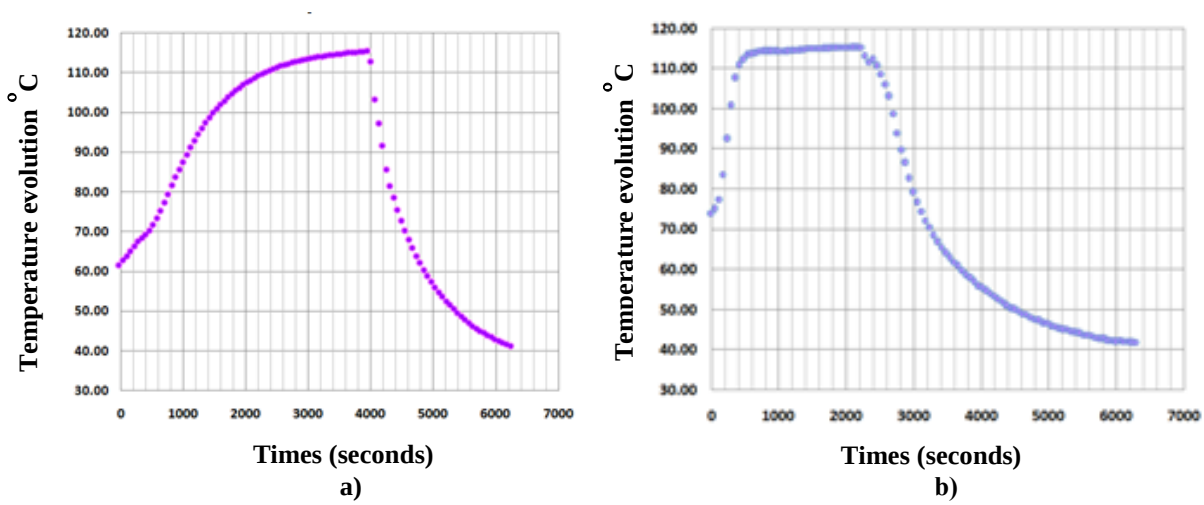


Figure 2 Temperature evolution in cold point a) brine and b) vinegar in immersion at 115°C

Cooling process (until a temperature of 40°C) presented an exponential decay for both products, being faster for brine product. These differences could be due to heat transfer mechanism that each product presents, for brine product treated with direct vapor and water immersion, presented heat transfer by conduction. Respect to products elaborated in vinegar, and treated with direct vapor and water immersion, presented a heat transfer by convection at the beginning and by conduction later. Similar results concerning to mechanisms of heat transfer by conduction have been reported by (7). This information is

also supported by results reported by (8) where they indicate that when suspended solids exist in a liquid food, heat transfer during canning process occurs by convection at the beginning and by conduction later.

Thermal properties

Table 1 show results related with thermal properties: Specific heat (Cp), density (ρ) and thermal conductivity (k) in function with temperature and chemical composition, calculated with equation (1). It is important to mention that behavior of these properties was similar for all the experiments, and for this reason results for the product escamoles in brine treated con direct vapor at 115 °C, were presented as an example. Results indicate that specific heat and density decrease as process temperature increases, while thermal conductivity increases as temperature increases. Similar results were reported by (9) during peas canning process. The ANOVA ($p<0.05$) indicated no significant effect of studied parameters on the convective heat transfer coefficient (h) ($9.34*10^9$ W/m^2°C), Cp and ρ, but they caused a significant effect on thermal conductivity. This one had a general average value of 1.71W/m °C and was affected by type of product and by the interaction between type of product and sterilization method.

Table 1 Thermal properties of products

Experiments	$h_{nlr}*10^{-9}$ (W/m^2 °C)	k_{rnl} (W/m °C)	k choi y okos (W/m °C)	Cp kJ/kg°C	ρ kg/m^3
Vinegar / steam / 115°C	9.366±1.01	2.5±1.1	0.643±0.05	3.77±0.01	984.78±4.475
Brine / steam / 115°C	9.381±0.902	0.607±0.02	0.635±0.02	3.77±0.03	987.65±0.04
Vinegar / immersion / 115°C	9.133±3.75	4.58±1.3	0.635±0.06	3.76±0.04	978.3±2.4
Brine / immersion / 115°C	9.92±0.417	0.7±0.036	0.636±0.05	3.74±0.02	991.24±1.36
Vinegar / steam / 121°C	9.651±0.039	1.7±0.35	0.645±0.01	3.76±0.06	977.84±1.44
Brine / steam / 121°C	9.682±0.062	0.937±0.45	0.645±0.05	3.79±0.3	979.9±3.31
Vinegar / immersion / 121°C	9.754±0.3	2.6±0.4	0.643±0.07	3.77±0.4	976.42±0.685
Brine/ immersion /121°C	9.77±1.51	0.893±0.144	0.639±0.03	3.74±0.05	980.52±1.91

h_{nlr} = heat transfer coefficient obtained by nonlinear regression, k_{nlr} = thermal conductivity obtained by nonlinear regression, $k_{Choi\ and\ Okos}$ = thermal conductivity obtained by Choi and Okos, Cp = specific heat, ρ = density.

Conclusions

In this work was possible to elucidate the heat transfer mechanisms and thermal properties during escamoles canning process. These results are the base to develop the canning process of escamoles and to obtain a new alternative of conservation and marketing of these products.

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Glass Transition Temperature And Water Activity Of Model Food Systems Analogous To Fruit Powder Composition

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Introduction

Food powders are one of the most important products in the food industry because they are easy to store for long periods, transported locally and for export, and used in innovative formulations producing the widest possible range of food and beverages (1,2). Common food powders that are readily available in the market include baby food, cheese, whey products, coffee, tea extracts, egg powder, spice and herb extracts and sugar-rich products such as honey and fruit juice powders which are predominantly in a glassy amorphous form (1,2). However, several changes in physical, chemical and biological characteristic of food powders occur during storage and distribution (3,4). From physical stability point of view, stickiness or cohesion, caking, sugar crystallization, loss of volatile compounds and loss of crispness are the most common problems in handling of powders containing amorphous carbohydrates (5,6). Amorphous sugars particles are in a meta-stable state and can undergo crystallization or structural relaxation to achieve an equilibrium with a rate of change that depends on the surrounding temperature and water content or relative humidity. Therefore, food powders are temperature sensitive and highly hygroscopic. Recently, the concepts of water activity coupled with glass transition temperature have been used in the literature to evaluate the storage stability since a product is most stable at its monolayer moisture content or at below the corresponding glass transition temperature (2). In the particular case of fruit powders, these are characterized by containing low molecular weight sugars such as fructose, glucose and sucrose with Tg values between 5 and 62 °C (2,3,5). Therefore, there is a need to measure and understand the transition states of model food systems including mixtures of fructose, glucose and sucrose, to envisage the glass transition of fruit powders based on their sugar contents that can be used for predicting and optimizing the stability of fruit powder during storage.

Materials and methods

Model systems

Analytical grade crystalline fructose and glucose (Sigma Chemical Co., St. Louis MO, USA, 99.5 % pure, molecular weight of 180.16) and crystalline sucrose (CTR Scientific Co., Monterrey México, 99 % pure, molecular weight of 342.30) were used in these experiments. The mass fraction of fructose (X_F), glucose (X_G) and sucrose (X_S) in the sugar mixtures was established using a user-defined distance-based experimental design for mixtures of the three components. A total of 16 model food systems including individual, binary and ternary sugar mixtures were analyzed. The moisture content of the dry model systems was varied by keeping the samples at different saturated salt solutions (P_2O_5, $CaCl_2*2H_2O$, K_2CO_3, $SrCl_2*6H_2O$) which produced water activity values between 0 and 0.70.

Differential scanning calorimetry (DSC)

The improved calorimetric methodology proposed by Saavedra-Leos et al., (7), was used in this study for the glass transition temperature measurement. In brief, the experiments were carried out by using a Differential Scanning Calorimetry (DSC Q200, TA Instruments, Lukens Drive, New Castle, Delaware

19720, United States) with a temperature and sensitivity accuracy of ±0.1 ºC and ±0.2 µW, respectively. Samples of about 16 to 20 mg were accurately weighed in Tzero® aluminum pans (TA Instruments) using an electronic microbalance (Mettler Toledo R-3769: XS205, ±0.01mg) and immediately and hermetically sealed using a Tzero® press pan sealer (TA Instruments). Samples were firstly equalized in the DSC a -15 °C for 5 min before starting the experiment. Then samples were continuously heated at 20 °C/min until the sample melted completely. A melting holding time of 2 min was sufficient to achieve a completely molten state. After the melting treatment, the samples were quickly cooled to -15 °C at 20 °C/min in order to achieve the amorphous state. A vitrification holding time of 2 min was used. Finally, the samples were reheated at 10 °C/min to 245 °C. The temperature values of the glass transition during reheating were recognized in this last stage as a step change of heat flow in the DSC thermograms using universal thermal analysis 2000 software, version 4.4A. Although onset, peak and endset Tg values were determined from the first derivative plot of DSC thermograms, only the Tg peak value is reported in this study.

Glass transition temperature and moisture sorption modeling

The experimental Tg data were subjected to a linear least square regression analysis using the adapted Scheffe's cubic polynomial equation (8) as follows:

$$Tg_H = \sum_{n=0}^{3}\left[\sum_{1\le i\le 3}\beta_{in}x_i + \sum_{1\le i<j\le 3}\beta_{ijn}x_ix_j + \sum_{1\le i<j\le 3}\gamma_{ijn}x_ix_j(x_i - x_j) + \beta_{123n}x_ix_jx_k\right]a_w^{\,n} \qquad (1)$$

where a_w is the water activity, n represents the power of a_w and x_1, x_2, x_3 are respectively, the mass fraction of glucose, fructose and sucrose in the model food system. If the values of xi are fixed, then equation (1) can be expressed in the form:

$$Tg_H = \sum_{n=0}^{3}\alpha_n a_w^{\,n} = \alpha_0 + \alpha_1 a_w + \alpha_2 a_w^{\,2} + \alpha_3 a_w^{\,3} \qquad (2)$$

Then equation (2) can be used to predict the relationship between glass transition temperature and water activity at any model food chemical composition, where α_0, α_1, α_2 and α_3 represents the regression coefficient of the model. On the other hand, the experimental data of moisture content as a function of water activity was modeled using the non-linear regression with the GAB equation.

Results and discussion

Experimental and predicted values using Eq. (1) regarding Tg of the 16 model food systems at the several water activities are plotted in three-dimensional response surfaces as shown in Figure 1. In all the cases, there was a good agreement between the experimental and predicted values. Equation (1) interpreted data variability with a determination coefficient (R^2) of 0.993 and with model significance (p) <0.0001. The shape of Figure 1 indicated that independent of the water activity value, the maximum glass transition temperatures were exhibited at the point which correspond to pure sucrose (Xs=1) and the minimum values of Tg were observed for samples containing pure fructose (X_F=1). It was observed that when water activity was increased, the glass transition temperature of model food systems decreased (Fig. 1b-1d). These observations are in agreement with those described by different authors (5,9). In this context, the experimental and predicted values using Eq. (2) of Tg versus water activity for some of the model food system evaluated were also plotted (results not shown). It was noted that equation (2) predicts a sigmoid relationship between Tg and water activity that was consistent with experimental results. Some authors (5,10), have also reported this sigmoid relationship in their experiments conducted with berry powders and spray-dried tomato, respectively. However, the linear relationship between Tg and aw proposed by Roos (11) has been extensively considered for the analysis of several food products in literature (9). Accordingly, this indicates the suitability of the proposed equation in this work to predict adequately the relationship of Tg versus of several sugar model food systems. The moisture adsorption isotherms of the model food systems obtained at 30 °C and the varying water activities between 0 and 0.7 were also analyzed (results not shown). All samples exhibited type "J" or type III isotherms which are representative of high sugar food products. At low water activities (0-0.43), the water sorption is not marked, but a sharp increase of water content is quite noticeable when a value of 0.7 was reached.

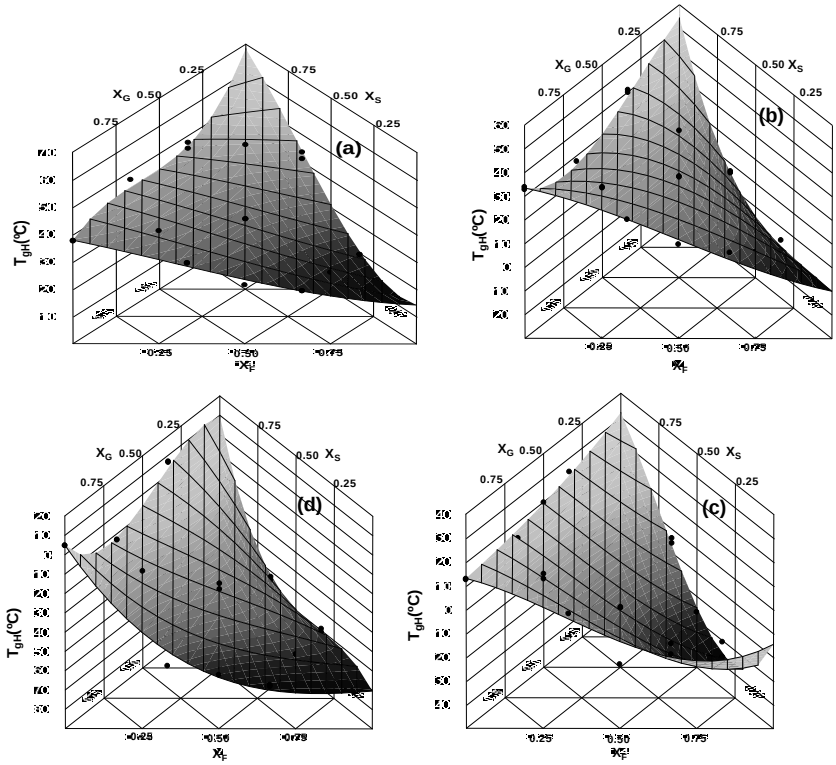

Fig 1. Three-dimensional response surface for the glass transition temperature measured at several water activities. (a) aw: 0, (b) aw: 0.224, (c) aw: 0.432, (d) aw: 0.71

Conclusion

Glass transition temperature was measured for different model food systems to cover a large number of possible fruit compositions including individual, binary and ternary mixtures of sugars as a function of water activity. Increments in water activity normally provoked decreasing in glass transition and a new expression was found to adequately describe the relationship between Tg and water activity. Concerning water sorption properties, all samples exhibited type "J" isotherms and the GAB model was found adequate to represent the experimental data throughout the entire range of water activity.

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Evolution Of Ascorbic Acid Degradation During Hot Air Drying Of Papaya Maradol (*Carica papaya*).

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Introduction

Papaya (*Carica papaya*) is an important crop widely grown in tropical and subtropical lowland regions, highly accepted worldwide. *C. papaya* is nutritive fruit, rich in vitamins A and C, that presents good organoleptic characteristics and it is fragile and perishable. However, Its fragility is a characteristic that limits large-scale exportation to anothers countries. For that reason, for producers its important keep the quality from harvest to the consumers, and one of the most important techniques used for its conservation is the drying.

The drying is one of the oldest forms of food preservation known to man and is the most important process for water removal from fruit and vegetables and for that, the most frequently method used for the preservation of food (1) Water is one of the main food components and has a decisive direct influence on the quality and durability of foodstuffs through its effect on many physico-chemical and biological changes (2). On the other hand, dry foods have lower costs for storage and transport compared to the fresh food (3).

Conventional air-drying is a simultaneous process of heat and mass transfer, accompanied by phase change (4). The mechanism of drying involves combined heat and mass transfer and, in most cases changes the properties of products (5), these property changes are not just physical but also chemical that in some case are undesirables. In the particular case of fruits, the dried is associated with loss of nutrients (6, 7).

In order to evaluate the severity of the treatment in a drying process has been used indicators of quality. Ascorbic acid is an important nutrient in vegetables, and its degradation has been widely used in food research as an indicator of quality because of its heat labile nature compared to other nutrients in foods (5, 8, 9, 10, 11). Depending on the severity of the treatment, it can cause varying degrees of loss of ascorbic acid during drying and the degradation can be accentuated by the effect of variables involucrated: temperature, air velocity and thickness. For that reason, the aim of this paper was to study the ascorbic acid degradation during drying, with a model that describes it depending of the variables process.

Materials and Methods

C. papaya samples were acquired on a local supermarket of Veracruz, Ver. Mexico. Drying kinetics were realized at two air velocities 1.5 y 2.5 m/s. The thickness of the slices fruit employed were 1.0 and 1.5 cm; the kinetics of degradation of ascorbic acid were realized at four temperatures (40, 50, 60 y 70ºC), each experiment was performed in duplicate, during process was evaluated the ascorbic acid content at specific times. Convective dryer (APEX model SSE17M) was used, which has an operating range of 40 to 120ºC with a maximum air speed of 2.5 m/s.

Destructive samples were obtained every 30 minutes for the first 3 hours, then every hour until to obtain a constant water activity during drying convective. The ascorbic acid content was expressed as a differential of concentration in relation to initial concentration. Ascorbic acid content was expressed as a differential of concentration in relation to initial concentration and a quotient concentration in time was established. Rate constant were determined with natural logarithm of the quotient concentration by slopes method.

Temperature samples were considered together with all dates, a matrix was created and by lineal estimation the model was obtained.
The ascorbic acid (AA) content was determined by HPLC and the moisture content was determinated by AOAC 1980 method (12). The HPLC system was constituted by a Varian Star 8800 pump. The run flow rate was 0.7 ml/min, with KH_2PO_4 1 % as the movil phase. Reverse-phase separation was attained an LC 18 column (150 mm 4.6 mm). The wavelength detection was read at 254 nm, using a waters 2487 UV detector. Samples were filtered through a 0.2 µm sterile acrodisc (Millipore) before into injection into the HPLC. The injection volume for each run sample was 50 µl. Samples were run by triplicate This data were analyzed, according to statistics analysis, the result showed that the variable which one most affected the degradation of ascorbic acid during drying convective, was the temperature. Simply first order degradation has been used for describing ascorbic acid degradation. A first order kinetic was assumed in order to evaluate the rate constant degradation, where the constant k was evaluated to different steps during kinetics for every time according to Arrhenius model.

Result and Discussion

Constants evaluated at different levels of moisture and temperatures (during every kinetics one) were adjusted with a linear regression to a model type Arrhenius (with a linearization for logarithmic transformation) to obtain the following:

$$\ln K = \ln Ko + \beta_1 \ln x + \beta_2 \frac{1}{t} + \beta_{1,2} \frac{X}{T} \qquad 1$$

Where the constant rate, K ($\min^{-1}$), X and T are moisture (g. gss^{-1}) and temperature (K) of the product, respectively. The equation 1, predicted a decrease on constant value while moisture was decreased, it behavior was observed by another investigators (9, 10), one example show when $X = 0.8$ y $T = 320$ K the result obtained is $k = 0.0020\ \min^{-1}$. These values are in the magnitude order as reported Goula and Adamopoulos (9) of $k = 0.0012\ \min^{-1}$ to $X = 0.8$ and $T = 323.15$ K in tomatoes, and by Uddin *et al.* (13) of $k = 0.00160\ \min^{-1}$ to water activity 0.81 and $T = 323.15$ K in kiwi. The effect of the air velocity it was not significant, this could be explained because a high air velocities produces an increase of temperature in the sample. In the table 1, as we can see there are shown the values of the parameters obtained, and with that there were calculated the constant rate in the process.

Table 1. Parameters of the model

Model	Parameters
$\ln K = \ln K_0 + \beta_1 \ln x + \beta_2\ 1/t + \beta_{1,2}\ X/T$	$\beta_1 = 0.255$ $\beta_2 = 2368$ $\beta_{1,2} = 191098$

The experimental results are presented in the figure 1, experimental data were analyzed with rate equations of different orders, and a first order equation is found to provide the best fit to describe the degradation of ascorbic acid under drying process. According to the experimental results, the ascorbic acid content decreases while the process going on and the deterioration rate has been found to be maximum at high levels of temperature.

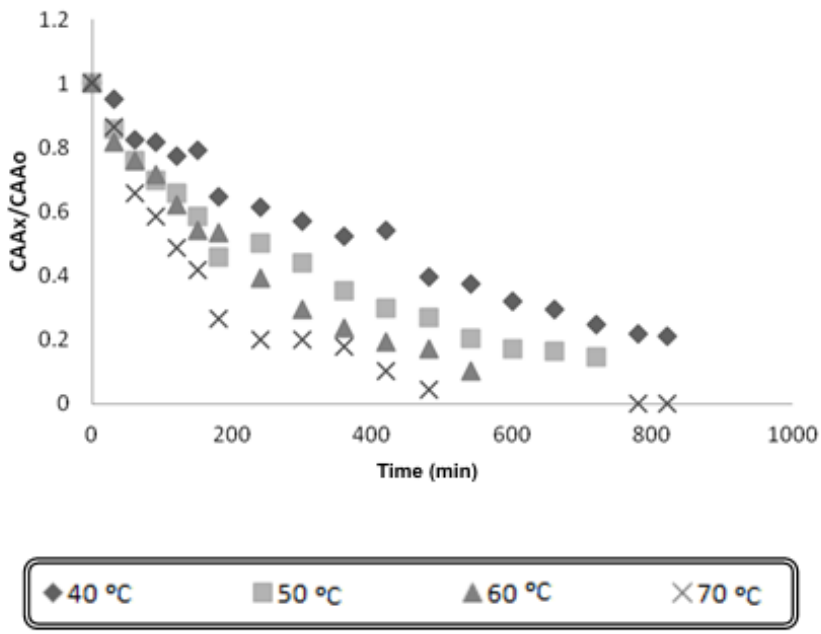

Figure 1. Experimental kinetics of ascorbic acid degradation at 40, 50, 60 and 70°C with thickness slices of 1.0 cm.

Figure 2, shows the drying kinetics obtained at 60 °C and air velocity of 1.5 m/s, the performance of the model vs experimental conditions was compared; the results showed a good prediction for the model. A continue line represents the performance of the model, in all cases studied it predicted adequately the degradation of ascorbic acid in papaya during drying process.

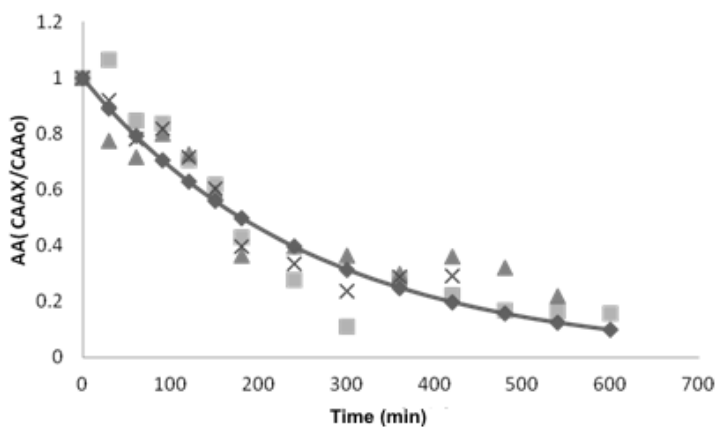

Figure 2. Experimental Kinetics of ascorbic acid at 60°C, *vs* prediction of ascorbic acid degradation with air velocity of 2.5 m/s.

From this results we can conclude that kinetic modelling realized was conducted to develop a functional tool, which in association with a heat transfer model, could predict ascorbic acid degradation in *C. papaya* and another fruits exposed to different heat processes under isothermal or non isothermal conditions.

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Tray Drying Of Banana (*Musa acuminate*) And Mango (*Mangifera indica*) Pretreated With Fructans

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Introduction

In recent years improvement of quality retention of dried by altering process conditions and pre-treatments, has been a major research goal. Pre-drying treatments such as addition of natural compounds are needed for fruits drying as the banana and mango in order to avoid physical-chemical changes. Drying can lead to browning reactions in fruit pulps (5) and pigment destruction (1). Moreover, high temperatures during drying can lead to total or partial destruction of the fruits antioxidants (vitamin C and carotenoids). Visual colour changes are considered a major sensory parameter and acceptable attribute of a food product. Many studies have shown that directly or indirectly, colour affects the acceptability and preference of food (4, 9, 12).

Mango (*Mangifera indica*) and banana (*Musa acuminate*) are ones of the most commonly consumed tropical fruits around the world. Mango and banana are appreciated by consumers as fresh fruit but also as an ingredient in processed products such as snacks, cereal, ice creams or fruit salads. Mango Manila and banana Roatan represent the most important variety produced and exported from Mexico, but it has a short post- harvest life.

Fructans are a large group of carbohydrates. Chemically fructans are polymers of fructose. Inulin and agavin are used increasingly in foods because it has unusual nutritional characteristics. The agave genere is one of Mexico's most important agroindustrial resources. It contains significant amounts of fructans with interesting nutritional and technological properties. Preliminary data on the impact of Agave fructans in obesity and diabetes areas present new niches for agavin's research as well as health and food applications. Inulin is a fructan that is linked by β(2-1) glycosidic bonds and contains either a terminal β-D-fructose or an α-D-glucose. The degree of polymerization (DP) of inulin is usually 2-60 units with an average DP-12. Inulin has attracted much attention in the food and pharmaceutical industries for its multiple benefits, such as dietary fiber (3, 7) and prebiotic nature (1, 6, 10). In this work, the influence of fructans on drying kinetics of thinly sliced banana and mango, and their physical characteristics during drying was investigated.

Material and Methods

Materials

The Mango (Manila var.) and banana (Roatan) fruits were obtained from fruits Agricultural Group located in the city of Actopan Veracruz, Mexico. Selecting was based on a similar ripening degree and fruits showing a skin colour more green than yellow. Banana and mango slices (3 cm of diameter and 0.2 cm of thickness) were used in the drying experiments which were carried out at air temperatures of 50 and 70 °C. The samples of fresh banana and mango were dipped into solutions containing inulin and agavin (0 and 10 % w/w) for 15 minutes, before drying. Quality evaluation was performed on all samples, including moisture content, water activity and color.

Drying equipment

Pilot plant dryer to carry up the tray drying of banana and mango samples was used. The vertical tray dryer used in this study is presented in Figure 1. It consists of a steel drying chamber that is connected to an electric heater at 4.5 kW, which was used to heat up the air to the desired drying temperature, which

was electronically controlled with a variation of ± 1 °C. The drying chamber is composed by ten trays (0.50 m wide and 0.75 m long) perforated through where flows vertically the drying air. The air velocity in dryer was measured with an anemometer at the outlet of the dryer. This was kept constant at 1 m/s adjusted during drying with a flow-valve.

Fig. 1. Pilot plant dryer used in tray drying of banana and mango samples

Experimental Design

The effect of different processing conditions on drying behavior of banana and mango was determined using a 2^{4-2} fractional factorial design for each fruit. The independent variables studied in the experimental design were: fructan type, air temperature, fruit, and fructan concentration. Table 1 shows the -1 and +1 levels and their relative values used in this study. The drying experiments were carried with control and treated samples at 50 and 70 °C at a constant speed of hot air.

Table 1 Independent variables and levels of study

Variable	-1	+1
Fructan type	agavin	inulin
Air temperature (°C)	50	70
Fruit	banana	mango
Fructan conc. (%)	0	10

The experimental runs with codified variables obtained from the 2^{4-2} factorial design are showed in Table 2. Experiments were made in duplicate and the mean values with standard deviations were reported.

Table 2 Operational conditions from experimental fractional design

Exp.	Fructan	Temp.	Fruit	Fructan Conc. (%)
1	-1	-1	-1	-1
2	-1	-1	+1	+1
3	-1	+1	-1	+1
4	-1	+1	+1	-1
5	+1	-1	-1	+1
6	+1	-1	+1	-1
7	+1	+1	-1	-1
8	+1	+1	+1	+1

Analytical methods

Product moisture content was measured with an infra-red moisture balance (MB35 HALOGEN, OHAUS) at 65 °C of samples containing approximately 1 g banana and mango sample. The results were expressed in terms of dry solid (d.s.). Water activity (a_w) of the banana and mango samples taken during drying process was determined at 25±1 °C using Aqualab water activity meter, series 3 TE, DECAGON, Washington.

The samples colour was measured by a colorimeter HunterLab (model MiniScan XE plus). The equipment was calibrated with white and black standards. The colour was determined by reflectance mode and expressed by L* (Luminosity), a (green-red) and b (blue-yellow) parameters. During each run of drying, samples were taken for colour measurements. Colour difference (ΔE) between the dried and fresh samples was calculated using following equation:

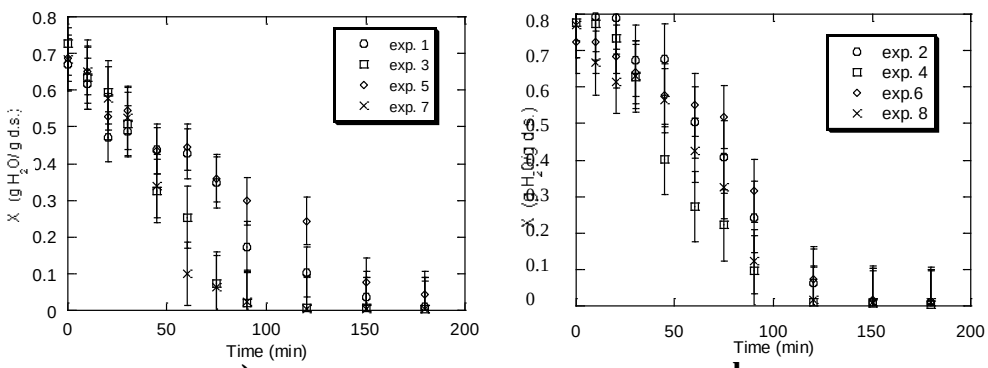
$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + \Delta b)^2}$$

Where, ΔL=L of dried sample-L of fresh sample, Δa=a of dried sample-a fresh sample and Δb=b of dried sample-fresh sample. Samples of 1 g were taken out from the dryer for moisture, a_w and color measurement.

In addition to the drying process, a sensory evaluation of dried banana and mango was performed. Twenty students of Instituto Tecnológico of Orizaba, México were selected as panelists of the sensory evaluation.

Results and Discussion

The drying kinetics of banana and mango were found to be greatly affected by the air temperature and fructan concentration during drying. In particular the temperature increment increases the drying constant and decreases the equilibrium moisture content of the dehydrated banana and mango samples (Figure 2). Reports found in the literature have shown too that, the drying velocity depends strongly on the air conditions and size of the material (12).

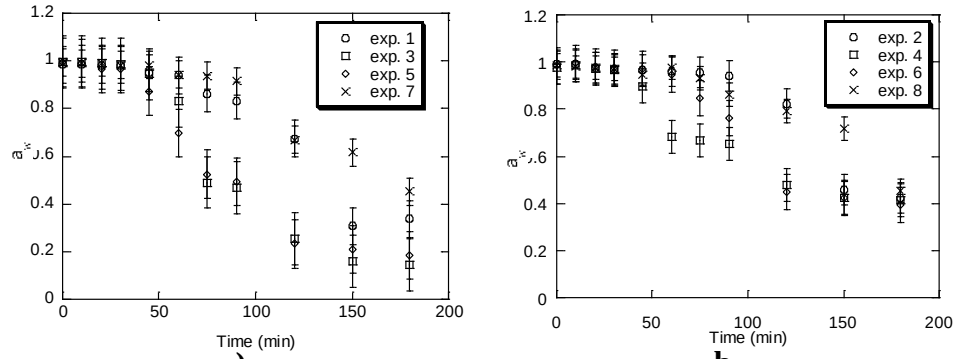


a) **b)**

Fig. 2. Drying kinetics: a) banana and b) mango

Water activity is an important factor in food preservation because the physical-chemical and microbiological alterations that can occur during processing and storing of a food product depend directly on this parameter (8). The effect of tray drying conditions on the water activity of banana and mango samples during the drying process is shown in Figure 3.

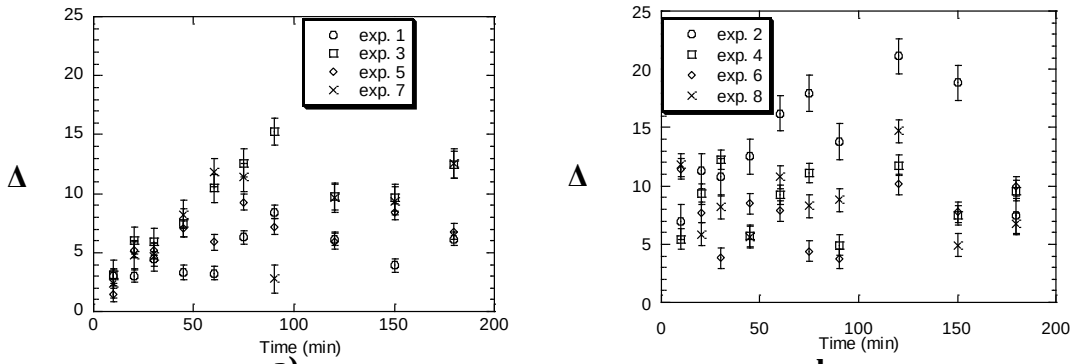
The analysis of variance (ANOVA) of the water activity showed significant difference (p=0.05) among treatments. It is observed that there was a significant difference in water activity between 0 and 10 % inulin samples. There was significant difference in water activity among the banana and mango samples dehydrated at two temperatures. As the water activity values of the dried banana and mango samples were lower than 0.50, they are considered safe and shelf-stable with respect to microbial growth. Figure 3 also shows that for the same drying time (for example 120 min), water activity in the product increases significantly decreasing the drying temperature.



a) **b)**

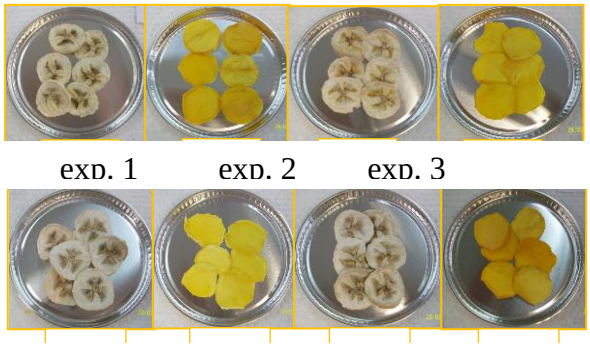
Fig. 3. a_w of samples during the tray drying: a) banana and b) mango

The Figure 4 shows the results of colour measurements (ΔE) of banana and mango samples during the tray drying. Banana and mango color was significantly ($p<0.05$) affected by the drying temperature of 70 °C. However, acceptable color was obtained when the fruits were dehydrated at 50 and 70 °C (Figure 5). Preferred colours of a dried product are those closest to the original colour of fresh sample. Colour is an important factor in order to accept a product (11, 12).



a) **b)**

Fig. 4. ΔE of samples during the tray drying: a) banana and b) mango.



exp. 1 exp. 2 exp. 3

exp. 5 exp. 6 exp. 7

Fig. 5. Colour of banana and mango samples dehydrated by tray dryer

The results of the sensory evaluation of dried samples obtained from various fructans concentrations compared to those from 0 % fructans were obtained. The highest score of overall acceptance was acquired when banana and mango were dried with fructans at 50 °C. At these conditions, dried samples also gained good scores for the acceptance of color and flavor. A decrease in the sensorial properties (color, flavor, taste and texture) was observed when the drying temperature reached 70 °C.

Conclusions

The drying velocity for pretreated samples of banana was higher than the drying velocity for the samples without fructan. Color (L, Hue, Chroma values) closest to the original color of fresh were obtained when the banana was dehydrated at 50-70 °C and pretreated with agavin (10%). It was observed that the samples pretreated and dried presented higher color retention than the samples dried without fructans. In general, the drying of thinly sliced banana and mango was influenced by air temperature, drying time and fructans.

Acknowledgements

The authors express acknowledge to the Mexican Consejo Nacional de Ciencia y Tecnología CONACyT (INFR-2011-03.173906) by the financial support of this research.

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Simulation And Validation Of Spray Drying Process Of Coffee Extract

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Abstract: In this work, a combination of simulation with experimental runs of spray drying process were developed in order to study the energy resources required to obtain a production of spray dried coffee's extract at given caffeine and moisture content. This model of simulation is in terms of heat and mass transfer coefficients and equilibrium relation for water among phases. The drying simulator was validated using spray drying trials carried out on pilot-plant scale. Simulations were developed at product flow (G_β) between 0.25 and 4.25 kg dry matter h^{-1}; input product moisture (X_β) between 0.5 and 3.5 kg water (kg dry matter)$^{-1}$, input air temperature (T_γ) between 145 and 220 °C; and air flow (G_γ) between 200 and 600 kg dry air h^{-1}. Simulation results suggest optimal conditions at initial product moisture of 0.35 g/g, 195 °C at input air, 200 kg dry air h^{-1}, and 4.38 kg dry product h^{-1}. The moistures of instant coffee (≤0.01 kg water (kg dry matter)$^{-1}$) obtained from the simulation were significantly slower than experimental ones. The moisture difference is explained in terms of product re-hydration during experimental treatments. In these conditions, for outlet temperature an average deviation of 0.00175 % between experimental and simulated were obtained. It appears that the suggested simulation model is adequate for predicting the spray drying of instant coffee.
Keywords: simulation, drying spray, coffee extract, energy

Introduction

Instant coffee, also called soluble coffee, is a beverage derived from brewed coffee beans. Its taste, flavour, aroma, and refreshing effect make it unique. When the coffee extract is discharged from the extractor, it has a solids content of 6-8 % TS, depending on how the extraction and aroma recovery is performed. To obtain a first-class instant coffee powder by spray drying, it is necessary to concentrate the extract (without the aroma fraction). Spray drying is a well-established method for converting liquid feed materials into a dry powder form.(1, 2) Spray drying is widely used to produce powdered food products such as whey, instant coffee, milk, tea, and soups, as well as health care and pharmaceutical products, such as vitamins, enzymes, and bacteria. (1)

Spray drying was reported as an appropriate preservation method for coffee extract and other food. Spray drying is however a high energy demand operation, requiring at least 2500 J g^{-1} of evaporated water, in the ideal process for pure water evaporation, and up to 3 or 4 times greater values in the real processes. A very common engineering method to save experimental runs, is the simulation of the process.

In recent years, computational fluid dynamics (CFD) has been increasingly applied to food processing operations.(3,4) For spray drying, CFD simulation tools are now often used because measurements of air flow, temperature, particle size, and humidity within the drying chamber are very difficult and expensive to obtain in large-scale dryers.

In this work, a combination of simulation with experimental runs of spray drying process were developed in order to study the energy resources required to obtain a production of spray dried coffee's extract at given caffeine and moisture content.

Material and methods

Materials and drying equipment

The coffee extract was produced by extraction from roasted coffee beans using only water as the means of extraction. This was obtained from a Mexican Industry (CAFIVER, S.A. de C.V.) located in the city of Orizaba Veracruz Mexico.

A VIBRASEC (model PSA5) spray drier was used (Figure 1). The setup consisted of a chamber of 2 m diameter and 4.3 m height. Ambient air is drawn into the drying chamber by means of a centrifugal fan. The air is heated by a group of electric resistances and introduced through an annular opening in the drying chamber ceiling. These electric resistances allow for an inlet air temperature of 300 °C. The spray system used was a rotator disc with a diameter of 0.15 m.

Fig. 1. Pilot plant dryer used in spray drying of instant coffee

Simulation

Luna et al. (2005) model was used in order to simulate spray drying process of coffee extract. This model of simulation is in terms of heat and mass transfer coefficients and equilibrium relation for water among phases. The internal heat and mass transfer coefficient are in function of particle transport properties, and external ones of flow regimen. That is, for spherical particles. The equations of model were called simulator, and programmed in Visual Fortran 2011 for windows. The fact that the simulator reproduces the experimental behaviour at different conditions depends of the adequate selection of thermophysical properties for air and coffee which was conducted (5).

Simulations were developed at product flow (G_β) between 0.25 and 4.25 kg dry matter h^{-1}; input product moisture (X_β) between 0.5 and 3.5 kg water (kg dry matter)$^{-1}$, and input air temperature (T_γ) between 145 °C and 220 °C. The simulation runs are listed in Table 1. Each run was carried out at three different air flow (G_γ): 200, 400 and 600 kg dry air h^{-1}. The drying simulator was validated using spray drying trials carried out on pilot-plant scale (Figure 1).

Table 1. Simulation runs of spray drying of coffee extract

Simulation runs	$T_{\gamma 0}$ (°C)	$G_{\beta 0}$ (Kg min^{-1})	$X_{\beta 0}$ (kg water kg d.s.$^{-1}$)
1	145	0.25	0.5
2	145	0.25	2
3	145	0.25	3.5
4	145	2.25	0.5
5	145	2.25	2
6	145	2.25	3.5
7	145	4.25	0.5
8	145	4.25	2
9	145	4.25	3.5
10	170	0.25	0.5
11	170	0.25	2
12	170	0.25	3.5
13	170	2.25	0.5
14	170	2.25	2
15	170	2.25	3.5
16	170	4.25	0.5
17	170	4.25	2
18	170	4.25	3.5
19	195	0.25	0.5
20	195	0.25	2
21	195	0.25	3.5
22	195	2.25	0.5
23	195	2.25	2
24	195	2.25	3.5
25	195	4.25	0.5
26	195	4.25	2
27	195	4.25	3.5
28	220	0.25	0.5
29	220	0.25	2
30	220	0.25	3.5
31	220	2.25	0.5
32	220	2.25	2
33	220	2.25	3.5
34	220	4.25	0.5
35	220	4.25	2
36	220	4.25	3.5

Analytical methods

Product moisture content was measured with an infra-red moisture balance (MB35 HALOGEN, OHAUS) at 65 °C of samples containing approximately 1 g coffee sample. The results were expressed in terms of dry solid (d.s.).

Results and discussion

Figure 1 shows some of the simulator results. The air temperature obtained by simulation was very close to the experimental. However, the moistures of instant coffee (≤0.001 kg water (kg dry solids)$^{-1}$) obtained from the simulation were significantly slower than experimental ones. The moisture difference is explained in terms of product re-hydration during experimental treatments.

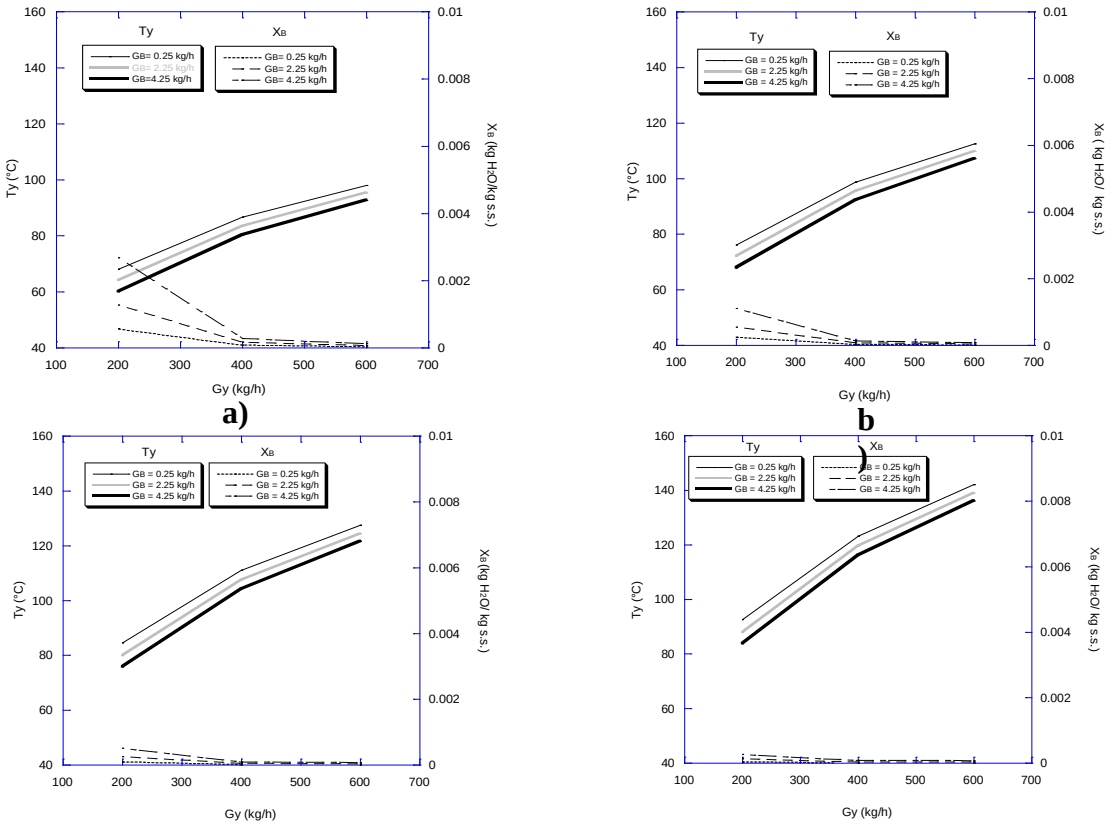
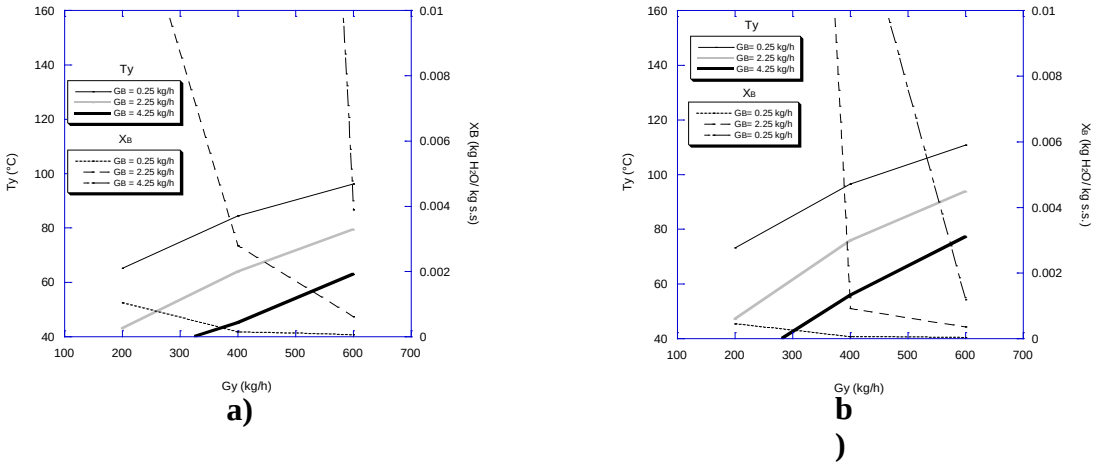


Fig. 1. Simulation for out air temperature and product moisture at $X_{\beta 0}$ = 0.5 kg H_2O (kg d.s.)$^{-1}$: a) $T_{\gamma 0}$ = 145 °C, b) $T_{\gamma 0}$ =170 °C, c) $T_{\gamma 0}$ = 195 °C and d) $T_{\gamma 0}$ = 220 °C



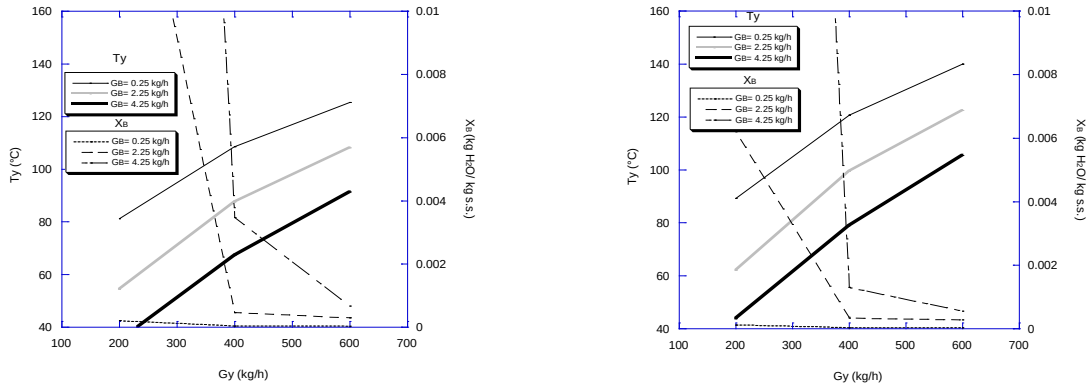


Fig. 2. Simulation for outlet air temperature and product moisture at $X_{\beta 0}$ = 3.5 kg H_2O (kg d.s.)$^{-1}$: a) $T_{\gamma 0}$ = 145 °C, b) $T_{\gamma 0}$ =170 °C, c) $T_{\gamma 0}$ = 195 °C and d) $T_{\gamma 0}$ = 220 °C

The experimental moistures were evaluated from the coffee powder collected in the separation unit after an air exposition. Like the air in our location has a relative humidity greater than 70 %, the instant coffee is fine and hot (closer than output temperature), and its moisture is slower than 0.001 kg water (kg d.s.)$^{-1}$, exists a significant rehydration before the moisture evaluation.

Table 2 shows validation results of spray drying simulation of coffee extract for some conditions listed in Table 1. Simulation results suggest optimal conditions at initial product moisture of 0.35 kg H_2O (kg d.s)$^{-1}$, 195 °C at input air, 200 kg dry air h^{-1}, and 4.38 kg dry product h^{-1}. In these conditions, for outlet temperature an average deviation of 0.00175 % between experimental and simulated were obtained. It appears that the suggested simulation model is adequate for predicting the spray drying of instant coffee.

Table 2. Validation results of spray drying simulation of coffee extract

Simulation runs validated	$T_{\gamma j}$ (°C) Exp.	Sim.	Average deviation %	$X_{\beta j}$ (°C) Exp.	Sim.	Average deviation %
10	76.14	71	0.00243	0.0249	90.24	7.23
11	74.68	71	0.00333	0.0245	86.40	5.18
12	73.23	71	0.00445	0.0260	82.88	3.14
19	84.65	79	0.00095	0.0179	94.69	7.15
20	82.7	79	0.00154	0.0181	91.49	4.68
21	81.23	79	0.00209	0.0180	88.38	2.82
28	92.68	87.5	0.00043	0.0100	95.7	5.92
29	90.72	87.5	0.00079	0.0110	92.81	3.68
30	89.24	87.5	0.00110	0.0100	89	1.98

Conclusions

In this paper, a methodology that combines empirical with mechanistic modeling was proposed for the simulation of spray drying of coffee extract. The best variables values reported are particulars for the stated constrictions, but the simulator is general for coffee spray drying. New optimal values may be obtained for other particular requirements, by using the same models.

Acknowledgements

The authors express acknowledge to the Mexican Consejo Nacional de Ciencia y Tecnología CONACyT (INFR-2011-03.173906) by the financial support of this research.

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Drying and Extraction Process of Lemongrass (*Cymbopogon Citratus*)

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Introduction

Cymbopogon citratus is one of the most used plants in Mexico folk medicine for the treatment of nervous and gastrointestinal disturbances. The usual way to use it is by ingesting an infusion made by pouring boiling water on fresh or dried leaves.The use of medicinal plants is part of a competitive market, which includes pharmaceuticals, food, cosmetics, and perfumery markets. In pharmaceutics, plant extracts are especially relevant due to the use of their active substances as prototypes for medicine development and as sources of raw material, to obtain both pharmaco and adjuvant. Medicinal plants are also used to obtain medicines made exclusively from plant extracts such as phytotherapeutic medicines, whose use is limited by various factors including: cultivation, harvest period, climatic factors, humidity, brightness, part of the plant, transportation method, storage, drying process and extraction process which may all modify the composition of the products, directly affecting safety and efficiency[1,8].

The post-harvesting process of medicinal plants has great importance in the production chain, because of its direct influence on the quality and quantity of the active principles in the product sold[9]. For this reason, adequate dryers are needed, using temperature, velocity and humidity values for drying air that provides a rapid reduction in the moisture content without affecting the quality of the active principles of medicinal plants. The drying process may also contribute to a regular supply and facilitate the marketing of plants, because drying improves the transport and storage[1].

Chemical changes are the most important in the post-harvest of medicinal plants and can be influenced by drying[4,2]. Moreover, drying can promote changes in product appearance (color) and smell, modifying the final quality. According to Lorenzi and Matos[5], drying of medicinal species is a preparation process, carried out to meet the needs of the pharmaceutical industry, which doesn't have the adequate conditions to use fresh plants on the scale required by industry.

The essential oils of medicinal plants are the constituents most sensitive in the drying process. The drying air temperature limits are determined according to the sensitivity of these substances, because the product temperature is increased during the drying process[6,11].The purpose of this work was to determine the influence of drying conditions on the physic-chemical characteristic of lemongrass and extraction process.

Material and methods

The specie (*Cymbopogon citrates*) utilized for drying process and extraction experimentation was cultivated at the growing area at the Orizaba, Ver. Mexico region. The harvest period was from February to November 2011.Blades of the leaves were cut in small parts (Figure 1), about 2, 4 and 6 cm2 surface area and dried at different temperatures, until establishment of the weights. Moisture content, water activity and Hunter color (L, Hue, and Chroma parameters) were determined for each treatment. To conduct the experiment, a randomized experimental design was used, with three repetitions. The drying treatments were arranged in a factorial (3x3) with three surface areas (2, 4 and 6 cm^2) and three drying air temperatures (40, 60 and 80 °C).

Pilot plant dryer to carry up the tray drying of lemongrass samples was used. The vertical tray dryer (VTD) used in this study, it consists of a steel drying chamber that is connected to an electric heater at 4.5 kW, which was used to heat up the air to the desired drying temperature, which was electronically controlled with a variation of ± 1 °C. The drying chamber is composed by ten trays (0.50 m wide and 0.75 m long) perforated through where flows vertically the drying air.The air velocity in dryer was measured with an anemometer at the outlet of the dryer. This was kept constant at 1 m/s adjusted during drying with a flow-valve.

Fig. 1. Lemongrass samples cut at different surface areas: a) 2 cm^2, b) 4 cm^2 and c) 6 cm^2

Product moisture content was measured with an infra-red moisture balance (MB35 HALOGEN, OHAUS) at 65 °C of samples containing approximately 1 g lemongrass sample. The results were expressed in terms of dry solid (d.s.).

Water activity (a_w) of the lemongrass samples taken during drying process was determined at 25±1 °C using Aqualab water activity meter, series 3 TE, DECAGON, Washington.

The samples colour was measured by a colorimeter HunterLab (model MiniScan XE plus). The equipment was calibrated with white and black standards. The colour was determined by reflectance mode and expressed by L* (Luminosity), a (green-red) and b (blue-yellow) parameters. During each run of drying, samples were taken for colour measurements. Colour difference (ΔE) between the dried and fresh samples was calculated using following equation:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + \Delta b)^2}$$

Where, ΔL=L of dried sample-L of fresh sample, Δa=a of dried sample-a fresh sample and Δb=b of dried sample-fresh sample. Samples of 1 g were taken out from the dryer for moisture, a_w and color measurement.In the following process a hydrodistillation and alcohol-distillation, during 2 hours, by laboratory equipment was utilized to extract the essential compounds. So, studies were conducted to show the effect of different conditions in the drying process on the amount and quality of extract of *Cymbopogon citratus*.

Results and discussion

The drying kinetics of lemongrass were found to be greatly affected by the air temperature and surface area during drying. In particular the temperature increment increases the drying constant and decreases the equilibrium moisture content of the dehydrated lemongrass samples (Figure 2). Reports found in the literature have shown too that, the drying velocity depends strongly on the air conditions and size of the material[3,7,12].

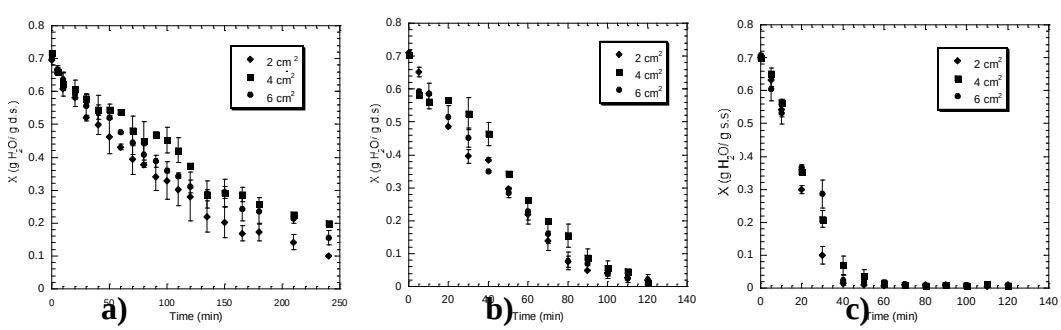

Fig. 2. Drying kinetics of Lemongrass samples dehydrated by tray drying: a) 40 °C, b) 60 °C and c) 80°C.

Water activity is an important factor in food preservation because the physical-chemical and microbiological alterations that can occur during processing and storing of a food product depend directly on this parameter[10].The effect of tray drying conditions on the water activity of lemongrass samples during the drying process is shown in Figure 3.

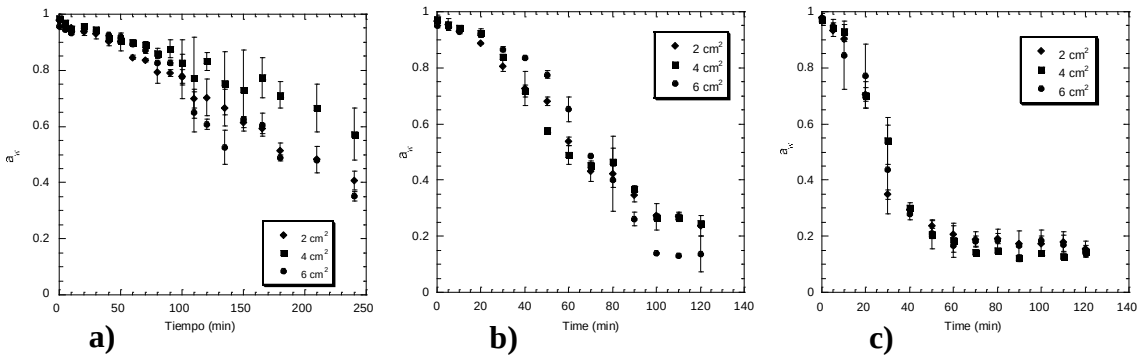

Fig. 3. a_w of lemongrass samples during the tray drying: a) 40 °C, b) 60 °C and c) 80°C.

The analysis of variance (ANOVA) of the water activity showed significant difference (p=0.05) among treatments at 40 °C. It is observed that there was a significant difference in water activity between 2 and 4 cm^2 surface area samples. There was significant difference in water activity among the lemongrass samples dehydrated at three temperatures. As the water activity values of the dried lemongrass samples were lower than 0.50, they are considered safe and shelf-stable with respect to microbial growth. Figure 3 also shows that for the same drying time, water activity in the product increases significantly decreasing the drying temperature.

The Figure 4 shows the results of colour measurements (ΔE) of lemongrass samples during the tray drying. Lemongrass color was significantly ($p<0.05$) affected by the drying temperature of 80 °C. Acceptable color was obtained when the lemongrass was dehydrated at 40 and 60 °C (Figure 5). Preferred colours of a dried product are those closest to the original colour of fresh sample. Colour is an important factor in order to accept a product[10].

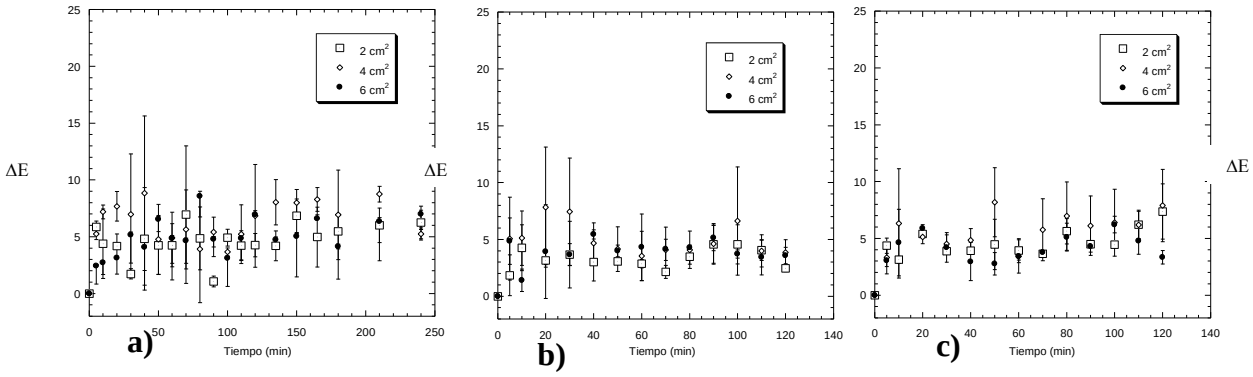

Fig. 4. ΔE of lemongrass samples during the tray drying: a) 40 °C, b) 60 °C and c) 80°C.

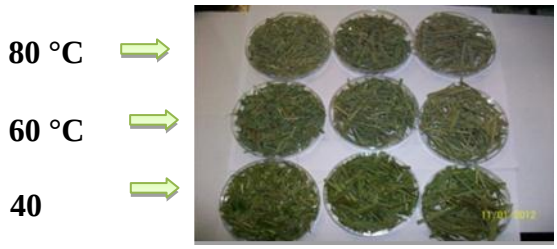

Fig. 5. Colour of lemongrass samples dehydrated by tray dryer

A higher amount of extract of lemongrass could clearly be collected with the lower drying temperatures (40 and 60 °C) and surface area (1 and 2 cm^2). The other conditions reduced the antioxidant activity of the dried lemongrass when compared to fresh lemongrass. The experimental values of the antioxidant activity (DPPH method) were 87.29 % and 63.6 % for the extract by hydrodistillation and alcohol-distillation respectively. In general, the statistic analysis of drying results showed significant effect on extraction degree of lemongrass based on the colour parameter (Table 1).

Table 1.Colour parameters of lemongrass extract.

Sample	Solvent	Colour			
		L	**Hue**	**Chroma**	**ΔE**
fresh	water	$15.4^{2.40}$	$83.5^{1.22}$	$6.61^{1.32}$	$33.7^{4.4}$
fresh	ethanol	$1.2^{0.13}$	$-50.8^{8.19}$	$0.80^{0.28}$	$40.1^{0.2}$
dried (80°C)	water	$13.1^{4.73}$	$69.3^{5.19}$	$7.53^{3.09}$	$30.6^{0.7}$
dried (80°C)	ethanol	$0.72^{0.18}$	$-45.9^{24.6}$	$0.64^{0.59}$	$34.4^{7.6}$
dried (60°C)	water	$17.4^{9.56}$	$64.8^{4.54}$	$10.9^{0.58}$	$37.6^{10.}$
dried (60°C)	ethanol	$1.60^{0.80}$	$-63.6^{0.21}$	$0.85^{0.02}$	$39.6^{2.9}$

The pH in fresh samples (time 0) remained comparable to lemongrass extract 2 h after processing but decreased significantly after 5 h (Figure 6). The pH in extract samples decreased between 5.5 and 6 after 10 h of process (Figure 6). pH was influenced significant ($p \leq 0.05$) by solvent and sample type of extraction process.

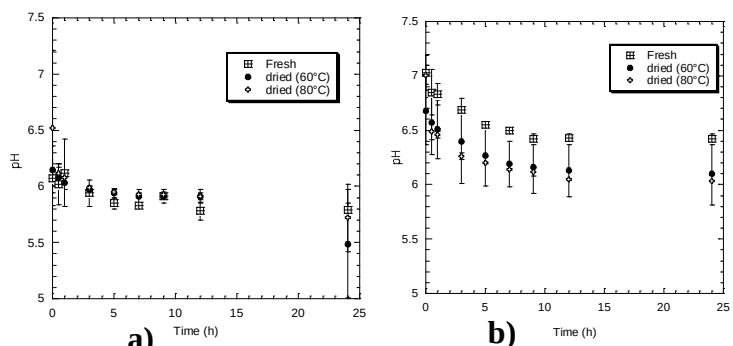

Fig. 6.Evolution of PH during the extraction process, a) diameter 0.15 cm, b) diameter o.45 cm and longitude 1.5 cm

The Figure 7 shows the results of colour of lemongrass samples during extraction process. The extract was more dark green through time in most experiments.

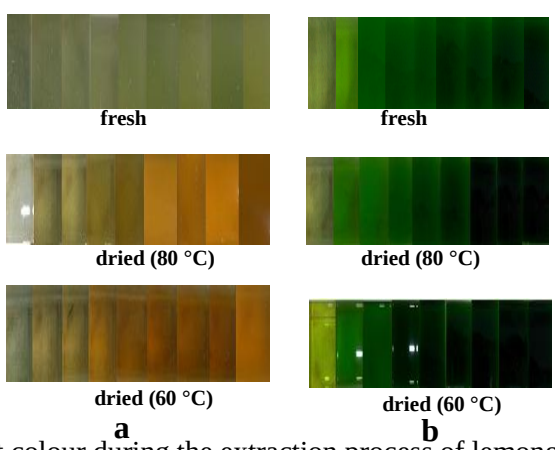

Fig. 7. Extract colour during the extraction process of lemongrass samples

Conclusions

A higher amount of extract of lemongrass could clearly be collected with the lower drying temperatures (40 and 60 °C) and surface area (2 and 4 cm^2). The other conditions reduced the antioxidant activity of the dried lemongrass when compared to fresh lemongrass. The experimental values of the antioxidant activity (DPPH method) were 87.29 % and 63.6 % for the extract by hydrodistillation and alcohol-distillation respectively. In general, the statistic analysis of drying results showed significant effect ($\alpha<0.05$) on extraction degree of lemongrass.

Acknowledgements

The authors express acknowledge to the Mexican ConsejoNacional de Ciencia y TecnologíaCONACyT (INFR-2011-03.173906) by the financial support of this research.

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Microwave And Conventional Drying Of Carrot Pomace

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Introduction

Recently food industry is being forced by governments to develop production without secondary residues. Therefore, in these industries there is a considerable emphasis on the recovery, recycling and upgrading of their by-products [1]. Since these by-products may still contain many valuable substances such as phytochemicals with antioxidant activities[2], could be converted into a range of commercial products [3]. For this reason, different researches have assayed different methods in order to obtain new raw materials products from fruit and vegetable processing by-products [4].

In recent years, the improvement of quality retention of dried products by altering drying process and/or pretreatment has been a research goal. Novel drying methods including vacuum, infrared, microwave, freeze- and osmotic drying has been used in different fruits and vegetables [5]. Microwave drying is an alternative method because of its uniform energy and high thermal conductivity to the inner sides of the material, space utilization, sanitation, energy savings, precise process control and fast startup and shutdown conditions [6].

Carrot (*Daucus carota* L.) is an economically important horticultural crop that has gained popularity in recent decades due to increased awareness of its nutritional value. China, Russia and the United States are the top 3 producers of carrots in the world, contributing with almost 50% of the world carrot crop (27,386, 535 tons in 2008). Mexico is the thirteenth carrot producer in the world [7]. Carrots do not supply a significant amount of calories to the human diet, but do supply nutrients in the form of phytochemicals, such as carotenoids, anthocyanins, and other phenolic compounds. Carrots contribute significantly to dietary vitamin A intake through α- and β-carotene and modestly to other nutrients, but research has also focused on carrots as a source of fiber [8].

The objective of our study was to obtain two different carrot pomace powders dried by microwaves and hot air, and to evaluate their carotenoid and fiber content of the carrot pomace, as well as its antioxidant activity.

Methods and Materials

1. Conventional and microwave drying.

Carrots were purchased from a local market and kept at 4°C prior to use. The carrot pomace was obtained from juice extraction (Juice extractor, Moulinex 753, Mexico) .It was obtained a 51.6% of juice and 48.4% of carrot pomace, which was dried in an air-oven (Linderbeg Blue M,) at 60°C during 8 h. The dried sample was finely grounded using a Krups GVX211 grinder and passed through a #40 mesh sieve (0.42 mm). In order to estimate the best microwave drying conditions (power and time) for carrot pomace, an orthogonal routable central composite design Response Surface Methodology was used. The independent variables (factors) were power (595-972 Watts) and time (15-20 min). The responses were moisture, temperature and color parameter (a*) of pomace. The sample dried under the optimum conditions of power and time determined by the routable central composite design Response Surface Methodology in the microwave oven was finely grounded using a Krups GVX211 grinder and passed through a #40 mesh sieve (0.42 mm).

3.Chemical composition

Moisture content was determined by gravimetric heating (110°C ± 1°C for 2 h) using a 2–3 g sample. Ash, protein and fat were analyzed according to AOAC. Dietary fiber (DF) was tested using the 985.29 AOAC methods [9]. DF fractions were obtained as indigestible residues after enzymatic digestion of non-DF

components; the insoluble residues were isolated by filtration, and soluble fiber was precipitated with ethanol. Dried residues corresponded to insoluble dietary fiber (IDF) and soluble dietary fiber (SDF), respectively.

4. Extraction of bioactive compounds

Samples (1g) were extracted with 20 ml of 80% acetone for 1 h with occasional stirring using a vortex mixer. The extract was centrifuged at 8000×g. The supernatant obtained was subjected for the estimation of bioactive compounds such as carotenoids, phenolic compounds and antioxidant activity [10]. The total carotenoid content was estimated according to the method described by Rodriguez-Amaya, 2001 [11].The absorbance of the extract was determined at 450 nm wavelength in a UV-Vis spectrophotometer (Genesys 10 UV, Thermo Scientific, USA).

5. Water holding and Oil binding capacities.

Water holding capacity was determined using the method outlined by Yu, Ahmedna & Goktepe, 2007 [12]. One gram of carrot pomace was weighed into a preweighed 15-mL centrifuge tubes. For each sample, 10 ml of distilled water was added and mixed using a Barnstead Thermolyne vortex at the highest speed for 2 min. After the mixture was thoroughly wetted, samples were allowed to stand at room temperature for 30 min, then centrifuged (Hermle z323k, Germany) at 3000 g for 20 min. The supernatant was decanted and the centrifuge tube containing sediment was weighed. Water holding capacity (grams of water per gram of protein) was calculated as WHC = (W2 - W1)/ W0, where W0 is the weight of the dry sample (g), W1 is the weight of the tube plus the dry sample (g), and W2 is the weight of the tube plus the sediment (g). Oil binding capacity was determined using the method of Yu, Ahmedna & Goktepe, 2007 [12]. One gram (W0) of carrot pomace was weighed into pre-weighed 15-mL centrifuge tubes and thoroughly mixed with 10 mL (V1) of vegetable oil (Patrona™ Corn oil) using a Vortex mixer. Samples were allowed to stand for 30 min. The carrot pomace–oil mixture was centrifuged at 3000 g (Hermle z323k, Germany) for 20 min. Immediately after centrifugation, the supernatant was carefully poured into a 10 ml graduated cylinder, and the volume was recorded (V2). Fat absorption capacity (milliliter of oil per gram of carrot pomace) was calculated as FAC = (V1 - V2)/W0. Triplicate samples were analyzed for each carrot pomace sample.

7. ABTS assay.

Total antioxidant capacity was evaluated using the method reported by Re, Pellegrini, Proteggente, Pannala, Yang & Rice-Evans, 1999 [13] with Trolox as the standard. A stock solution was prepared by reacting 7 mM ABTS with 2.45 mM potassium persulfate to generate the ABTS cation chromophore. This solution was diluted with absolute ethanol until reaching an absorbance of 0.7 ± 0.02 at 734 nm. A sample of 10 µL of carotenoid extract was added to 990 µL of ABTS solution and the reaction was followed over a seven minute time course. Total antioxidant activity or capacity was calculated relative to the reactivity of Trolox as the standard under the same conditions. The results were expressed as µmol Trolox equivalent/g of carrot pomace DW.

8. Statistical analysis.

Samples were analyzed in triplicate. Results are expressed as mean values ± standard deviations. Response surface data were analyzed using the Design Expert version 7.0 statistical program. Data from carrot pomace powders were processed using the software Sigma Stat (version 3.1 Systat Software Inc. San José California, USA). The analysis of variance (ANOVA) followed by Tukey test multiple comparison were used to contrast groups.

Results and Discussion

Surface response plots for moisture (M), temperature (T), and color (C) of carrot pomace dried in microwave oven are shown in Figure 1 A-C. The equations that describe the surface response behavior for moisture, temperature, and color of the carrot pomace dried in microwave oven were the following:

$M= 9.41-21.67A-5.61B+1.01AB+11.67A^2+4.42B^2$ (Eq.1)

Where M is carrot pomace moisture, A is drying time (min) and B is microwave oven power.

$T=77.44+25.80A+11.16B+9.25AB+16.10A^2+11.42B^2$ (Eq.2)

Where T is temperature, A is time (min), and B is microwave oven power (W)

$C= 16.16-3.46A-2.51B-0.22AB-1.35A^2-2.20B^2$ (Eq.3)
Where C is color, A is drying time (min), and B is microwave oven power (W)

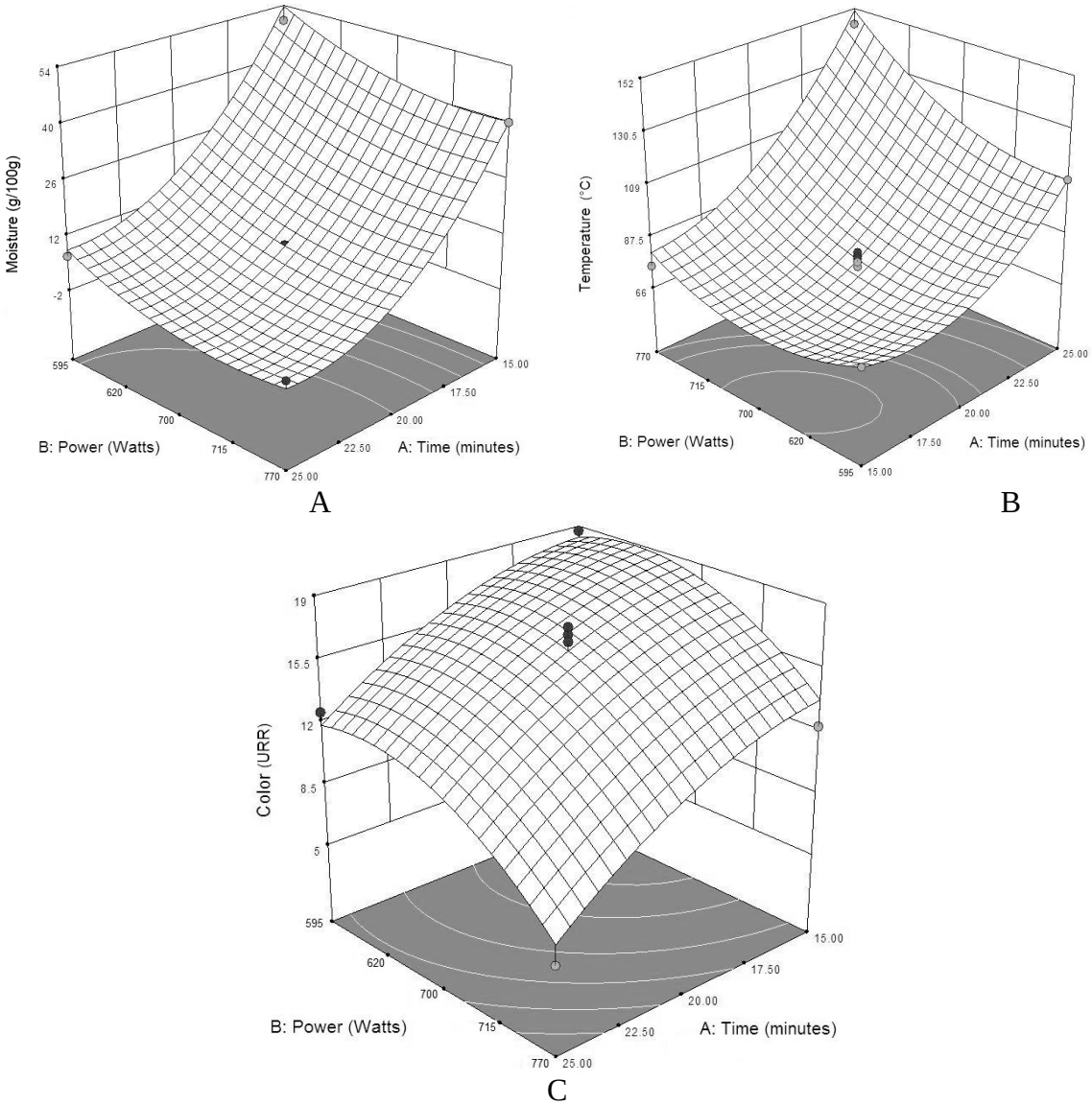

Figure 1. Effect of time and power on the A) moisture, B) temperature and C) color of carrot pomace dried on microwave oven.

In order to obtain the best process conditions of the microwave oven drying process, the three equations were processed with the numerical optimization function of the Design Expert version 7.0 software. Optimization was done looking for minimum moisture, the maximum color and lower product temperature values of carrot pomace in the microwave oven, with a desirability of 0.977. Optimum drying conditions were obtained at a power of 700 W and a time of 20 minutes.

The microwave dried powder had 8.73 g/100g of moisture and the conventional powder had 9.62g/100g of moisture. No significant differences ($p>0.05$) were found in protein, ashes, fat, dietary fiber and carbohydrates content among dried pomaces. These values are comparable to the values reported by [14] for carrot pomace and higher than the values reported by [15] in passion fruit, pineapple and guava by-products. Total dietary fiber content of the carrot powders was 64.35±3.50 g/100g dry weight (DW) for

the microwave powder and 64.15±3.15 g/100 g dry weight (DW) for the conventional powder. The microwave powder contained a higher amount of carotenoids than the conventionally dried carrot pomace powder (599.04 ± 6.52 mg/100g vs. 507.25 ± 4.23 mg/100g DW, $p<0.05$)

Water retention capacity was 15.41± 0.8 for microwave powder and 16.17 ± 0.45 g H_2O/g for conventional powder, and oil retention capacity was 2.94 ± 0.18 mL oil/g for microwave powder and 3.08 ± 0.05 mL oil/g for conventional powder.

On the ABTS assay, the microwave powder presented the highest antioxidant capacity (474.21 ±52.02μMol trolox/g vs. 352.58 ±28.09 μMol trolox/g, $p< 0.05$).

Conclusion

The results obtained showed that carrot pomace could be used as an alternative to obtain a carrot powder that may be employed in the formulation of different foods.

Acknowledgements

This research was financed by SIP-IPN project 20120075, and the student received grants from COFAA-IPN and CONACyT

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Drying Characteristics of Huauzontle Seeds in a Fluidized Bed Dryer

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Abstract

Strains of the Mexican pseudocereal Huauzontle (*C. berlandieri spp. nuttalliae*) have been selected recently for commercial production as a broccoli-like vegetable, and it can be found under cultivation in several agricultural centers of Mexico. The present work is an attempt to assess the drying kinetics of Huauzontle seeds in a fluidized bed dryer in order to increase the knowledge of the theory of drying and storing of this kind of products. The drying experiments were performed at different temperatures of the drying air (150 and 100 ^{o}C), using variable air velocity of 2.06, 1.81, and 1.59 m/s. Results showed that high air velocity and high operating temperature enhanced the kinetics of drying of the seeds. The moisture loss from the seeds was described by the Fick's diffusion equation, and based on the obtained results the effective moisture diffusivity was estimated, getting a value in the range from 2.8 to 8.4 x 10^{-9} m^2/s. The relationship between the temperature and the effective moisture diffusivity was described adequately by means of Arrhenius-type equation. The activation energies calculated were between 12.89 to 17.22 kJ/mol. ANOVA test reported a significant interaction at $P<0.05$ for both temperature and air velocity on moisture diffusivity. Results are in good agreement with literature for other crops and the information found in this research can be useful in the design, simulation of drying equipment, and the exploitation of seeds in several applications.

(Keywords: Huauzontle seeds, fluidized bed drying, mass diffusion coefficient)

Introduction

Fluidized bed drying has been used widely to process different kinds of food and agricultural products (1), where the conditions are favorable for a rapid heat and mass transfer thanks to its many advantages, such as high rates of drying (as consequence of an excellent contact between the drying medium and the samples), high thermal efficiency and uniform drying procedure resulting in very low residual moisture content in the materials and in a rapid drying (2). Although a large number of fluidized bed drying works have been reported on different kinds of fruits and grains (3), no data is available on its use with Huauzontle seeds. The pseudocereal Huauzontle (*Chenopodium berlandieri spp. nuttalliae*) can be found under cultivation as an irrigated row crop in several agricultural centers of central Mexico (4). Today, there is very little scientific information about chemical and functional properties of Huauzontle seeds and none knowledge about drying of their products. In this work, fluidized bed drying experiments of Huauzontle seeds were performed and the effects of various drying conditions on the drying kinetics were investigated in order to increase the knowledge of the theory of drying and storing of this kind of products.

Materials and methods

Experimental set-up

A pilot-scale fluidized bed dryer consists of a cylindrical borosilicate glass drying chamber, which has an inferior diameter of 0.1 m, superior diameter of 0.15 m and a height of 0.35 m. An air supply line from an air bower with control valve was used to generate the air needed for drying while an air heater at 2 KW

was used to heat the air to the desired drying temperature (±1°C). A fine wire mesh was spot welded over the distributor plate to arrest the flow of solids from the fluidized bed to the air chamber. A computer controller was used to control the whole drying unit, including the air blower and the air heater.

Material

The raw mature seeds were collected in crops from Cholula-Puebla, Mexico. These were cleaned manually to remove any foreign matter. The moisture content of the seeds at this point was 9%. Huauzontle seeds were soaked in water at temperature of 90 ^{o}C for 1 h. At this point the moisture of the seeds was approximately 85%.

Experimental methods

Experiments were performed to compare the drying rates by using variable drying conditions, inlet air temperatures of 150 and 100 ^{o}C and inlet air velocities of 2.06, 1.81 and 1.59 m/s. A 100 g of soaked Huauzontle seeds was introduced into the equipment after ensuring the steady temperature and the air flow rate. Huauzontle seeds were sample at 3 min intervals and were analyzed for moisture content. The moisture content of the samples was determined by drying in a convective oven at 110 ^{o}C for 3 h.

The effective moisture diffusivity of the seeds was calculated using the mathematical Fick's second diffusion model (*Eq.* 1). The solution to this equation, developed by Crank (5), could be used for various regularly shaped bodies such as rectangular, cylindrical and spherical products:

$$MR = \frac{X(t) - X_e}{X_0 - X_e} = \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}exp\left[-n^2\pi^2 Deff\frac{t}{r^2}\right] \quad Eq.1$$

where *MR* is the moisture ratio, *Deff* is the effective moisture diffusivity (m^2/s), *r* is the equivalent radius of the seed (m), *t* is the time (s), *X(t)* is the theoretical moisture content at any time *t*, X_e is the equilibrium moisture content and X_0 is the initial moisture content. When n = 1, equation 1 states as follows (*Eq.* 2):

$$MR = \frac{6}{\pi^2}\exp\left[\frac{-\pi^2 Deff}{r^2}t\right] \quad Eq.2$$

It could be rewritten in a logarithmic form as shown in *Eq.* 3.

$$lnMR = ln\frac{6}{\pi^2} - \frac{\pi^2 Deff}{r^2}t \quad Eq.3$$

The effective moisture diffusivity (*Eq.* 4) was calculated from the slope of a straight line, plotting experimental drying data in terms of *ln(MR)* versus drying time (6):

$$Deff = \frac{mr^2}{\pi^2} \quad Eq.4$$

Subsequently, the effective moisture diffusivity can be related to the temperature of fluidized bed drying by simple Arrhenius equation as shown in the below equation (*Eq.* 5):

$$Deff = A \cdot \exp(-E_a/RT) \quad Eq.5$$

where *Ea* is the activation energy (kJ/mol), *A* is the Arrhenius pre-exponential factor (m^2/s), *R* is the universal gas constant (8.314 kJ/molK) and *T* is the absolute air temperature (K). The values of the activation energy are represented through the slope of each linear relationship (*Eq.* 6):

$$ln(Deff) = ln(A) - \frac{E_a}{RT} \quad Eq.6$$

The activation energy is determined from the slope of the Arrhenius plot, *ln (Deff)* vs. *1/T* (*Eq.* 7).

$$E_a = mR \quad Eq.7$$

Statistical analysis

To verify the statistical significance of the studied parameters, means and standard deviation of three measurements were determined. Where it was appropriate, differences between groups were tested by 2-way ANOVA using SPSS v11.0 software (SPSS Inc. Chicago, IL). Significant differences (*P* values < *0.05*) were assessed using Tukey`s test (7).

Results and discussion

The changes in experimental *MR* as function of drying time of soaked Huauzontle seeds at different inlet air drying temperatures and inlet air velocities are presented in Figure 1. It can be seen from Figure 1 that drying was fastest, as expected, when using an inlet air temperature of 150 ^{o}C at an air velocity of 2.06 m/s (*P<0.05*). This is reasonable because the mode of the moisture removal is by internal diffusion and the driving force is the temperature difference between the center of the particle and fluidizing air (8).

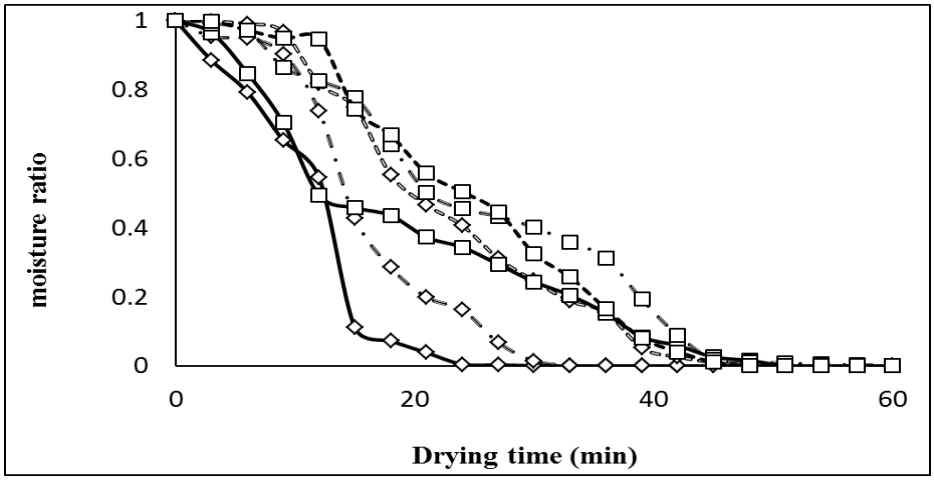

Figure 1. Moisture ratio versus drying time of germinated Huauzontle seeds at different inlet air temperature and inlet air velocity. *Inlet air temperature symbols*: ◊) 150 ^{o}C, □) 100 ^{o}C. *Inlet air velocity symbols*: ——) 2.06 m/s, —·—) 1.81 m/s, and -------) 1.56 m/s

The moisture ratio determined was reduced exponentially with drying time as expected; this behavior is typical of some biological materials. Based on the obtained results in this work, the effective moisture diffusivity (*Deff*) was estimated getting a value in the range from 2.8 to 8.4 x10^{-9} m^2/s (Table 1). As expected here, *Deff* values of dried samples of soaked Huauzontle seeds increased significantly (*P<0.05*) with increasing drying air temperature and inlet air velocity. Corn, wheat and rice present *Deff* values ranged between 0.11 x10^{-10} to 5.3 x10^{-10} m^2/s, 5.1 x10^{-10} to 2.2 x10^{-9} m^2/s and 1.3 x10^{-10} to 3.2 x10^{-9} m^2/s, respectively (9). Generally, the effective moisture diffusivity is used as consequence of limited information on the mechanism of moisture transfer during drying and complexity of the process. The use of Fick's diffusion equation to represent the drying kinetics in a fluidized bed assumes all the solids in the fluidized bed to be exposed to uniform conditions. The moisture in the solids is assumed to be uniformly distributed initially and the model predicts the varying moisture concentration profile in the solids with respect to time. Irrespective of the kind of dryer, the diffusion coefficient estimated serves as an indicator for the estimation of the drying kinetics and often comparison of the performances of different dryers is based on the estimated diffusion coefficient (10).

Table 1. Evaluated effective moisture diffusivity values (m^2/s) at different drying conditions.

	Inlet air velocity (m/s)		
Temperatures	2.06	1.81	1.56
150	8.4 x10^{-9} a	6.0 x10^{-9} c	4.4 x10^{-9} e
100	2.8 x10^{-9} b	3.7 x10^{-9} d	5.3 x10^{-9} f

Means +/- standard deviation in columns and rows with different letters are significantly different (*P*<0.05 by Tukey´s test).

The effect of temperature on effective moisture diffusivity has been described using Arrhenius-type relationship to obtain the activation energy (*Ea*) from experimental data of the effective diffusivity. The result shows a linear relationship between (*ln Deff*) and (*1/T*) or an Arrhenius-type relationship with a R^2 of 0.6507, 0.8517 and 0.93 for inlet air velocities of 1.56, 1.89 and 2.06 m/s, respectively. The activation energies calculated from the linear regression were 12.89, 17.22 and 16.52 kJ/mol, respectively. These values are useful for equipment design and scaling in the studied temperature range.

Conclusions

In this study, the drying behavior of soaked Huauzontle seeds in a fluidized bed dryer was studied at drying air temperatures ranging from 100 to 150 ^{o}C and fixed inlet air velocities of 2.06, 1.89 and 1.56 m/s. The calculated effective diffusivities ranged from 2.8 to 8.4 x10^{-9} m^2/s, increasing with increase both in temperature and in inlet air velocity of the sample. The activation energies calculated from the linear regression were 12.89, 17.22 and 16.52 kJ/mol, respectively. The experimental data on fluidized drying of soaked Huauzontle seeds exhibit that the moisture extraction rate depend significantly on the inlet air temperature rather than in the air mass flow rate. The information found in this research can be useful in the design, simulation of drying equipment, and the exploitation of seeds

Acknowledgements

The authors thank the Consejo Nacional de Ciencia y Tecnología (CONACYT) for financing the research project-101498.

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Thermal Analysis In *pre* And *post rigor mortis* In *Longissimus dorsi* Of FESC Line Rabbit Meat.

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Introduction

Most people are accustomed to consuming beef, pork, fish and birds, but we have little knowledge about rabbit meat nutritional properties, which is becoming a new nutrition and taste alternative on the Mexican tables in a very different preparation styles.

Meat and meat rabbit products may be considered functional food, because of their numerous compounds. For example, the Loin (*m. Longissimus dorsi*) composition is: water, 74.6±1.4 g/100 g; protein, 22.4±1.3 g/100 g; lipid, 1.8±1.5 g/100 g ash, 1.2±0.1 g/100 g and offer excellent nutritive and dietetic properties. Along with high protein content, rabbit meat also contains high essential amino acid levels (EAA). The lean meat portion (water and protein) is relatively constant with a decreasing trend from the middle to back and front pieces of the carcass. Mineral content is around 1.2–1.3 g/100 g meat. Furthermore, rabbit meat could contribute to the intake of vitamin E, minerals such as Ca, Mg, K, and omega-3 fatty acids (FAs), all of which are often under-consumed by adults.

In the last 50 years, rabbit meat world production has increased to 1.6 million tons in 2009. China is currently the world leading producer (700,000 t/year). Italy (230,000 t/year), Spain (74,161 t/year) and France (51,400 t/year) are the main rabbit meat producers in Europe. In Mexico the common rabbit races are the white New Zealand, California, Chinchilla, butterfly, Satin Red, and some others like the black Aztec.

The muscle conversion to meat is a very complex process. It carries out a series of interdependent processes like oxidation and proteolysis; these changes develop the meat qualities attributes [4]. In the absence of ATP, actin and myosin combines permanently, there is rigor mortis, which arises after the death of the animal. The blood flow interruption deprives the oxygen muscle; the cellular respiration is paralyzed; the redox potential changes and anaerobic glycolysis arise. In these conditions, the ADP is converted into lactic acid.

The lactic acid formation causes a pH decrement, which inhibits various enzymes activities. Finally, though glycolysis stops glycogen stores are not exhausted, and ATP content approaches zero, that is when the actin and myosin form irreversible actomyosin complex. The drop in the pH and the bonds formation between the actin and myosin bring out electrical modifications and changes over the proteins configuration.

Near the isoelectric point (pH 5.5), the protein chains tend to approach and create a partially crystalline structure; this motivates a decrease in water retention capacity, affecting the meat texture [1].

After death, if the animal is not cooled quickly, the pH decrease at a too low level; this can motivate a proteins denaturation and a large part of the water in the miofibrilar structure is ejected from the intercellular spaces or even exuding out of the cell, with meat texture damage.

FES-C rabbit line was developed in la Facultad de Estudios Superiores Cuatitlán- UNAM, from august 2002 to july 2003.The reproductive parameter responses of 5 cycles were created from the races white New Zealand, California and Chinchilla (Valencia, 2005) with the aim to improve meat quality. Not many

researches were made in this line around quality parameters. The aim of this work was to evaluate the effects of the pre and post rigor mortis processes, over the transition temperatures corresponding to proteins denaturation in rabbit *Longissimus dorsi*, by calorimetry during the muscle to meat conversion.

Materials and methods

Raw material:
M. *Longissimus dorsi* from in line rabbits produced in the FESC-UNAM institution with 70 ± 2 days of age and 2.1 Kg in weight were used in this work. *Pre rigor mortis* samples were used 2 hours after slaughtering and *post rigor mortis* after 48 hours. pH was measured (ORION model 3-Star) and used as a selection criteria for the muscles (pH 5.53± 0.13%). After selection, the muscles were stored at 4°C. Proximate composition was also determined for fresh meat with water content 64.82±1.50% b, protein 20.5±2.54%, lipids 6.038±0.52% 1.13±0.07%. Determinations were done in triplicate.

Modulated DSC measurements:
Samples were analyzed using a differential scanning calorimeter with temperature modulation (MTDSC 2920, TA Instruments, New Castle, U.S.A.). Cooling was carried out with a refrigerated cooling system RCS. The cell constant calibration was performed with indium and heat capacity with sapphire. The Universal Analysis Software V. 4.4A was used to analyze the thermograms. 20.8 mg ±0.28% of meat was placed in hermetic aluminum DSC pans (TA Instruments, New Castle, U.S.A.). Samples were analyzed in triplicate by heating in the modulated DSC furnace in nitrogen atmosphere at a rate of 5°C min $^{-1}$ with temperature modulation of 0.8°C/60s. Thermal decomposition data for the samples were collected over a temperature range 30 to 90°C.

Results and Discussion

The conversion of muscle to meat is essentially a biochemical and structural process after animal slaughter. Pearce et al, 2011 [5], working on the water distribution in meat using NMR, establishes that the glycogen levels drop and the ATP decreases to critically low levels. In this situation, the myosin heads start to become permanently bound to the actin filaments, which result in formation of the actomyosine complex, muscle become inextensible with a longitudinal shrinkage that reduces the spaces with important consequences for water mobility. Also, after depletion of ATP there are formation of actomyosin complex and the decrease of pH cause alteration of the structure in the myofibrilar proteins [2]. During the conversion of muscle to meat, inmobilized water, likewise free water, can be mobilized due to the alteration of the muscle cell structure [3].
Figure 1 shows the behaviors of the total heat flow for samples in *pre and post rigor mortis*. The observed differences are due to the consequences of the conversion process, particularly in the area of myosin denaturation, with a width to the half height of endotherm at 17.36 ° C for *pre rigor mortis* and 12.78 ° C in *post rigor*. This indicates a more time used to carry out the transition in the first sample with a lower enthalpy required and important changes in myosin denaturation zone during the conversion process. At temperatures close to 80 ° C, a second transition is observed with a similar enthalpy values for both samples as shown in table 2.

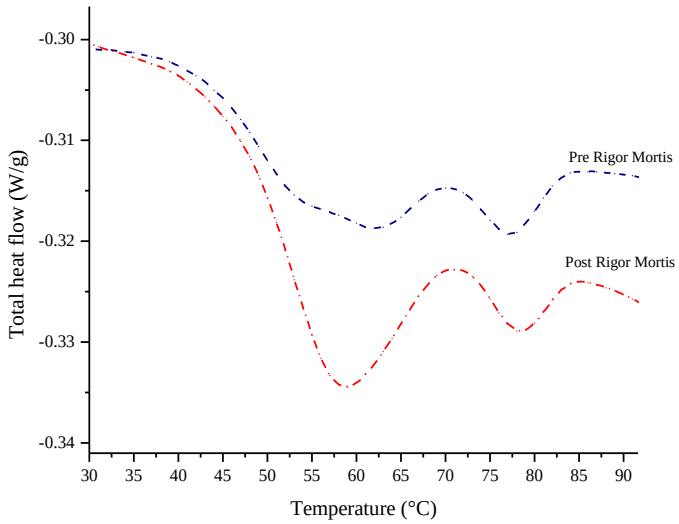

Fig. 1 Total heat flow in samples of *pre and post rigor mortis* in FESC line rabbit loin (*Longissimus dorsi*).

Table 2. Maximal temperature and enthalpy of *pre* and *post rigor mortis* samples in Rabbit *longissimus Dorsi*.

Onset °C	Maximum °C	Stop °C	Enthalpy J/g	Sample
45.33	54.93	69.42	1.400	Pre rigor mortis
71.87	77.38	84.71	0.4211	Pre rigor mortis
48.4	57.9	70.59	2.5430	Post Rigor Mortis
72.98	78.26	84.84	0.4290	Post Rigor Mortis

Figure 2 shows the derivative of the total heat flow in which is observed a stability of the transitions temperatures about 75 ° C in both samples. Differences are evident in the range of 50 - 55 ° C which corresponds to the myosin denaturation zone. Apparently in the convertion process of muscle to meat, this protein presents a different behavior due to changes in the muscle cell structure, water mobility and changes in pH.

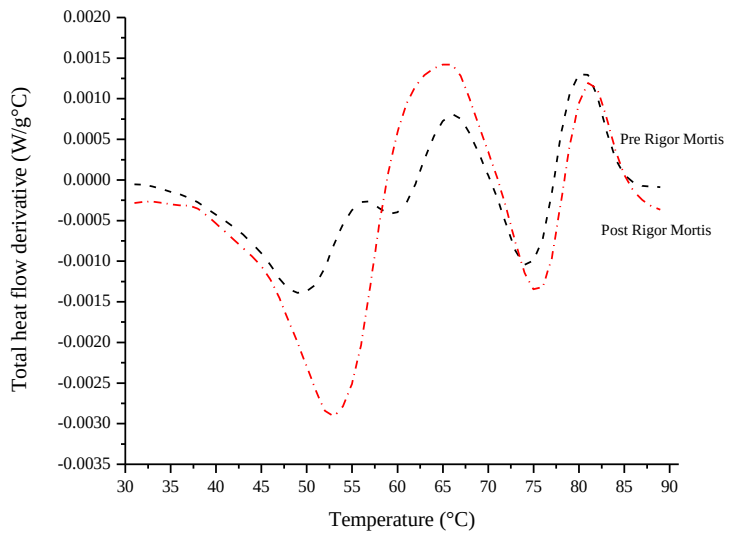

Fig 2. Derivative of total heat flow of Rabbit samples in *pre* and *post rigor mortis* status.

The heat capacity is a property of materials which characterizes them and for food this depends on the water content, their mobility and structural changes at the molecular level of its components. Figure 3 presents the heat capacity profile for samples of rabbit loin *pre* and *post rigor mortis*, observed a continuous increment from 2.0 to 2.5 J/g°C in the range of 40 to 90 ° C. Changes in the transitions temperatures are seen in 54°C and 82°C in good agreement with those reported for myosin and collagen denaturation. The Cp to these temperature ranges are located in 0.18 J/g°C and 0.067 J/g°C respectively for *pre rigor mortis* and 0.136 and 0.067 J/g°C in *post rigor mortis* samples, being the most important change in the myosin denaturation area.

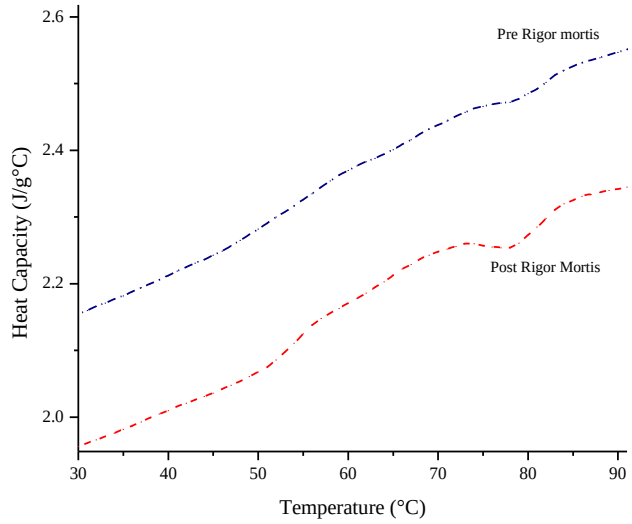

Fig.3. Heat capacity in *pre* and post *rigor mortis* in line FESC rabbit muscle (*Longissimus dorsi*)

Acknoweledgments

Authors grateful to the DGAPA-UNAM, PACIVE-UNAM for the financial support for this research through the grants numbers IN204506, IT202312 and NCOS-01. Also to Dr. Crisóforo Mercado M. for the technical support in this work.

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Heat And *Momentum* Transfer Simulation For Tomato Puree In A Concentric-Pipe Heat Exchanger

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Tomato puree is a typical product in the tomato processing industry and has an economic significance in Sinaloa. Tomato puree is a shear thinning fluid, which is subjected to unit operations involving heat transfer in heat exchangers. Therefore, it is necessary to evaluate system pressure drop and outlet temperature of tomato puree in the heat exchanger. These parameters have a direct influence on the power requirements, in energy consumption and in the equipment design at industrial level. The aim of this work was to simulate the pressure drop and outlet temperature for tomato puree in a concentric-pipe heat exchanger, using temperature dependant heat and *momentum* transport properties.

Materials and methods

Tomato paste was used as feedstock, which was obtained from *Productos Industrializados del Noroeste* enterprise. Initial soluble solid content of tomato paste ranged from 19 to 21 °Brix and was subsequently diluted to achieve a tomato puree concentration of 12 °Brix. Thermophysical properties [thermal conductivity (k), specific heat capacity (Cp), and bulk density (ρ)] and rheological properties [consistency index (K) and flow behavior index (n)] were determined as a function of temperature (20, 30, 40, 50, and 60 °C).

Thermal conductivity (k) was evaluated by the linear heat source method. A heat flow is provided from a heat source located inside of the sample and the temperature change at a known distance was measured (1). The k value was obtained by regression analysis between temperature (T) and the natural log of time ($\ln t$), using the following equation:

$$T - T_1 = \frac{Q_L}{4\pi k}\left[\ln\left(\frac{t}{t_1}\right)\right]$$

where $Q_L = I^2R/L$ is the supplied heat per unit of length (W/m), I is current intensity (A), R is probe resistance (Ω), and L is probe length (m).

Specific heat capacity (Cp) was obtained using a differential scanning calorimeter (DSC2920 TA Instruments, USA). Sapphire was used as a reference material, sample size (m) was approximately 20 mg and heating rate (dT/dt) was 20 °C/min (2). The absorbed energy was recorded on a thermogram. For each temperature, the amount of heat flow (dQ/dt) absorbed to increase the temperature is proportional to the enthalpy change (ΔH), which is obtained from the difference between the baseline and the line corresponding to the sample as shown in the following equation:

$$\frac{dQ}{dt} = E\Delta H$$

Where E is a calibration constant which is obtained from the reference material. The behavior of a liquid at constant pressure is equalized with the previous equation:

$$dQ = mCpdT \qquad Cp = \frac{E}{dT/_{dt}} \frac{\Delta H}{m}$$

Bulk density (ρ) was calculated by the difference of volume methodology. The sample was equilibrated to the appropriate temperature and its density was calculated from the ratio of mass (m) and volume (V) as follows:

$$\rho = \frac{m}{V}$$

Rheological parameters, consistency index (K) and flow behavior index (n), were measured using a viscometer (Brookfield, DV-II, USA). The behavior of non-newtonian fluids is described by the power law model:

$$\tau = K\dot{\gamma}^n$$

Shear stress (τ) and shear rate (γ) were calculated using the recorded value from the alpha viscometer scale (0 – 100), rotation speed (N) and conversion factors $[(k]_{\alpha_\tau}, k_{N_\gamma})$ which depend on the number of used spin or flow behavior index (3). For this purpose, the following equations were used:

$$\tau = k_{\alpha_\tau}\alpha \qquad \gamma = k_{N_\gamma}N$$

Rheological parameters were obtained by a regression analysis between log τ and log γ, where n and K are obtained from the slope and the ordinate at the origin, respectively:

$$\log(\tau) = n\log(\dot{\gamma}) + \log(K)$$

Heat and *momentum* transfer simulation was performed using the FLUENT software for the numerical solution and the GAMBIT software for creating the mesh. A triple pipe heat exchanger was simulated using the following diameters and length: D_1= 0.0127 m, D_2= 0.0254 m, D_3= 0.0381 m, L= 3m. Hot water was flowing inside the inner pipe and the external annulus, while tomato puree was flowing in the central annulus in countercurrent to water inlets. Inlet temperatures of puree and water were 25 and 85 °C, respectively while input speed of both streams was 0.05 m/s. Heat and *momentum* transport properties, as function of temperature, were input to the FLUENT software, and velocity profile (axial and radial), field of pressure and temperature profile were simulated.

For evaluating the effect of temperature on tomato puree thermophysical and rheological properties, a completely random design was used. Levels of the factor were 20, 30, 40, 50, and 60 °C. Fisher test was applied as a multiple comparison test (α = 0.05).

Results and discussion

The k values showed an increase from 0.604 to 0.754 W/m °C as temperature was increased from 20 to 60 °C (Figure 1). These results are in agreement with those reported by Drusas and Saravacos (1985) for

tomato paste 27-44 °Brix (4). They found that conductivity increased from 0.460 to 0.660 W/m °C, as temperature increased from 30 to 50 °C. These authors also noted that as soluble solids content increased, thermal conductivity decreased.

Specific heat capacity also showed an increase from 3327 to 5760 J/kg °C as temperature rose from 20 to 60 °C (Figure 2). In a study made by Aparicio (5), specific heat capacity of tomato paste (29 °Brix) increased from 3716 to 4001 J/kg °C as temperature rose from 0 to 60 °C.

Bulk density (ρ) showed no significant difference (α=0.05) at the different studied temperatures and the average bulk density was 1023 kg/m^3.

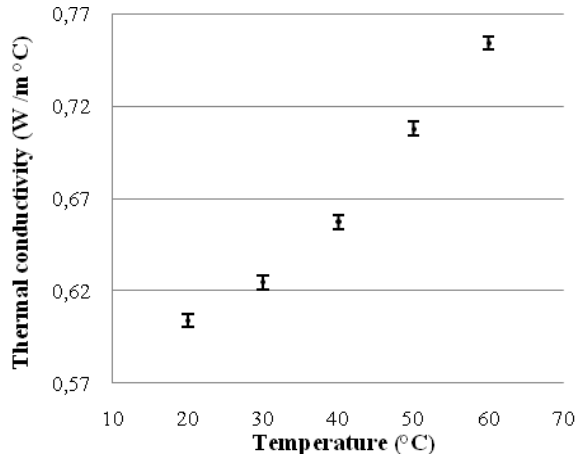

Figure 1. Thermal conductivity of tomato puree (12° Brix) at different temperatures (LSD=0.008)

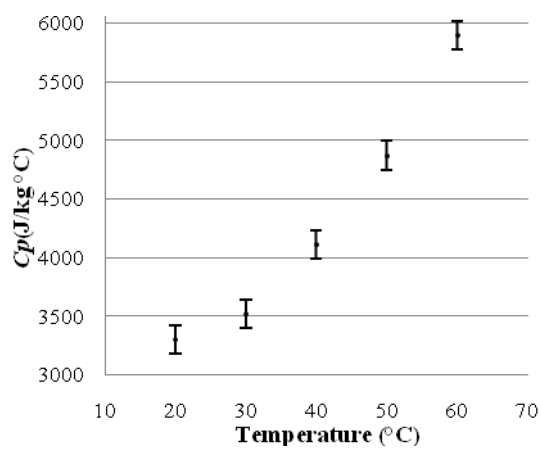

Figure 2. Specific heat capacity of tomato puree (12 °Brix) at different temperatures (LSD=245.22)

The flow behavior index (n) showed no significant difference (α=0.05) and the average value was 0.26 (Figure 3), which is typical of a pseudoplastic fluid; while the consistency index (27.4 to 36.4 Pa s^n) decreased as temperature rose (Figure 4).

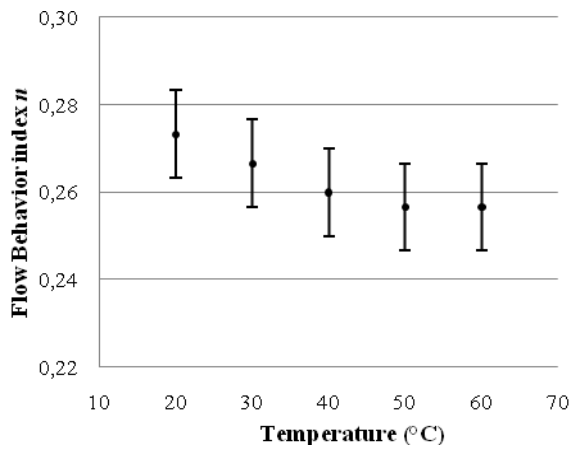

Figure 3. Flow behavior index for tomato puree (12 °Brix) at different temperatures (LSD=0.02)

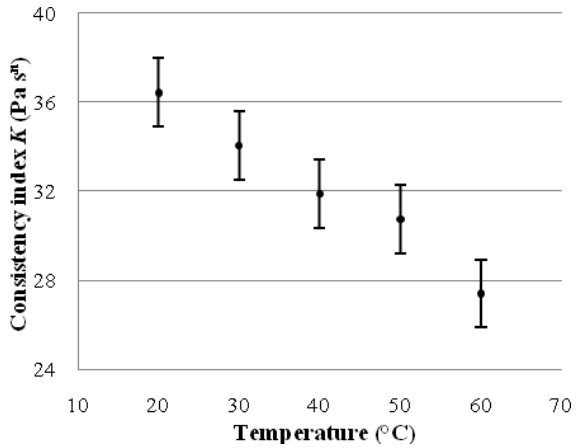

Figure 4. Consistency index for tomato puree (12 °Brix) at different temperatures (LSD=3.07)

Rao et al. (6) determined the rheological parameters of tomato puree (11 °Brix). They observed that as temperature increased from 76 to 120 °C the *n* value decreased from 0.27 to 0.23, and consistency index also decreased from 7.0 to 5.5 Pa s^n. The difference in the range of values for the consistency index (*K*) may be due to the enzyme inactivation method used. Thermophysical and rheological parameters are shown in Table 1.

Table 1. Thermophysical and rheological properties for tomato puree (12°Brix)

Temperature °C	***ρ*(kg/m^3)**	***k*(W/m °C)**	***Cp* (J/kg °C)**	***n***	***K* (Pa s^n)**
20	1021 [a]	0.604 [a]	3327 [a]	0.27 [a]	36.43 [a]
30	1023 [a]	0.625 [b]	3552 [a]	0.27 [a]	34.07 [ab]
40	1024 [a]	0.657 [c]	4102 [b]	0.26 [a]	31.89 [bc]
50	1023 [a]	0.708 [d]	4805 [c]	0.26 [a]	30.74 [c]
60	1023 [a]	0.754 [e]	5760 [d]	0.26 [a]	27.42 [d]

Note: Different letters in the same column indicate significant difference

The velocity profile was fully developed at 0.12 m from the inlet, and although it was not symmetrical, the maximum speed (0.0747 m/s) was reached in the annulus radial center. (Figure 5). Although the velocity profile was completely developed at this point, the temperature profile had just begun to develop (Figure 5).

The temperature profile (Figure 6) was asymmetric. The coldest point was found at 0.57 mm from the annulus radial center and an increase of 22.2 °C was found throughout the pipe at the coldest point. The pressure drop was 43.9 kPa.

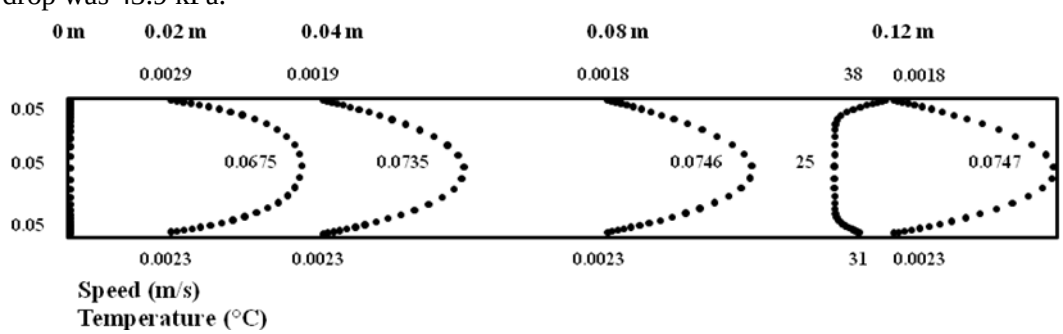

Figure 5. Velocity profile development

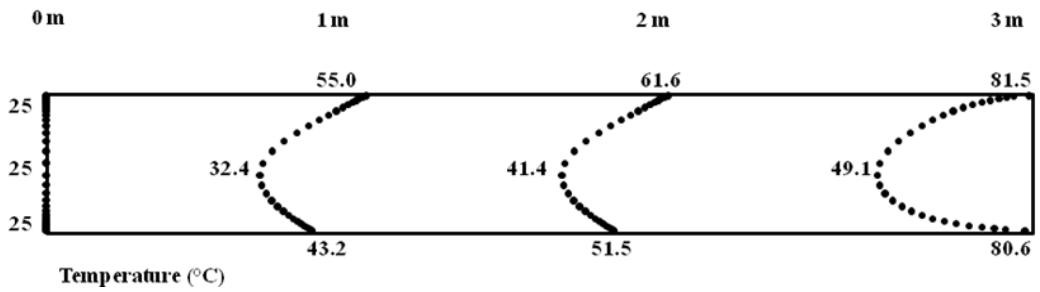

Figure 6. Temperature profile development

Conclusion

Thermophysical and rheological properties of tomato puree were characterized. Thermal conductivity and specific heat capacity showed similar trends to those reported in the literature, a similar situation occurred with consistency index and flow behavior index. These simulated results will be useful for the estimation of the pumping system, the power consumption and the pipe heat exchanger design when tomato puree is used as feedstock.

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Convective-Drying Optimization Of Apple Slices Based On Physicochemical Parameters

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Drying is widely used for food preservation, including fruits and vegetables. Water activity is reduced at appropriate levels to minimize chemical and physical changes during storage of the dried food, inhibiting the growth of microorganisms and decay [(1)]. Apple provides appreciable amounts of vitamin C and fiber. The drying process has the advantage of prolonging shelf life of fruits. The objective of this work was to obtain the best process conditions for the convective drying of apple slices cv. Granny Smith based on color, firmness and rehydration rate parameters.

Materials and methods

Apples (*Malus domestica* cv. Granny Smith) were obtained from a local market in the city of Culiacan, Sinaloa, selected based on color and shape uniformity and absence of defects. They were washed and sliced (Torrey slicing, RB-250) at a uniform thickness, which ranged from 2.6 to 5.4 mm according to the experimental design. The slices were blanched at 80 °C for 1 min in a 0.1 % calcium chloride solution (J.T. Baker) and then immersed for 5 min in a 0.1 % 4-hexyl-resorcinol solution (Sigma-Aldrich) [(2)].

Convective drying was accomplished in a hot air tunnel dryer built from fiberglass. The heating system consisted of four tubular electrical resistances of 1000 W each and the speed of the dryer's fan (S&P extractor, CK.-50, Chicago, IL) was regulated through a rheostat [(3)]. Apple slices were placed in acrylic trays inside the tunnel dryer [(4)].

Response surface methodology (RSM) was used to optimize the convective drying process. A rotatable central composite design with five levels of variation was selected as experimental design. The process variables were drying air temperature (51.9 to 73.1 °C) and slice thickness (2.6 to 5.4 mm). Air velocity was fixed at 1.5 m/s [(5)].

Water activity (a_w) was measured with an Aqualab hygrometer (Aqualab CX-2, Decagon, Pullman, USA) previously calibrated with neutral distilled water (a_w = 1.00) and a saturated NaCl solution (a_w = 0,753 at 25 °C). Drying of apple slices was finished when the water activity was less than 0.6 for each treatment.

Total color difference (ΔE) was evaluated using a tristimulus colorimeter (Minolta Chromameter CR-200, Osaka, Japan). Six apple slices were selected from each treatment and three shots were performed on the surface of each apple slice (fresh or dried) to record the L*, a*, and b* color parameters. Total color difference was calculated using the square root of the sum of the squared differences between the chromatic parameters of dried and fresh samples [(4)]. Therefore, color of fresh apple slices was selected as reference and the total color difference indicated how far the color of dried slices was from that of fresh samples.

To calculate the rehydration index (RI), dried apple slices were immersed in water at 40 ºC for 30 min and then placed on blotting paper to remove the excess water on the surface. Rehydration index was calculated from the ratio of the mass of adsorbed water to the mass of the dried sample and expressed as percentage. Five replicates were performed for each of the 13 treatments [(4)].

Shrinkage (S) was calculated from the ratio of the volume difference between the fresh and dried sample to the fresh sample volume and expressed as percentage. Volume of the samples was determined by toluene displacement using a 10 mL graduated cylinder. Five replicates were accomplished for each treatment [(4)].

Firmness (F) was evaluated according to the methodology reported by Marangoni [(6)] with some modifications. A digital force gauge (Chatillon, E-DFE100, USA) was used to determine the maximum compressive force to penetrate the fruit tissue (5). The test was performed in five slices per treatment and three readings in each slice were taken.

Drying curves were built plotting the moisture content (kg of water/kg dried solids) against time (min). The sample mass was recorded every minute with an electronic balance (Sartorius, TE1502S, Germany), through an interface (interface kit Sartorius, PTYDO01) [(7)].

The critical moisture content (*Xc*) was statistically evaluated assuming that moisture content showed a quadratic behavior as a function of time, $X = \beta_0 + \beta_1 t + \beta_2 t^2$. A multiple linear regression analysis was performed to estimate the β coefficients. First, all moisture content and time data in a treatment were considered in the regression analysis. Then, final data were removed one by one and the significance of the β's was evaluated every time a data point (X, t) was removed. When the coefficient of the quadratic term, β_2 was no significant ($\alpha > 0.05$), the most suitable model for adjusting the remainder experimental data was a straight line, as expected for data corresponding to the constant-rate drying period. Critical moisture content corresponded to the moisture content at the end of the constant-rate drying period [(7,8)].

In the constant-rate drying period, drying rate (N) was calculated using the slope of the drying curve ($-dX/dt$) and the ratio between the dry solid mass and transfer area [(7)]. The mass transfer coefficient (k_Y) was calculated from the ratio of the mass flux (N) to the difference between absolute humidity at the adiabatic saturated temperature (Ys) and absolute humidity of air stream (Y) [(9)].

Apparent diffusivity (D_{app}) was calculated by solving the Fick's second law for unidirectional diffusion in an infinite slab, using 10 terms of the series. For each moisture content this series was solved for the D_{app} using the second order Newton-Raphson's method for finding of roots [(7)].

In the optimization of the convective drying process, the Design Expert 2008 software, version 7 was used [(10)]. Total color difference (ΔE), rehydration index (RI) and firmness (F) were selected as response variables and shrinkage was not selected because it showed little variation. Optimization criteria were taken as low values of ΔE and high values of RI and F.

Results and discussion

Total color difference (ΔE) values ranged from 8.3 to 13.1, where smaller values corresponded to shorter processing times (73.1 °C and 4 mm) and higher values were related to longer processing times (55 °C and 5 mm). In all treatments, ΔE was relatively low, which could be due to the pre- treatment applied to the fresh apple slices. Blanching in the calcium chloride solution and immersion in 4-hexylresorcinol may have decreased enzymatic browning caused by the polyphenol-oxidase (PPO), as in the presence of 4-hexylresorcinol the substrates of PPO form inclusion complexes. When the substrate is located inside the cyclodextrin cavity, PPO is not able to oxidize it, resulting in inhibition of the enzyme activity by decreasing the availability of its substrate [(11)].

Rehydration index values ranged from 265 to 519 %. This response variable did not show a significant variation ($\alpha = 0.05$) with temperature, but it decreased as the slice thickness increased. This could be attributed to the fact that at higher thickness there is less cell damage. A similar behavior was obtained by

other authors, who reported that at smaller thickness cell damage is greater and this is reflected in higher rehydration rates [1,4].

The smallest value for shrinkage (77%) was obtained for the highest thickness treatment (62.5 °C and 5.4 mm) and the highest value (84%) was found for the lowest thickness treatment (62.5 °C and 2.6 mm). These results are consistent with the 79.3 to 81.3 % shrinkage reported by other authors in the convective drying of apples at temperatures from 50 to 70 °C [12].

Firmness values ranged from 8.3 to 11.3 N, observing that this range is small. Temperature did not exert a significant effect ($\alpha = 0.05$) on firmness. Leeratanarak [13] also reported a lack of effect of temperature on firmness. As the thickness increased the firmness values decreased, which could be due to the fact that at higher thickness the cell damage caused by the process is smaller.

The highest value of the ratio of critical moisture content to initial moisture content obtained was 0.88 and corresponded to 55 °C and 5 mm, while the lowest value was 0.54 related to 62.5 °C and 2.6 mm. As temperature increased, the critical moisture content decreased; this could be attributed to a higher water mobility as temperature rises, allowing a higher evaporation before the change of drying periods. At higher thickness, the critical moisture content was higher indicating that surface saturation was lost faster, probably due to the fact that inner water mobility was not enough to keep surface saturation [7,8,14].

The highest value of mass flux (N) was 5.93×10^{-04} kg of water/m^{2}s in the treatment of 70 °C and 3 mm, and the lowest was 3.99×10^{-04} kg of water/m^{2}s at 55 °C and 5 mm. As temperature increased, the mass flux also increased. This behavior may be due to an increase in the ability of air to hold water vapor as temperature rises [7,8]. The mass transfer coefficient (k_Y) values ranged from 2.27×10^{-02} to 7.92×10^{-02} kg of water/m^{2}s ΔY. These coefficient values increased as temperature increased. On the other hand, as thickness increased the mass transfer coefficient decreased. This could be attributed to a longer distance that water needs to move to reach the sample surface producing lower water removal rates.

Apparent diffusivity (D_{app}) values ranged from 3.45×10^{-12} to 5.40×10^{-09} m^2/s. These results were similar to those reported by Dikbasan [15] who obtained apparent diffusivity values of 7.7×10^{-10}, 6.3×10^{-9}, 1.35×10^{-9}, and 1.42×10^{-9} m^2/s at 45.6, 53.4, 60.5, and 65.3 °C, respectively, during the dehydration of apple cv. Granny Smith. As temperature increased apparent diffusivity also increased. This is in agreement with reports by Simal et al [16] who found higher values of apparent diffusivity at 60 °C (1.27×10^{-09} m^2/s) than at 40 °C (5.31×10^{-10} m^2/s).

Optimal process conditions were 51.9 °C and thickness of 3.7 mm. Predicted values for ΔE, rehydration index (RI) and firmness (F) were 12.7, 407.3%, and 9.8 N, respectively. Overall desirability was 0.92. Predicted values corresponding to the optimal process conditions were validated and the experimental values obtained were ΔE = 11.78, RI = 388.46%, and F = 10.3 N. These results were close to the predicted ones. At these conditions, values for N and k_Y were 4.03×10^{-4} kg of water/m^{2}s and 5.8×10^{-2} kg of water/m^{2}s ΔY, respectively and apparent diffusivity ranged from 8.94×10^{-11} to 3.79×10^{-9} m^2/s.

Conclusion

Total color difference was mainly affected by thickness and this was associated to drying process time. Temperature showed no significant effect on rehydration index and firmness. The highest rehydration index (519.4%) and the lowest firmness values (8.3 N) of dried apple slices were obtained at the lowest thickness, which can be attributed to a higher cellular damage. Mass flux and local mass transfer coefficient increased as drying temperature increased, this could be due to the fact that in the constant rate drying period the main resistance to mass transfer is the external resistance.

Optimal drying conditions were 51.9 °C and an apple slice thickness of 3.7 mm, with an overall desirability of 0.92.

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Thermal Analysis Characterization In Apple Osmotic Dehydration

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Introduction

The possibility of developing schemes of minimal processingfruit, today raises broad prospects for industrial application in the manufacture of pre-processed raw materials to be used later, or to obtain final products. In this sense, a possible method to process the apple is osmotic dehydration (OD) with sugar solutions, working at mild temperatures to preserve its properties. This reduces the water and enzyme activity, with minimal changes in product characteristics [1].

The OD is the extraction of water from a product which is immersed in a hypertonic solution at specific time and temperature. This extraction is due to the driving force that is created by the solution high osmotic pressure or low water activity (a_w) or the concentration gradient between the solution and the solid [2].

In dehydrated foods, the solid matrix formed by food polymers and other compounds, may be in an amorphous metastable state (very viscous glass or more liquid-like rubber), that can becomeplasticized as a consequence of the water content or temperature increase. Mostly, the foodamorphous state can be achieved by water elimination. The change from the glassy to the rubbery state occurs as a second-order phase transition at atemperature range known as the glasstransition temperature (*Tg*) [3]; is a kinetic transition that can be measured by heatcapacity (*Cp*) changes, by a drastic change in molecular rotational mobility or by an abrupt change in hydrogen bonding [4]. A tendency to explain the foods processing and storage behavior, is considering them like waterplasticized polymeric systems; so, with Modulated Temperature Differential Scanning Calorimetry (MTDSC) technique is possible to obtain complex heat capacity information, in isothermal as well as in non-isothermal conditions, and the heat flow signal decomposition on its reversible and non-reversible contributions, allow the study of complex systems [5].

Both thermodynamic and kinetic parameters are important in food processing and stability.Thermodynamic parameters are represented by water activity (a_w); kinetic properties can be represented byviscosity and related to the *Tg*. Thus, in a food system passing from high to intermediate and low moisture content, the prevailing control mechanism will gradually change from thermodynamic to kinetic [6]. Studies on the relationships between structure and properties in food [7] have underlined that, under many common circumstances, the thermodynamic activity of water is far less relevant to processing and storage than structure-related properties, which can restrict the mobility and diffusion of reactants.

Thus, the aim of this study lies in the thermodynamic changes and kinetic behavior duringstarking apple OD in 45% sucrose solution at 65°C, evaluated by MTDSC.

Materials and methods

The apples were acquired in a local market and classified according to its ripening state. The average characteristics of the used apples were: firmness = 70± 3 N, Bx = 14.5±0.2 °, acidity = 0.23±0.03 g malic acid/100 g, moisture = 85±1.2 %. Once washed and peeled, the apples were cut in slabs to get 1 cm^3 cubes. Osmotic solutions were prepared at 45% sucrose (w/w), and worked at 65° C during three hours. The samples immerse into this solution were removed every 30 min and drained for 5 min before weighting. The samples moisture was found in an oven at 70° C until constant weight, for initial and at each experimental time. The soluble solids concentration was determinate with a digital refractometer (Sper Scientific 300003, AZ, U.S.A.).

With sample moisture and weight data was possible to obtain osmotic parameters: the percentage of water loss (WL) and solids gain (SG), [8]:

$$WL = \frac{M_0 X_0^w - M_t X_t^w}{M_0} x100 \quad (1)$$

$$SG = \frac{M_t X_t^{ts} - M_0 X_0^{ts}}{M_0} x100 \quad (2)$$

where M_0 is the initial sample mass, M_t the sample mass at time t, X^w_0 the initial water concentration, X^w_t the water concentration at time t; X^{ts}_0 the initial total solids concentration and X^{ts}_t is the total solids concentration at time t.Both parameters are expressed as g/100g of fresh sample.

Each experimental run, consisting of the osmoticdehydration of 21 experimental units (cubes) over 3 h, was conducted in triplicate. The water fraction (Xw) foreach experimental time during OD was consideredto be the response variable. Statistical analyses wereperformed using MINITAB 15. A two-way ANOVAanalysis was applied within experimental runs (each timerepetition) to verify the mean equality, which was confirmedwith an a posteriori Tukey test. Another one-wayANOVA was applied among runs (replicates) to obtainstatistically valid mean values for the entire experimentalseries. We then obtained and compared the 95%confidence intervals to confirm the average behavior.Only the mean values were used to compute the osmoticparameters.

For thermal analysis, a mass fraction of each sample (±16 mg) was placed in hermetic alluminum pans in a MTDSC 2920 (TA Instruments, NJ, U.S.A.) previously calibrated with Indio and sapphire, for constant cell (1.0538) and heat capacity constant (1.1806), respectively; an empty pan was used as reference.After stabilization at -50° C, the samples were heated to 40 °C at 5°C/min in a nitrogen atmosphere at 60 ml/min. The freezing procedure dictates the structure within the sample, which then dictates thermal behavior. The Tg range was evaluated as well as the ice melting enthalpy (ΔH), the melting point (T_m) and Cp changes in each thermogram by the Universal Analysis software V. 4.4A. The ratio of this enthalpy by the pure ice fusion enthalpy was the amount of unfrozen water ($Wg´$). The freeze-concentrated solution in the fruit ($Cg´$), was obtained by extrapolating to zero the fusion enthalpy as a function of the Brix index [9].

Gordon and Taylor empirical equation (Eq. 5) was used to predict Tg as a function of water content:

$$Tg = \frac{x_s Tg_s + k x_w Tg_w}{x_s + k x_w} \quad (3)$$

where Tg, Tg_s, and Tg_w are glass transition temperatures of the samples, solid matrix and water, respectively; x_s and x_w are the corresponding percent of solid and water contents and k (4.7) an empirical parameter [10]. Glass transition temperature of pure water was taken as Tg_w = -135° C.

Results and Discussion

Only during the third repetition were found and eliminated atypical values. The ANOVA analysis among replicates, with α=0.5, confirm the mean equality. From the interval plot comparing the repetitions and replicates can be concluded that, in spite of the variability, the confidence interval overlapped, so it was possible to work with the mean values.

The osmotic parameters and dehydration kinetics are shown in Fig. 1; about 49% water was lost and 19% solids were gained in the same process. WL and SG were higher in the initial phase of osmosis. With progression of time, the osmotic driving potentials for moisture and solute transfer decreased. This may be due to structural changes caused by the rapid loss of water and uptake of solids near the surface, leading to compaction of these surface layers and increased mass transfer resistance for water and solids [11, 12].

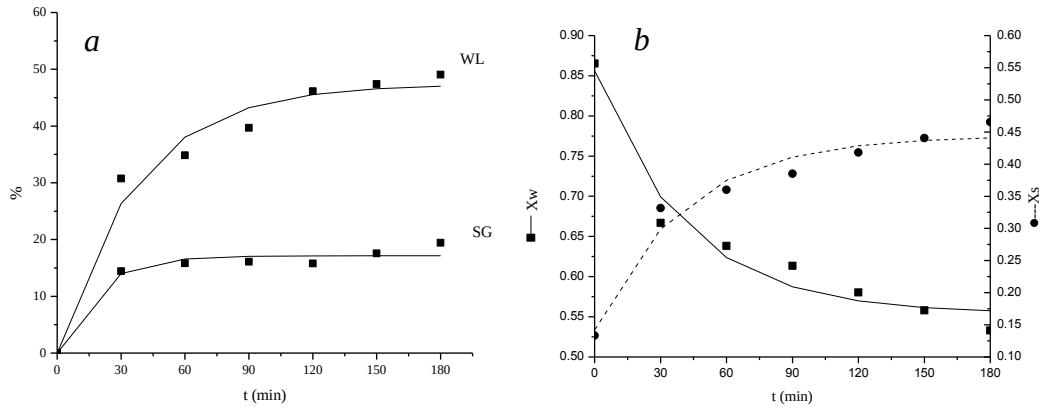

Figure 1. a) Osmotic parameters: water loss (WL) and solid gain (SG), b) dehydration kinetics for water fraction (Xw) and solids fraction (Xs).

Generally,the massand volumeof samplesduring the ODdecreaseuntil itreaches a minimum valueat a so-calledcompositionalequilibration time(t_c); after thispseudo-equilibrium situation, the mass and volumeof the sampleincreaseagain[13].This is because the mechanical stressstored inthe food matrixduring dehydrationdueto contraction,is released asexpansionof the sample (relaxation). This phenomenonproducespressure gradients inthe matrix, causing the external fluid flow in the structure; this mass transportoccurs byahydrodynamic mechanism(HDM) alongthe intercellular spacesand occasionallyacrossthe cell wall[14].Therefore,water lossdecreases withconsequent reductionin the solids gain,as shownat 90 and150minof the process; the same effect can be observed in the water and solid fraction kinetics (fig. 1b).

Figure 2 shows the MTDSC thermograms at each osmotic dehydration time, where can be seen that the *Tm* decrease as a function of process time and water content. Below a water content threshold, whose value depends on the chemical and physical structure of the polymeric matrix, the water molecules adsorbed cannot form ice crystals in the matrix. Beyond this threshold, the water adsorbed crystallizes at a temperature lower than the normal-water-melting point, which decreases with the decrease in matrix water content [15]. The ice fusion enthalpy also decrease when the sucrose concentration into the fruit increase, because there is less freezing water (Table 1).

The small endothermic peak observed for high moisture contents immediately before fusion is known as antemelting (*Tg´*)[16] and has been observed during phase transitions of biological products, suggested that it was due to molecular layers of liquid, which form on the crystals surface; another explanation for antemelting endothermic peak could be the formation of a limited region of an eutectic mixture, melting at lower temperatures.

It is also importantto note that forcertain water content,the thermogramsshowasmall exothermic peak(T_d), which is the resultof the freezable water crystallization thatremainsunfrozenwithin the matrix, a phenomenon known as "devitrification."Whenthe sampleis heatedabove the*Tg*, the water crystallizesif is not boundwithsucrosebyhydrogen bonds [17].This phase separationreducesthe water contentin the amorphous phaseand therefore,increasesthe*Tg*.

Althoughfreezablebound wateris presentin the samples,the crystallization processwas progressivelyinhibited, probably due to the increasingviscosityof the liquid phaseof the product, by the high solid concentrationin the amorphous matrix. This water contentencloses themass fraction ofnon-freezablewater(*Wg'*) whichincludes bothfree waterand the not crystallized freewateradsorbed onthe solids matrix.

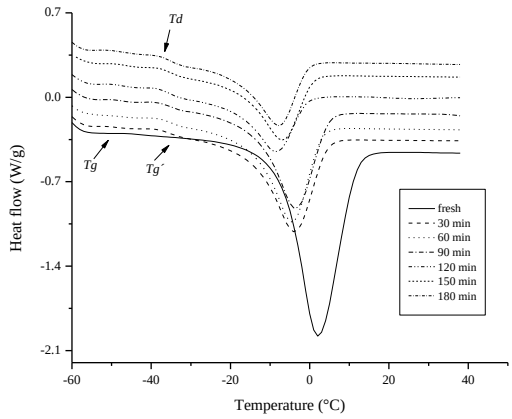

Figure 2- MTDSC thermogram at different dehydration times

Table 1. Values obtained from MTDSC at different solid content (°Bx) during apple OD

t (min)	°Bx	Moisture (%)	a_w	Tm (°C)	ΔH (J/g)	Tg (°C)	ΔCp (J/g/°C)	Tg´ (°C)	Wg´
0	12.0	86.52	0.897	1.95	267.2	-56.00	0.123	-29.43	0.798
30	34	66.69	0.574	-4.01	150.6	-47.22	0.111	-32.64	0.450
60	37	63.83	0.524	-4.63	143.5	-44.52	0.183	-34.05	0.428
90	42	61.35	0.483	-3.74	152.1	-45.91	0.109	-34.69	0.454
120	44	58.03	0.430	-8.73	94.29	-46.25	0.094	-35.04	0.281
150	45.5	55.80	0.395	-7.07	107.4	-44.80	0.136	-35.04	0.321
180	50	53.29	0.359	-7.94	103.7	-43.03	0.186	-33.11	0.310

The second-order transitions (*Tg*) were identified in the derivative heat flow signal, as an endotherm, and the heat capacity changes (*ΔCp*) associated, were obtained from the increase in the reversible *Cp* signal (table 1).

Analyzing the behavior in reversible *Cp*, shown in Figure 3a, changes due to the thermodynamics of the system can be seen. Since *ΔCp* is associated with molecular mobility during the *Tg*, samples with higher moisture content have higher changes, given the water plasticizing effect. However, the *ΔCp* is also related to the level of aggregation/reorganization of molecules, then the higher values for samples with less moisture (Table 1), means that they probably are in a glassy state with a higher molecular disorder [18].In general, the*ΔCp*decreases withthe Tgincrease (Table1), suggesting that there is an associationwith the hydrogen bondsstrength inthe glassy state, the greater thebond strengthgreater changein heat capacity[19].

During dehydration, whichminormoisture contents are reached, there is a decrease inreversible *Cp* wherethe glass transitionends, since hydrogen bondformation between water-sucrose, which in turndetermine the water availabilityfordeterioration reactions, i.e.a_w. So that represents a good stability parameter [20].

When plottingthenon-reversible*Cp* as a function of temperature(Fig. 3b), can be observed the sample kinetic behavior,associated withincreasedmobilityofthe molecules. The increment is relatedto the outflowof unfreezable water from theglassymatrixand the descent with the ice formation.Sothat, at lower moisture contentwas obtained alower melting temperature,as a resultof ice formation dependence over time.Similarly,inthearea where*Tg'*, the increase and decrease ofnon-reversible*Cp*are indicative ofatimeand/or temperature-dependent event, so is considered as arearrangementofunfrozen waterin the freeze-concentrated matrixthat crystallizesbefore melting(devitrification)[21].

In this manneris explained, as willhave lessfree water andhighersucrose concentration during dehydration, the increased viscosityof the liquid phaseinhibits the crystallizationduring the sample cooling.Temperaturesabove the*Tg*allow mobilityandmolecularrearrangementin the crystalline state, depending on moisture content.Then, thefreezable waterwhich remained unfrozendue to hindered crystallizationduring quenching crystallizes during the heating.

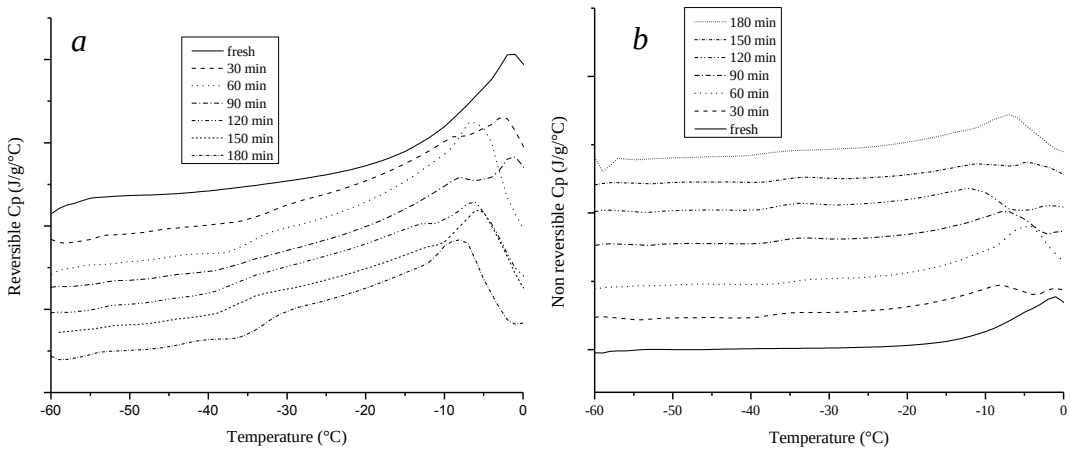

Figure 3. Changes in a) reversible Cp and b) non revesible Cp during OD

The *Tg* at room temperature was predictedfromthe experimental data obtainedduring coolingof the samples,using the equation ofGordonand Taylor.The moisture contentinthe solid matrix at which the applehas a changefromglassy to therubberystate at room temperature (*Tg*≈24 °C),was identified as thecritical moisture content(1.11g water /gdry solid); at the freeze-concentrated solutionconcentration(*Cg'*) in the fruit (0.473 solid fraction), corresponds with anon-freezablewater(*Wg'*) value of 0.527 water fraction.

Then, ifthe phase transitionsgovern changes inthe physical stateof the materials,the glass transitionrepresentsavery important parameterin the stabilityof products, or ratherthe *Cp* changes.Therefore,finding the relationshipbetween it andthe moisture contentwill be usefulto determinethe storage conditions, and moreover,the process conditionsof a particular product.

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Rehydration Of Cooked-Lyophilized *Longissimus dorsi* Pork

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Abstract.

Food preservation processes have among others of their main objectives, to avoid structural changes that could be reflected on their sensory attributes. In this sense, freeze-dry is a not food aggressive dehydration process, although it is necessary to assess the impact that it has according to manage conditions and composition of foods to be preserved in this way. In this study we determined the effect of cooking and freezing, or both, prior to freeze-drying of *Longissimus dorsi* muscle in rehydration phenomena, evaluating meat Aw before and after treatments, as well as their behavior depending on the use of different buffer solutions (tetrasodium pyrophosphate ted pigs, weighing approximately 100 kg were used, preparing two batches of cut cubes of about 1cm^3 made from this meat. First lot was subjected to cooking in a convection oven at 250 °C for 12 mor sodium tripolyphosphate) or water. *Longissimus dorsi* muscle from 6 months old castrain and high velocity air and the second batch was left without heat treatment. Aw of both batches of fresh and cooked meat cubes were determined, and subsequently were frozen at -70 °C in a Ultrafreezer and then lyophilized at -49 °C and 0.035mBar. For samples rehydration, different buffer solutions or water at 70 °C and at 90 °C during 6 minutes were used. There was no difference found on the ANOVA results when using either of the two salts in meat rehydration, both in Raw- lyophilized or in cooked- lyophilized, although it is recommended that this should be done at 70 °C whereas at 90 °C rehydration phenomenon changed, due to coagulation of the proteins.

Keywords: [*Longissimus dorsi*, cooked, lyophilized, rehydration]

Introduction

In food processing, one of the sectors that has evolved has been the conservation. Among the main objectives of preserving food, is to prevent their structural changes are reflected in the sensory attributes affecting quality is difficult to define exactly what consumers conceive for meat quality, but current trends require admit three different concepts of quality: 1) organoleptic (sensory), 2) nutritional quality and 3) hygienic quality (safety). The freeze-dry preserves the quality of the meat, since these three concepts, since a dehydration process is not harmful to biological systems where the water is removed by sublimation under controlled conditions of pressure and temperature, whereby is necessary to apply a pre-freezing process depending on a number of factors at specific conditions (such as freezing rate, the size and shape of the crystal, etc.) will have a beneficial or detrimental impact that the dehydration process (1). It is also necessary to control the conditions of rehydration of the flesh, as it should recover some properties similar to those he had earlier in the process, among which water activity (Aw), water holding capacity (WHC), resistance to deformation and the thermodynamic properties of proteins, that is why the phosphates play an important role in the rehydration of freeze-dryed meat, since they act as pH regulators, buffering agents and sequestering agents (2).

Methods and Materials.

In this work was used *Longissimus dorsi* muscle of pork, castrated male 6 months of age, weighing approximately 100 kg, were subsequently cuts 1cm^3 geometries, using knife sharpener and vernier. Below 50% the batch obtained was oven Turbo Convection Oven Flavorwave mark for firing at a temperature of 250 °C, high air velocity and a time of 12min. Lots of meat (fresh and cooked) frozen in ultrafreezer

chamber REVCO Ultima II brand, at -70 °C for 24 hours. After freezing the samples were placed in the vessels of the lyophilizer (Labconco mark model Freezone 4.5) and were freeze-drying for 24 hours with a temperature of -49 °C and a pressure of 0.035mBar.
Rehydration of 5 lyophilized different cubes of meat (raw or cooked), was carried out with tetrasodium pyrophosphate / sodium chloride (0.15% / 2%) (w / v) sodium tripolyphosphate / sodium chloride (0.15% / 2 %) (w / v) and a third batch in water. 80mL were placed in a reconstituting agent grill heating at 70 and 90 °C, with stirring, when the desired temperature was reached, samples were immersed and weighed every 30 seconds for a period of 6 minutes. Rehydration was determined in percentage of weight gain in accordance with the monitoring was given to each of the samples from their raw and lyophilized state. Determinations were made for raw, cooked, lyophilized, freeze dried and rehydrated cooked samples to: Water Activity (Aw) through a dew point hygrometer Decagon Devices model Pawkit mark (5), resistance to deformation, with a manual penetrometer FT327 Fruit PressureTester mark pattern with a tip diameter of 11mm, and it is expressed in pounds force (kgf) Thermal analysis Differential Scanning Calorimetry with temperature modulation (TMDSC) with a TA Instruments calorimeter TMDSC brand 2920 series, with a constantan disk as heat transfer element, nitrogen was used as purge gas at a constant flow of 60 mL• min^{-1}. The objective of those determinations was to analyze the thermodynamic behavior of meat proteins during the processes and it was unable to obtain the denaturation temperatures, energy requirements (enthalpies) and changes in the specific heat at constant pressure (Cp) of myosin, actin that are primarily responsible for the CRA in flesh and collagen that is primarily responsible for resistance to deformation. The method used was as follows: Equilibrium to 10°C, modulation to 0,796 °C every 60s, and Isotherm for 120s, Ramp 5 °C / min to 150 °C. The meat was encapsulated in hermetic aluminum pans (for the case of fresh and cooked, the amount was for 3.6mg and 22mg for lyophilized) (4). All tests were performed with 5 replicates to ensure statistical reliability and for the case of the experimental design was used an analysis of variance (ANOVA) with a 2-way least significant difference test honestly (DMSH) commonly called Tukey test and a 5% significance (6), which relate the cooking process, and rehydration with restorative agents 3 to 2 different temperatures and its direct effect on the resistance to deformation as a parameter of quality.

Results and Discussion

The results of water activity (Aw) obtained for samples of meat cooked- freeze-drying or freeze-drying shown in Table 1 where it can be seen that the cook process at 250 °C enables a reduction of 10% in Aw, freeze-dry other hand promotes greater removal of water (approximately 84%) and to obtain a more stable product with longer shelf life (3). Regarding the rehydration phenomenon shown in Figure 1, Aw values of the rehydrated samples, which shows that irrespective of the temperature and type of reconstituting agent is used, always recovers the initial water activity.

Table 1. Aw Values

Sample	Aw
Raw	0.88
Cooked	0.80
Raw-lyophilized	0.14
Cooked-lyophilized	0.078

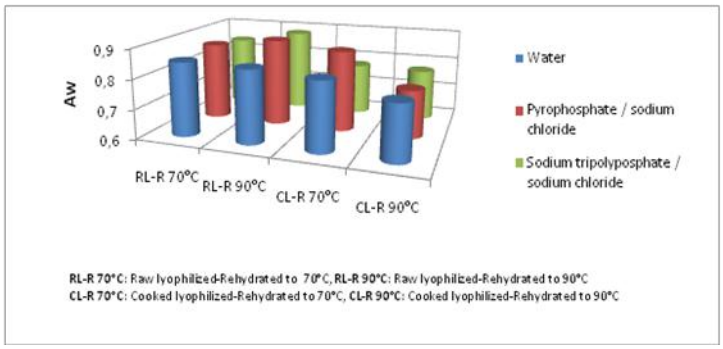

Figure 1. Aw of rehydrated samples of meat

In the case of the evaluation of the process of rehydration was taken into account variations in weight of the samples was due to the water content of the meat, which was done by the monitoring the weight of each sample (meat cubes) and thus when rehydrated, one would obtain the percentage of water gain. Table 2 shows that in no case fully recovers the initial water content, or varying the temperature or the type of

restorative agent and that for the case of the cooked meat before being freeze-drying not recovered beyond 45% of its initial water content, indicating that the thermal process is affecting the structure of proteins responsible for water retention capacity in the meat and that even using buffering agents such as phosphates, are not having a good effect against this functionality due to the proteins to change their structural conformation, are changing their functionality, and in the case of samples which did not undergo a thermal process prior to freeze-dry, it can be seen that the percentage of gain by weight water, is greater compared to that spent by firing, this is probably because the water is interacting structures of different shape, if so, that when subsequently frozen and freeze-dry to this distortion exists partial proteins due to the high osmotic pressure generated in the feed and it affects the ionic strength of the meat probably causing a phenomenon being registered partial denaturation by freezing and effects of this change also to the structures of some protein, change its functionality and that is why this is reflected in their ability to reinstate water in its structure, following two different behaviors in the phenomenon of rehydration. The deformation resistance for fresh meat is 1.68kfg, which is the reference value because it is meat that has not undergone any process, whereas when it undergoes a process for cooking to the above conditions, the value of deformation resistance increases to 3.16kgf, indicating that there is a change in the structure of the meat, making it more rigid and resistant to suffer alterations.

Tabla 2. Water absorbed

Reconstituting agent	Freeze-dry meat (g)	Cooked and freeze-dry meat (g)
Water at 70°C	48.75	43.35
Water at 90°C	45.05	38.77
tetrasodium pyrophosphate at 70°C	55.44	24.32
tetrasodium pyrophosphate at 90°C	48.96	36.20
sodium tripolyphosphate at 70°C	46.63	38.67
sodium tripolyphosphate at 90°C	40.25	32.59

Figure 2 shows the influence of the cooking process, the process of freeze-dry and rehydration at 2 different temperatures (70 and 90 °C) 3 along with restorative agents, which clearly shows that in either case recovers the initial conditions had either fresh meat or meat that has undergone only cooking, whereas according to the analysis of variance and the Tukey test, there is no difference between using either of the two different salts rehydrate phosphate to either 70 or 90 °C freeze-dried meat, since both generate statistically similar values in resistance to deformation when rehydrated. Particularly when rehydrating with water in any of the two temperatures, it provides a lower resistance to deformation compared with solutions containing sodium chloride and phosphates.

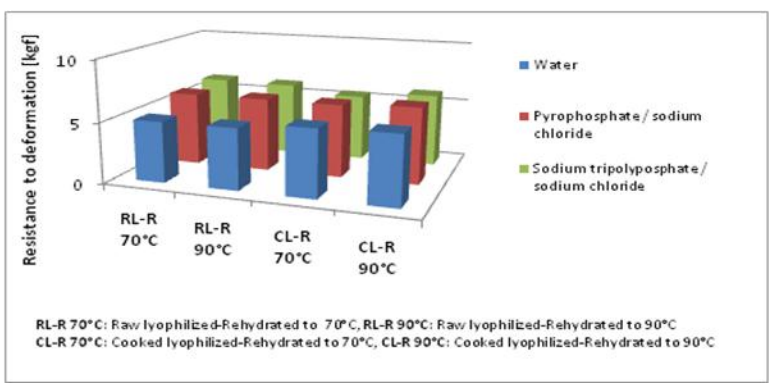

Figure 2. Resistance to deformation of the lyophilized and rehydrate samples with two buffer solutions at two different temperatures

Thermal analysis Differential Scanning Calorimetry Modulated Temperature (TMDSC) can obtain several responses which case only for this is taken into account transitions (denaturation temperature) and energy expenditure to denature the changes in Cp that is how to represent the thermodynamics major structural

changes that occur during the thermal analysis. For fresh meat and cooked thermogram was obtained (Figure 3) heat flow shows an idea of what is happening in the kinetic thermal and structural and meat samples which are reflected graphically endothermic reactions that occurred during the test, which are translated as protein denaturation and according to Kazemi, 2009, who had different types of tests to pork quality by Differential Scanning Calorimetry (DSC) identified denaturation intervals myosin, near of 56 °C, actin, about 79 °C and collagen about 65 °C, which according to experiments carried out with the values obtained by the kit, (Table 3) values denaturation and energy, data reported by Kazemi.

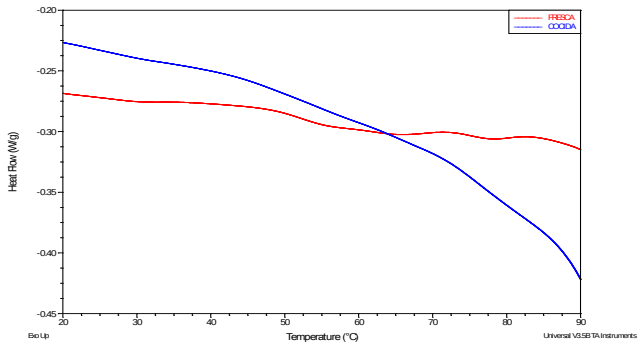

Tabla 3 Values of Calorimetry (TMDSC)

Protein	Denaturation temperature (°C)	Enthalpy (J/g)
Myosin	55.07	0.1797
Colagen	65.34	0.2329
Actin	77.88	0.2060

Figure 3 Thermogram of raw and cooked meat

The thermal behavior of cooked meat, it can be seen that is very different from raw meat, this because the cooking process and to significantly alter the protein, which is reflected in the transitions are nearly invisible on the chart, but they exist and are being practically the same temperatures and with the same energy expenditure. The freeze-dried meat was also monitored following thermodynamics proteins through a heat flow thermogram, which is shown in Figure 4, and can observe a different trend thermogram of Figure 3 wherein not reach actually visualize regions by protein denaturation effects of the absence of water, this is indicative of no existence of water to interact with the components of the meat, the reactions are carried out first with energy costs well above those recorded for fresh meat and cooked, but note that according to this graphic, these energy costs are due to bound water is being removed from the meat, freeze-dried meat for this phenomenon starts at 94.04 ° C and requires about 72.24J/gy for cooked meat, lyophilized starts at 86.87 ° C and requires 90.9J / g.

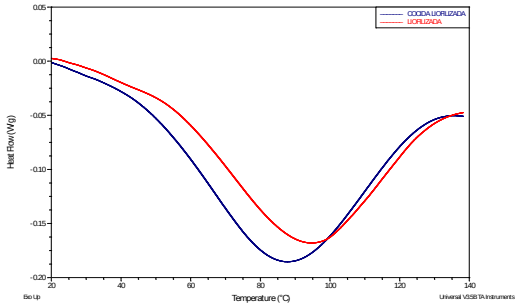

Figure 4. Freeze-dried and cooked- freeze-dried meat thermogram

For both cases were also changes in the Cp (Figure 5), showing graphically that they occur in proteins during heating and to test whether you can actually recorded temperatures where the transition are major changes in the structures. In fresh meat can be seen that the 42.86 °C is registering a major change which is attributed according to the thermograms, the effect produced first denature the protein in question (myosin), being that for the case cooked meat, the most important change is that temperature range, if not in your case is to 65.6 °C, where the protein is dominating the process of structural change is apparently mainly collagen.

In the case of the lyophilized samples can be noticed the movement of the largest curve, this occurs around 100 to 110 °C and is due primarily to the absence of water in the samples generated in the rearrangement reactions of structure and secondly that because at these temperatures bound water is being removed and energy costs are high, it can confirm that if there are changes in the structure, which relate to the endothermic reactions shown in the thermograms which confirms being carried out at these intervals protein denaturation temperature and with such energy expenditures.

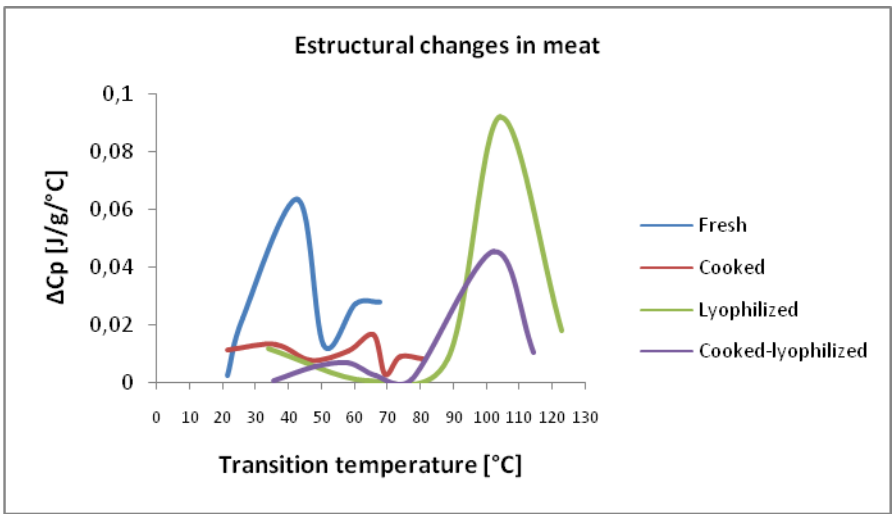

Figura 5 Cp changes in the meat samples

Aknowledgements

DGAPA-UNAM: PAPIME PE-202010, PAPIIT IT 202312, PACIVE 2010, 2012

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Precooling By Hydrofluidizationand Nanocapsule Coating Application In Fresh-Cut Fruit

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Introduction

Hydrocooling is one of most efficient methods for chilling horticulture products, because it covers the total area of fruit and lower the cooling timescompared with other methods. One valuable variant of these methods is the hidrofludization which consists in recirculate the cooling medium across the product at low temperature. Thenthe cooling medium is pumped through orifices or nozzles until a container, creating fluid bed underwater currents. The rate flux produces water agitation and increases the transfer of head to food [2]. The hydrofluidization is considered an emerging technology based on a high turbulence and superficial velocity in the cooling medium, which enhances cover the whole surface or the product and increase the superficial coefficient heat transfer. Also it is possible to add substances in the cooling medium as antioxidants, flavors, nutraceuticals, etc. [3]. Recently, it has been evaluated as method to apply coatings in particular those that includenutraceuticals or dispersions with nano or microcapsules. The coating application(e. g. on fresh-cut fruit) is a complicated issue.In general, the common coating techniques are immersion, spray and distribution with a brush. The immersion and spray techniques have important disadvantages such as coating difficult to apply and low uniformity coating. In this work,ahidrofluidizator was used as an alternative to apply coatingson fresh-cut fruit at low temperature. The main purpose of this study was to evaluate the effect of the orifice size (perforated tray) during chilling on the heat transfer coefficients and effectiveness of applying a coating with α-tocopherolnanocapsules.

Methods and materials

The study was carried out adapting and characterizing the hidrofluidizator shown in the Figure 1. Initial testwere performed with water in order to optimize the system. Then the nanocapsules were evaluated. Two orifices diameters were evaluated: 2 and 4 mm.Red Globegrapesand peard'Anjou were used as model fruits. Theywere acquiredin a commercial state ofmaturity, afterthey were selectedtakinginto account: color, shape (sphericalgeometry)and dimensions. The batches (n=3) were weighed(0.5 Kg) andthe diameterwas measuredusing adigital caliberto ensurebatch to batch consistency. The fruits wereminimally processed, the skinswere removed from thegrapes and the pears werepeeledand thenbothwere cut in spheres (d=1.67cm for grapes and d=2.5 cm for pears).Variationfactorswere the typeoffruit with3 replicationsandthediameterofthefluidizingholes(2 and4 mm). All experiments were performed in triplicate. Output variableswerethe flow rateat the outletofthe holes (Re) and thecooling time.

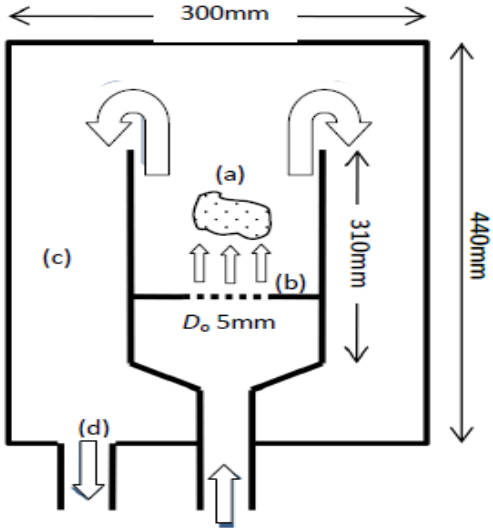

Figure 1. Hidrofluidization System: (a) containing tank, (b) perforated tray, (c) overflow, (d) recirculator.[1]

Nanocapsulas preparation

Thenanocapsuleswere preparedbytheemulsification-diffusion method[4]. In 20 mL of organic phase(ethyl acetate,saturatedwithwater)was solubilized the poly-ε-caprolactone and α-tocopherol in40 mL of anaqueousphase(saturatedwithsolvent) was dissolved the poly-vinyl alcohol (5g/100ml). An emulsionwas prepared dispersion the organic phase into the aqueous phase using a mechanic stirrer(4000 rpm/10min), the diffusion of the solvent from the intern phase to the extern is carriedout by adding180 mLofwater, leading thenanocapsules formation.The solvent was removedby evaporation at30°Cand93.325Pavacuum. The excessof surfactantwas eliminated byultracentrifugation at25.000g for30 minutes. The nanocapsules were characterized by theirparticle size,polidspersiónindexandzetapotentialusing alaserscatteringtechniqueat an angle of90° (Malvern Zetasizer,NanoSeriesLtdFrance).

Rheological characterization

The rheological characterization of the model fluid was made in a HaakeRehoWin 323 rheometerusing concentric cylinder geometry (Z-40 $_{DIN}$). The test was performed to the flow conditions used by the pump shear (3,560rpm and 400s^{-1}). The data were plotted in and ascend and descendway and the curves were used to determine the behavior of the fluid as function of shear time during the hidrofluidization operation.

Determination of cooling parameters

Two areas were selected to obtain thermal history. The thermocouples (type K) were introduced and the temperatures were recordingin a digital CheckTemp (HANNA Hi 98509). The cooling parameters were determined to solve the transfer cooling equation. The cooling coefficient was determined by: $\frac{T_P - T_m}{T_i - T_m} = je^{-(CC)(t)}$

The convective coefficient was calculated in function of the Re and Nu.

Experimental Desing

The effect of the orifice size (2 and 4 mm) and fruit type (grape and pear) on the convective superficial coefficient were analized with a factorial design 2^2. The data treatment was performed in Minitab Release 14®USA.

Results and discussion

The nanocapsules had a particle size of 247 nm, polydispersion index of 0.18 and zeta potential of -37mV, it was established that the system was submicronic with narrow distribution and physically stable without agglomerates.

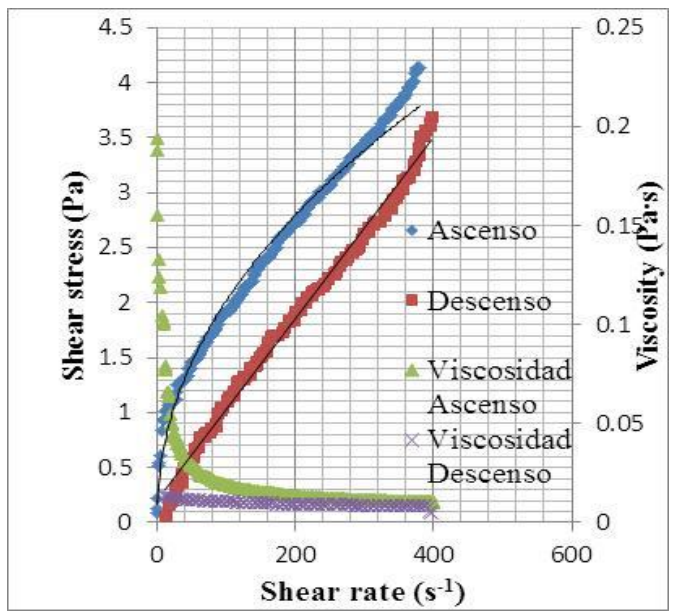

Figure 2.Rheological comportment of model system.

Rheological characterization

Figure 2shows the curvesof ascend and descend forthe shear stressandthe viscosity change. The modelhad athixotropic behavior witha fluidizerrisecurvethatfits with the power model andthen decrease withNewtonianbehavior.Thisdifferenceis attributedto changes influid viscosityas a functionof time,probably due totheaction ofpropylene glycol andxanthangumstructurecausing areductioninthecohesionof the polymer chainstructureopeningshearedsothattheviscositydecreasesdramatically.

Thecentrifugalpumpdrivinghidrofluidizatorat 372.8s-^{1}(3.560 rpm) carriesthe fluid tothe bottom chamberto 4.2s, this means thatduring this timethefluid shearcausedby the pump issufficient tochangeitsapparent viscosity(from 10 cPto 8cP).

Cooling parameters

The thermal conductivityof the grapeand pearwere of0.50and 0.43kJ/kg°Cwiththermaldiffusivityof 1.81E-7 and 1.25E-7 m^2/srespectively. Table1 shows theparametersfor hidrofluidization by coolingthe grapeand pear spheres(5-6min). The useofthe 4mm orifice plateextendedthe cooling times due to that theturbulence generatedwithin the chamberis less than theconstraintcausedbytheholesof2 mm.Thelagfactor(*j*) shows that thecooling by hidrofluidizationnot followNewton's law, this means thatthis processshould be treatedin a different wayit need to consider theproduct properties[6].Grapecoolinginthe chamberat different ratesofcoolingshowedcoefficientsof 2.6E-3 to 3.4E-3 and alagfactor(*j*) from 1.04 to1.07 withair velocities1and2 m/srespectively.Thelagfactor(*j*) obtainedwas significantly higher. This behavior was attributed tothe turbulence generatedby thehidrofluidizador.

Table 1. Cooling parameters in the hidrofluidizer with nanocápsules as fluid.

Fruit	Porus diameter	Time 1/2 of Cooling (min)	Time 7/8 of cooling (min)	*j*	Bi	h_m (W/m^2°C)
Grape	2 mm	2.08	5.42	1.2153	13.4	805.08
	4 mm	2.53	6.00	1.2494	8.1	485.32
Pear	2 mm	1.78	4.74	1.2135	17.5	602.33
	4 mm	1.93	4.98	1.2025	10.5	363.10

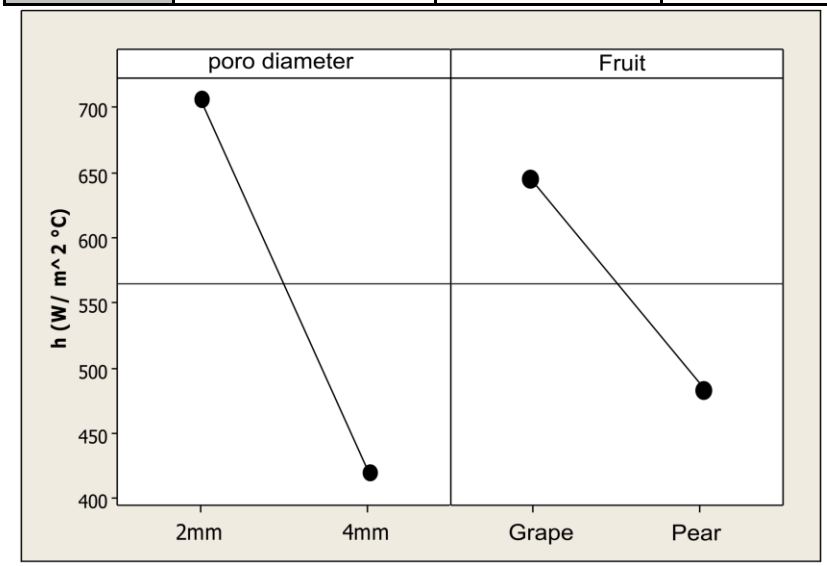

Figure 3

Figure 3. Effect of variables on superficial coefficient

Figure3 shows thedependenceofsuperficial heat transfer coefficientson the diameterofthe holesandthe typeoffruit. It is evidencedthatthehigherspeedis reachedwiththe2 mm plateforthe grape.The cooling ratedepends onthe temperature changebetweenthe cooling mediumandtheproduct, the fruit geometry andthe structureof the product.Lucas et al., (1998)[6] presented somevaluesofsuperficial heat transfer coefficientsin differentcoolingsystems. Fornaturalconvectionprocesses obtained coefficientsof210 to 290W/m^2°Candforced convectionprocessvaluesof680 to 2.600W/m^2°C.

Fikiin(1992)[7]reported for hidrofluidization process,valuesaboveof900 W/m^2°C in fish and vegetables freezing processes [8].

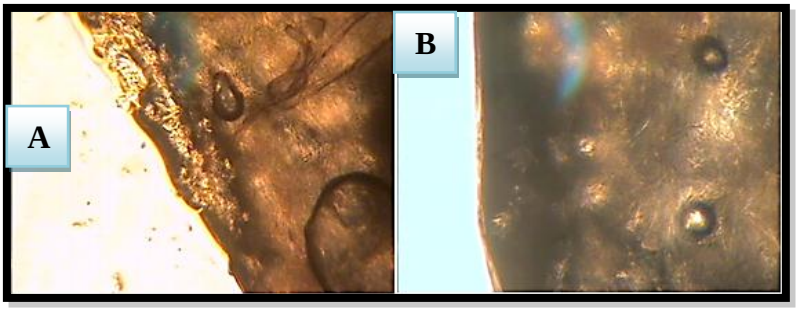

Figure 4. Micrographs with samples without treatment a) pear; b) grape (20 X).

Figure 4 shows the coating micrograph of fruit withouttreatment. These micrographs were used for comparing with the samples coated.

Figure 5 shows micrographs with samples coated, they evidenced an uniform and continuouscoating.This layer represents aresistancetothe heatlossincreasingthecoolingtime. Hidrofluidizationtime cooling 7/8withgrapeand 2 mm platewas 325s(5.4min)andthepearwas 284s (4.7min). The lowfluid viscosity, porosity and turbulencesinthechamberdo notlimitethe coating layerformedaroundthe pear.

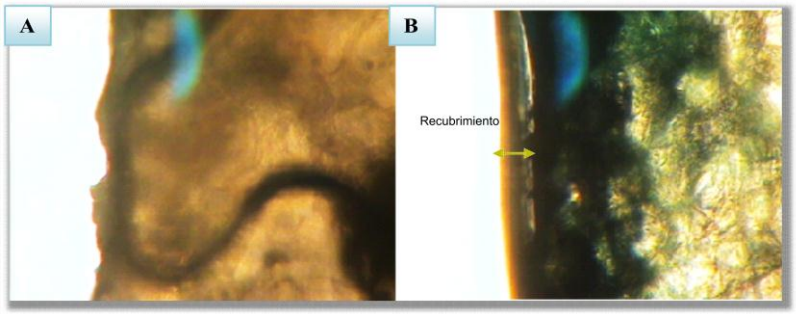

Figure 5. Micrographs after hydrofluidization process
a) pear coating; b) grape coating all at 20 X

Conclusions

The dispersion of nanocapsulesusedas cooling mediuminthehidrofluidizaciónhadbeneficial resultsforthe operation considering that thesuperficial coefficient heat transferwere obtainedbetween363.10and805.05W/m^2 °C.The coating formationduring cooling hidrofluidization depended of the time,the cooling medium viscosity, the porosityof the fruitand the turbulencecreatedduring the operation. The highconvective coefficients obtained with perforation of 2mm allow shorter cooling times.The evaluationofthetopographyofthe fruitsestablished thatthe formation of thecoating depends onthe rheological propertiesof the fluidmodelandthe physical characteristicsand surface of cool product.

Acknowledgements
The authors acknowledge the financial support for this work from PAPIME: PE203711;PAPIIT:IT231511 and IN222411-3.

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Physicochemical Characterization Of Banana Starch Particles Subjected To Different Degrees Of Acid Hydrolysis

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Introduction

Solid particles of nanoscale and microscale dimensions are becoming recognized for their potential application in the formulation of novel dispersed systems containing emulsified oil or water droplets, these systems are commonly called “Pickering emulsions”. The application of particles as a long-term stabilizer is important for the development of novel food, due to that the number permitted of emulsifiers in the food industry is low (1), these particles could develop emulsions as a method of protection for many compounds as odors, flavors, vitamins and polyphenols mainly, which are susceptible to environmental factors such as light, pH, temperature, etc. Recently, some food-grade microparticles have been used as particle emulsifier, among them microparticles from cellulose (2), chitin nanocrystals, (3) and hydrophobically modified starch particles (4). However, there still is little research work involving emulsions stabilized by food-grade particles. Starch particles are receiving reasonable attention due to their inexpensiveness, abundance, biocompatibility, biodegradability and nontoxicity. Most starches are traditionally isolated from maize and wheat, but a growing interest for obtaining functional starches from non-conventional sources like tubers, roots and some fruits has risen, as they provide different and unique functional properties that allow their application in novel food products or the improvement of existing ones. Furthermore, acid hydrolysis can be used to produce starch particles of different sizes with different physicochemical and functional properties. Amorphous zones of starch suffer rapid hydrolysis under acid treatment, while crystalline areas undergo only negligibly slow hydrolysis. The acid hydrolysis of starch produces particles that can be used as fillers in polymeric matrices to improve their mechanical and barrier properties and as emulsion stabilizers (5). The aim of the present study was produce particles of banana starch by means of an acidic treatment and to evaluate some morphological and physicochemical characteristics.

Materials and methods

Unripe fruits of banana (*Musa paradisiacal* L.) of the “Macho” variety were bought in a local market in Cuautla, State of Morelos, Mexico. Sulphuric acid (98 %) was obtained from J. T. Baker (Mexico City, Mexico). Deionized water was used in all the cases.

Starch isolation

Starch was isolated from unripe banana (*Musa paradisiacal* L.) according with the procedure reported by Casarrubias-Castillo et al. (6).

Acid hydrolysis

Acid hydrolysis was carried out according to the method reported by Kim et al. (7) with slight modifications: starch (15g, dry basis) was dispersed in a 3.6 M aqueous sulphuric acid solution (100 mL), the dispersion was stirred and kept at 40 °C for different periods of hydrolysis (1, 3, 7, 9, 15, 20 days).

The suspension obtained was centrifuged at 6000 rpm for 15 min (40°C) and the precipitates were washed with distilled water until reaching neutral pH. The precipitated starch particles were air-dried at 35 °C and stored at 4 °C.

Scanning electron microscopy (SEM)

Native and acid hydrolyzed starch particles were mounted on carbon sample holders using double-side sticky tape and were observed using a scanning electron microscope JSM Jeol Scanning Mycrosoft 7600f (Akishima, Japan) with the GB-H mode at 1 kV accelerating voltage.

Surface tension

The surface tension of starch particles dispersions was measured using the du Nöuy ring method as reported by Li et al. (8).

X-ray diffraction (XRD)

X-ray diffractograms of native starches and the acid hydrolyzed starch particles were obtained with a Siemens D-5000 diffractometer with Cu Kα radiation using the procedure described by Kim et al. (7). At a target voltage and current of 40 kV and 30 mA, respectively. The scanning range and rate were 3° - 30 °C (2θ) and 1.0°/min, respectively.

Differential scanning calorimetry (DSC) analysis

Gelatinization properties of native and acid hydrolyzed starch particles were analyzed using a DSC Q1000 (TA Instruments, New Castle, DE, USA) with the procedure reported by Espinosa-Solis et al. (9), with minor modifications. In a standard TA hermetic aluminium pan 3.0 mg of starch and deionizer water were added to make a 33 % solid dispersion. The pan was sealed and allowed to equilibrate for 2 h at room temperature within the DSC unit cell was then raised at 5 °C /min increments to 140 °C. An empty pan was used as the reference and the DSC was calibrated using indium. All measurements were done by triplicate.

Results and discussion

Scanning electron Microscopy study

The native and acid hydrolyzed starch particles obtained at different hydrolysis times were observed by SEM (Fig. 2). After 1 day of hydrolysis only some starch granules presented slight erosion on the surface; after 3 days of hydrolysis an increase in surface erosion was observed, but maintaining the shape of the original starch granules.

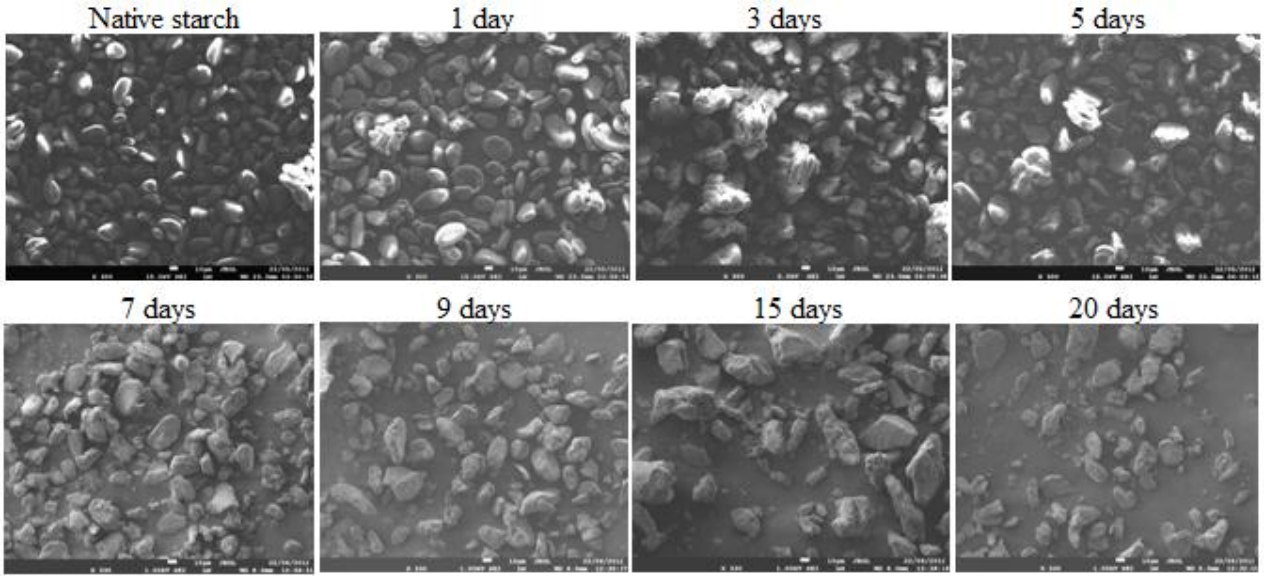

Figure 2. SEM morphology of acid hydrolyzed starch particles

Longer hydrolysis times (5 days and thereafter), the fragmentation of starch granules could be observed, and this pattern increased at longer hydrolysis times (up to 20 days).
Micrographs revealed that until 5 days of acid hydrolysis some residual granules are observed. The number of starch particles increased as longer acid hydrolysis times were achieved, although even after 20 days of hydrolysis some smaller fragments of starch granules are observable. These starch fragments can be associated to initial starch isolation, where a wide variety of shapes and sizes were obtained, that eventually led to an heterogeneous hydrolysis of the granules.. Additionally, the stirring during the acid hydrolysis process favoured the fragmentation of the starch granules (10).

Surface Tension

The starch particles hydrolysed for 20 days at a concentration of 0.05 % produced a surface tension of 68.4 mN/m. The surface tension decreased to 44.7 mN/m when the concentration was increased to 3.0 %. This surface tension reduction, although lower than that reported for surfactants or proteins, strongly suggests that these particles adsorb strongly at the interface, showing substantial surface activity (11).

X-ray diffraction study

X-ray diffraction patterns of native banana starch and the hydrolyzed particles for different times are shown in Fig. 3.

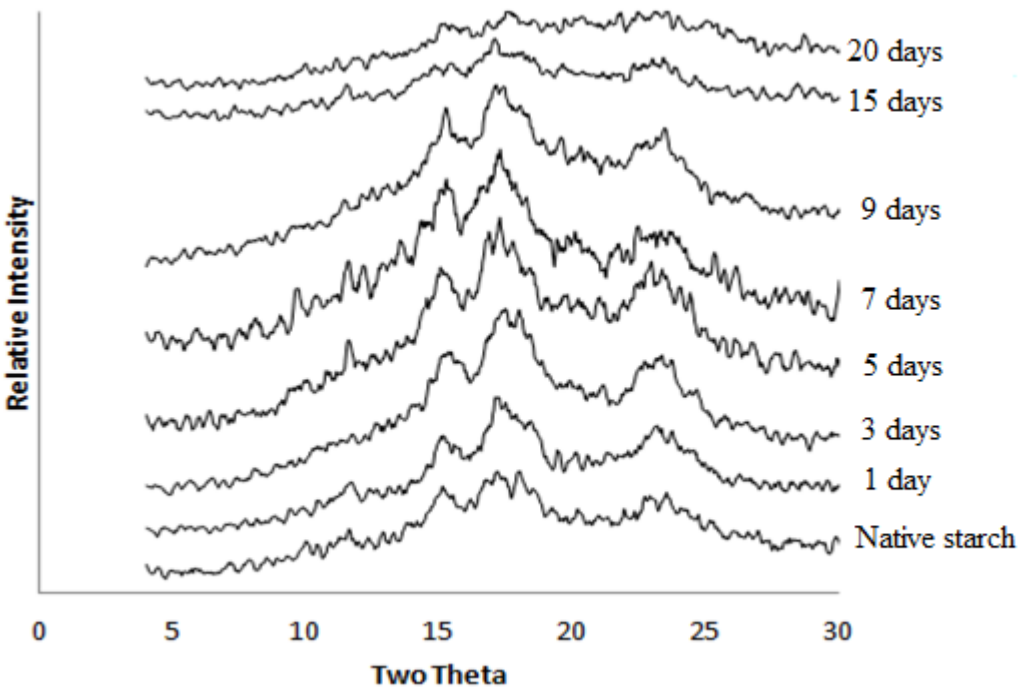

Figure 3. XRD patterns of native and acid hydrolyzed starch at different times

The intensity of the peaks increased with the acid treatment duration (between 1 and 7 days), as the crystalline regions continually increased as the amorphous regions dwindled due to the hydrolysis process. From day 9 up to 15 days, amorphous regions continually dwindled, but the intensity pattern tended to flatten out because a distribution of crystalline regions occurs. After 20 days, none of the crystalline regions dominates, resulting in a quasi-crystalline structure.

DSC analysis

Temperatures (To, Tp and Tc) and enthalpy (ΔH) of the phase transition for native and acid hydrolyzed banana starch are shown in Table 1.

Table 1. Thermal transition properties of native and acid-hydrolyzed starch

	Hydrolysis Time (days)	T_o (°C)	T_p (°C)	T_e (°C)	ΔH (J/g)	T_e -T_o (°C)
Native starch	---	74.6 ± 0.5[ad]	87.6 ± 0.6[a]	101.6 ± 0.9[a]	15.1 ± 0.4[a]	27.3 ± 0.5[a]
Hydrolyzed starch particles	1	71.2 ± 0.3[b]	88.1 ± 0.2[b]	103.9 ± 1.1[b]	14.9 ± 0.2[a]	32.7 ± 0.8[bc]
	3	72.8 ± 0.5[c]	89.2 ± 0.3[c]	104.8 ± 0.8[b]	14.8 ± 0.1[a]	32.0 ± 0.6[b]
	5	73.4 ± 0.1[c]	91.3 ± 0.4[d]	105.3 ± 0.5[bc]	13.5 ± 0.3[bc]	31.9 ± 0.3[b]
	7	74.6 ± 0.5[a]	93.6 ± 0.2[e]	106.7 ± 0.7[c]	14.6 ± 0.2[a]	32.1 ± 0.2[b]
	9	75.1 ± 0.4[d]	95.3 ± 0.7[f]	108.6 ± 0.6[d]	13.2 ± 0.2[b]	33.5 ± 0.7[c]
	15	74.8 ± 0.6[d]	97.4 ± 0.4[g]	111.3 ± 0.9[e]	13.8 ± 0.3[c]	36.5 ± 0.1[d]
	20	----	-----	-----	-----	-----

a) Values are means ± SD of three replicates. Significant differences in each column are expressed as different letter ($p<0.05$).

b) T_o, T_p and T_e = onset, peak and endset temperatures; ΔH = enthalpy change, T_e -T_o = gelatinization temperature range.

The samples hydrolyzed for 1, 3 and 5 days presented significantly lower To, whereas Tp and Te Showed a significant increase as hydrolysis time increased, and enthalpy changes were non-significantly different at any hydrolysis time compared to the native counterpart. These results are in agreement with the microscopy study, where longer hydrolysis times produced less lenticular structures displaying smooth surface erosion. Nevertheless, at this time the X-ray diffraction pattern was very similar to that of native banana starch. This pattern is in agreement with that determined in normal and waxy maize starch hydrolyzed for 5 days (7). At longer hydrolysis times (between 5 and 15 days) an increase in To was found, but they were similar to that of native banana starch. Respect to Tp and Te, the values increased when hydrolysis increased, but the enthalpy value kept constant. The range of the phase transition (Te-To) increased with the acid treatment, indicating heterogeneity in the particles sizes formed during the hydrolysis. The increase in Tp and Te with the acid hydrolysis is related with the removal of the amorphous regions of native starch granules, where the remaining crystalline regions melts at higher temperature, but the content and perfection of the double helices of amylopectin was not altered, as no changes in enthalpy values were observed. DSC data agree with results obtained by SEM and X-ray diffraction after 5 days of hydrolysis. Based on the above results it can be concluded that during the first 5 days of hydrolysis, banana starch granules were removed of their amorphous zones. At longer hydrolysis times the breakup of their crystalline areas was achieved, and also smaller starch particles were obtained. The starch particles in suspension tended to reduce the surface tension with increasing concentration in the suspension, this indicates that particles are surface active and could therefore behave “like-surfactants”, opening the possibility of using them as stabilizers in emulsions, however this requires further study.

Acknowledgements

The authors acknowledge the financial support from the Institute of Science and Technology of the Mexico City (ICyTDF) through the project PICSO-11-64.

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Effect Of Relative Humidity On Glass Transitions In Garlic (*Allium sativum, L.*) During The Drying Process

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Abstract

The glass transition temperature (Tg) was evaluated in order to be used to establish the limits of the temperature variation of the process conditions of drying, since the Tg indirectly reflects the structural damage of the material caused by the drying. This work evaluated the effects of three drying temperatures (40, 50 and 60°C) and three relative humidity (20, 30 and 40%) on the glass transition temperature of garlic (*Allium sativum, L.*). A differential scanning calorimeter was used to determine the Tg in samples of garlic and the statistical analysis with ANOVA. The structural changes during drying were studied by Scanning Electron Microscope. The values of glass transition temperature in garlic were found from 45 to 50°C. With 5% of significance that the relative humidity has significant effect on the glass transition temperature of garlic. It was observed that the degree of shrinkage of garlic during high temperature drying is greater than at low temperature drying. The aim of this work was to study to relative humidity effect on glass transitions garlic (*Allium sativum, L.*) during the drying process. This information shows that the glass transition temperature decreased with the increment of moisture content and it was not highly influenced by process conditions.

Keywords: Garlic, Drying, Glass Transition Temperature.

Introduction

Habitual consumption of fruits and vegetables provides significant benefits for human health (1). Garlic (*Allium sativum*) is a food ingredients widely used in our gastronomy. Is besides to be used like food, has been used as medicinal plant for 4000 years for a variety of ailments including headache, bites, intestinal worms and tumors (2). Evidence from several investigations suggests that the biological and medical function of garlic is mainly due to their high organo-sulphur compounds content (3).

Differential Scanning Calorimetry (DSC) is the most popular thermal analysis technique in which the heat flow rate difference into a substance and a reference is measured as a function of temperature, while the sample is subjected to a controlled temperature program. It is a common feature of these techniques that the various characteristic temperatures, the heat capacity the melting and crystallization temperatures, and the head fusion, as well as the various thermal parameters of chemical reactions, can be determined at constant heating or cooling rates.

The glass transition temperature (Tg) is the temperature beyond which the long-range translational motion of the polymer chain segments is active. At this temperature (on heating) the glassy state changes into the rubbery or melt state. Below Tg, the translational motion of the segments is frozen, only the vibrational motion is active. Formally, the glass transition resembles a thermodynamic second-order transition because at the glass transition temperature there is a heat capacity jump. But this heat capacity increase does not take place at a definite temperature as would be required by equilibrium thermodynamics, but rather in a temperature and relative humidity range exposed. The Tg of a product can be used as a reference in order to modify processing conditions and halt the drying process at process at the appropriate point in order to maintain the quality of the foodstuff during the storage.

The aim of this work was to study to relative humidity effect on glass transitions garlic (*Allium sativum, L.*) during the drying process. This information shows that the glass transition temperature decreased with the increment of moisture content and it was not highly influenced by process conditions.

Methods and Materials

Sample Preparation. Garlic (*Allium sativum, L.*) was obtained from a single lot provided by Walmart Super Market in West Lafayette, Indiana, USA. Longitudinal slices with a thickness of 2 ± 0.5 mm were cut using an adjustable steel slicer. The samples were placed on plastic meshes (25cm × 25cm), and then placed inside humidity chambers ($KC_2H_3O_2$; a_w=0.20, $MgCl_2$; a_w=0.327, K_2CO_3; a_w=0.431) in tree ovens 40, 50 and 60°C of temperature. Sliced garlic samples were removed from the humidity chambers for glass transition determination.

Moisture content. The moisture content of the garlic slices were determined gravimetrically (4) and moisture loss was expressed in terms of percent dry basis.

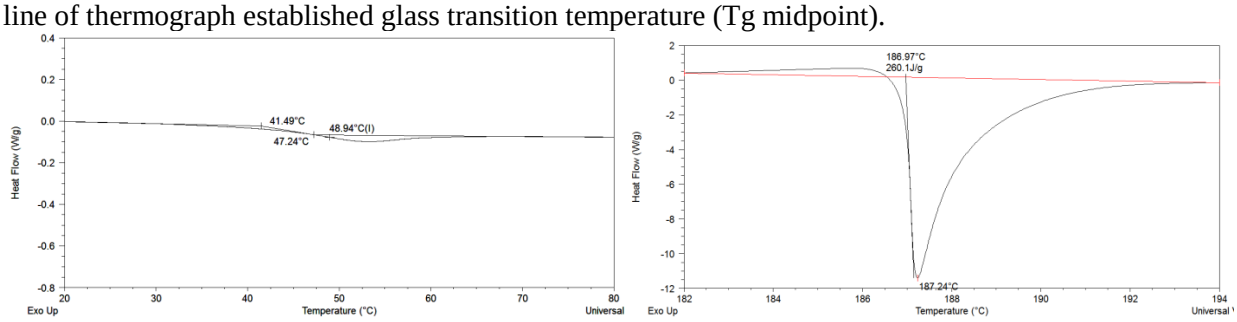
Scanning Electron Microscope SEM. The microstructure of the material was observed from dried samples that had been stored in plastic bags. The cellular structure of the samples was observed using a low-pressure vacuummetric scanning electron microscope (JEOL JSM-6360 LV, Tokyo, Japan) operated at 5 kV in secondary electron mode. The samples were first vacuum-dehydrated and then coated with a 10 nm-thick layer. No chemical fixation was used.

Modulated Differential Scanning Calorimetry (MDSC). The glass transition temperature was obtained using an MDSC (Q2000, TA-Instruments) and Universal Analysis 2000 4.1D software. The samples were ground with a mortar and pestle, and 5 ± 0.5 mg was hermetically sealed in aluminum pan. An empty pan was used as reference. The scanning range was from -40 to 100°C. The heating/cooling rate used in this study was 5°C/min with a modulation of ±0.5 °C/30seg in amplitude.

Statistical Analysis. The experimental design was completely randomized with factorial structure, where the first factor corresponds to the temperature (40, 50 and 60°C) and the second factor to the relative humidity (20, 30 and 40), with two repetitions per treatment. An ANOVA test was used to detect the treatment differences.

Results and Discussion

Figure 1shows some exemplary DSC thermographs obtained during the tests. The Tg was read in average from 45 to 50°C. The melting point was read out as the maximum peak whereas the change in the base line of thermograph established glass transition temperature (Tg midpoint).



a) **b)**

Figure 1 DSC thermographs in garlic: a) Glass transition temperature b) Melting temperature

The results of the different DSC scan are summarized in Table 1. Glass transition temperature also depends on the moisture of the tested materials. After a phase transition, the molecular mobility of foodstuffs is slower, and the product is more brittle than it was prior to the transition. As a result of this effect, Tg can be used as a reference to evaluate quality degradation occurring at a given point in the drying process. Some studies have found that the drying temperature and the thermal history of the material affect the Tg of foodstuff samples (5) (6).

Table 1 Summary of results

Previous work Lopez et al (2011)	Sample	Xeq (gH_20/gdm)	Tg (°C)	Tm (°C)
49.65	40°C 20%RH	0.003	49.15	187.92
	40°C 30%RH	0.004	48.35	185.54
	40°C 40%RH	0.014	47.15	184.09
46.62	50°C 20%RH	0.002	48.65	190.6
	50°C 30%RH	0.006	47.35	189.15
	50°C 40%RH	0.013	46.15	188.2
45.13	60°C 20%RH	0.009	48.40	193.54
	60°C 30%RH	0.015	47	191.46
	60°C 40%RH	0.037	45.9	190.34

The effects of drying on the cellular structure are shown in Figure 2. Well defined cellular structure was found in raw garlic (6), whereas a large amount of cracking and loss of tridimensional cellular structure was found in samples dehydrated ad high temperatures. Damage in the cellular structure of garlic (cellular cracking) may result in the loss of important nutraceutical properties (7).

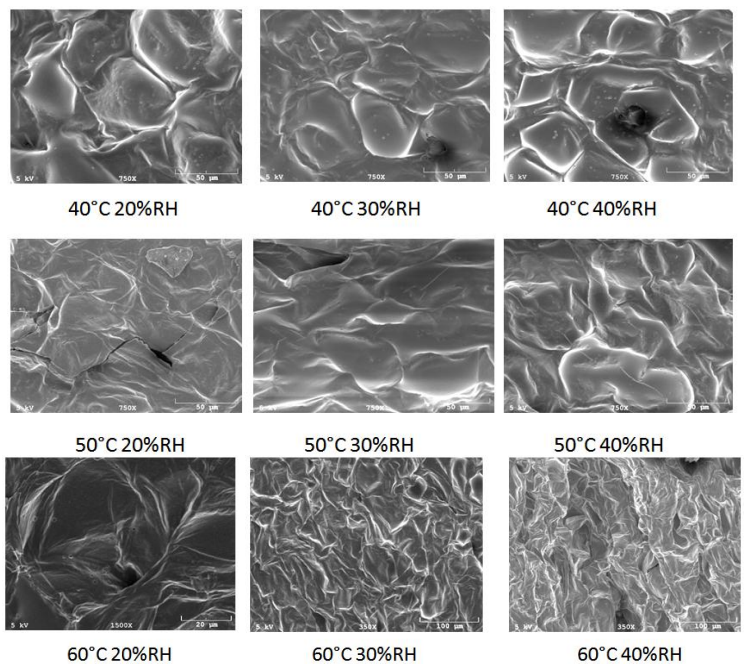


Figure 2 Microstructure of garlic in different conditions

It was observed that the degree of shrinkage of garlic during high temperature drying is greater than at low temperature drying. In addition, the effects of case hardening may affect the formation of pores (8), especially at high temperatures. Water elimination has been found to be accompanied by shrinkage; this results in an increase in apparent density and porosity during the drying process (9).

Conclusion

The glass transition temperature is dependent on the moisture content of the tested material, which imposes that for preserving the structure stability during the storage. Micrograph analysis confirmed that porosity and density at the end of the drying process were related to cracking in cellular structure. Finally

with 95% of confidence show that the relative humidity has significant effect on the glass transition and melting temperature of garlic during the drying process.

Acknowledgments

The authors would like to acknowledge grant to the Consejo Nacional de Ciencia y Tecnologia (CONACyT) and Comite Tecnico de Prestaciones a Becarios (COTEPADE) of the Instituto Politecnico Nacional.

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Microwave Drying Of Red Pepper Strips: Optimization

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Introduction

Drying is used in the food industry to extend product shelf life by reducing water activity. Microwave drying is a viable technological alternative to generate dried foods with a good quality. In addition, it induces a reduction in drying time and hence an energy saving. The aim of this study was to optimize the microwave drying method using red pepper strips cv. California, based on nutritional and physicochemical parameters.

Methodology

Red pepper (*Capsicum annuum* L) cv California was bought in a local market in Culiacan, Sinaloa. Peppers were selected based on color and shape uniformity and absence of defects. They were washed and cut into strips of 1 cm × 5 cm. Pepper strips were immersed in a 0.001% butylated hydroxyanisole, 0.1% acacia gum, 2.5% potassium carbonate and 1% peanut oil solution for five minutes [(1)].

A commercial microwave oven (Frigidaire, FMY141D1PGMM, Chihuahua, Mexico) was used for microwave drying. It was modified to manipulate the surface temperature of the sample and the air extraction rate [(2)].

The selected process variables were food surface temperature (45 to 50 °C) and extracting air velocity (1 to 2 m/s) and the response variables were physicochemical parameters [rehydration index (RI), total color difference (ΔE)] and nutritional parameters (capsanthin, β-carotene and vitamin C contents).

Water activity was measured with an electronic hygrometer (Aqualab CX-2, Decagon, Pullman, USA) previously calibrated with neutral distilled water (a_w = 1.00) and a saturated NaCl solution generating a relative humidity of 0.753 at 25 °C. Based on a preliminary study, drying of pepper strips was finished when the water activity was less than 0.6 for each treatment.

To evaluate the rehydration index, dried red pepper strips were immersed in water at 40 °C for 30 min. They were placed on blotting paper to remove the excess water on the surface. Rehydration index was calculated from the ratio of the mass of adsorbed water to the mass of the dried sample and expressed as percentage. Four replicates were performed for each treatment [(3)].

Chromatic color parameters (L*, a* and b*) in fresh and dried samples were measured with a tristimulus colorimeter (Minolta CR-200 Chromameter, Osaka, Japan). Colorimeter calibration was performed using three readings on the white card, where the device recorded the predefined values of Y = 93.50, x = 0.3139 and y = 0.3196. Total color difference (ΔE) was calculated by the square root of the sum of the squared differences between the chromatic parameters of the fresh and dry samples [(4)]. Therefore, color of fresh pepper strips was selected as reference and the total color difference indicated how far the color of dried slices was from that of fresh samples.

The content of β-carotene and capsanthin were measured by HPLC. A dried sample of 0.25 g was added to 5 mL of acetone and stirred in an Ultraturrax for 2 minutes. The solution was filtered through a 0.45 µm filter. A 1 mL aliquot was placed into an amber vial for analysis by HPLC (Agilent ChemStation, G 1322A, Japan) using a YMC carotenoid column (250mm × 4.6mm × 5 microns), 30°C, an initial mobile phase of terbutilmetileter (15%), methanol (81%), water (4%) and a flow of 0.7 mL/min until reaching the final mobile phase of 79% terbutilmetileter, 17% methanol and 4% water with 0.8 mL/min after 45 minutes. Detection was performed at 447 nm and 484 nm for β-carotene and capsanthin, respectively. An injection of 100 µL and a run time of 80 min (5). The same methodology was used to determine β-carotene and capsanthin in fresh samples, except that the amount of sample used was 2.00 g.

For quantification of ascorbic acid, two grams of sample were mixed with 100 mL of cold deionized water, liquefied for 1 min, filtered through a fine mesh and then filtered again using a 0.45 µm filter, followed by C_{18} Sep-pak cartridges (Waters). An aliquot of 1 mL was analyzed by HPLC (Agilent ChemStation, G 1322A, Japan) using an ODS column SPHERECLONE (250mm x 4.6mm x 5 microns), 11 °C, mobile phase trifluoroacetic acid (TFA) at 0.1%, flow of 0.95 mL/min, wavelength of 254 nm,10 µL injection and a running time of 10 minutes (6). The same methodology was used to determine the ascorbic acid content in fresh samples, but the amount of sample used was 22.7 g.

For construction of the drying curve, the mass sample was recorded every minute automatically with an electronic balance (Sartorius, TE1502S, Germany), through an interface (interface kit Sartorius, model PT YDO 01) and using a computer program (Sartorius Data Transfer Software, version 4.0 for MS-DOS). Drying curves were constructed from the data generated in each of the experiments, plotting the moisture content on dry basis (X, kg water/kg ss) versus time (t, min) (7).

Critical moisture content (Xc) in the sample was determined using multiple linear regression, which proposed a quadratic model ($X = \beta_0 + \beta_1 t + \beta_2 t$) was searched and the regression coefficient (β_2) was not significant ($p > 0.05$), the last value considered to fulfill this condition corresponded to the critical moisture content.

The flux mass (N) was calculated from the product of the slope of the drying curve (dX/dt) and the dry solids and the drying area ratio (8). The local mass transfer (k_Y), was calculated as the ratio of the flux mass and the difference in saturated moisture air (Y_s) and humidity of the drying air (Y) (9).

To find the values of effective diffusivity (D_{eff}), first moisture unreached fraction was calculated FHNA, then it was introduced into the Fick's second law solution of the equation for the unidirectional infinite plate diffusion and the Fourier number (Fo) was determined by numerical methods; finally, the effective diffusivity was determined of the following expression: ($F_0 = D_{eff}t/L^2$).

Drying treatments were obtained from a rotatable central composite experimental design with five levels of variation. Response surface methodology was used to perform the optimization based on physicochemical parameters [rehydration index (RI), total color difference (ΔE)] and nutritional parameters (β-carotene, capsanthin and vitamin C content). It is noteworthy to mention that the criteria considered for optimization were minimum values of ΔE and maximum values of RI, β-carotene, capsanthin and vitamin C contents.

Results and discussion

Experimental RI values in red pepper strips ranged from 115 to 165%; the maximum rehydration index value was desirable, which was obtained at high air speed drying. In microwave heat is generated inside the samples, probably by inducing the state change of internal water favoring cell damage. It is well known that the rate of hydration is related to cellular damage (10) and during microwave drying of chili pepper, most of the damage was caused at high air speeds.

Experimental values of ΔE in dried red pepper strips in microwave drying were found in a range of 9.7 to 14.4, which is relatively low compared with those reported by other researchers. The desired value in this study was a minimum value of total color difference, which was achieved at intermediate air velocities (1.5 m/s) and high temperature (51 °C), due to the shorter exposure time to microwaves.

During microwave drying, β carotene content was found in the range of 0.4 to 1.7 mg/g (bs); the highest content of β-carotene was obtained by processing under mild conditions (44 °C and 1.5 m/s). The experimental values expressed as percentage of retention of capsanthin in microwave drying were found in the range of 18 to 44%. The highest concentration of capsanthin was obtained under mild process conditions with a surface temperature of 45 °C and air velocity of 1 m/s; this concentration decreased as the process conditions became more drastic, with the lowest concentration reached at 51 °C. This behavior was expected since the pigment tends to degrade as the temperature increases. The values of vitamin C content in microwave dried red pepper strips were found in a range of 7.4 to 15.8 mg of vitamin C/g (bs). Maintaining the highest content of vitamin C was the main interest of this study, which was obtained using mild processing conditions (45 °C and 1 m/s).

Optimal conditions for microwave drying were a temperature of 45 °C and air speed of 1.5 m/s.

Red pepper drying under optimal conditions consumed 289 min, with a process time saving of 26% compared to the convective drying; this can be explained by the efficient way heat is generated inside strips by the microwave.

Microwave dried red pepper strips using optimal conditions showed a RI of 159.5% and ΔE of 12.0. Capsanthin; β-carotene, and vitamin C contents were 300 µg/g (db), 0.8 µg/g (db), and 1.0 mg/g (db), respectively. Positively stand rehydration index and the content of capsanthin values.

Under optimal conditions, microwave drying of red pepper strips generated the following engineering parameters: drying rate 2.59×10^{-3} kg water/m^2s, mass transfer coefficient k_Y = 0.97 kg water/m^2sΔY and range of effective diffusivity from 2.08×10^{-12} to 7.00×10^{-9} m^2/s.

Conclusions

Prediction models obtained for the physicochemical parameters are good predictors because the average experimental of ΔE and RI fall within the confidence interval of the predicted value. Moreover, the models obtained for nutritional parameters (β-carotene, capsanthin and vitamin C contents) were poor predictors because it is difficult to model with living beings, because of their rapidly changing characteristics.

Microwave drying of red pepper strips reduces the drying time, increases retention of color and favors sample rehydration, which is important from the viewpoint of quality and productivity.

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Influence Of Fructooligosaccharides Synthesis On The Glass Transition Temperature Of Sucrose Rich Fruit Juices

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Introduction

Nowadays consumers are aware that foods directly contribute to their health. For this reason the consumption of foods and beverages containing functional prebiotics and probiotics is a current global consumer trend. Fortification of foods with novel functional ingredients such as prebiotics like fructooligosaccharides(FOS) is a recent development in this direction (1). FOS areused in the pharmaceutical and food industries as functional sweeteners. FOS with low polymerizationdegreehave better therapeutic properties than those with a high polymerization degree (2). These present properties such as low caloric values, non-cariogenic, decrement of triglycerides and cholesterollevels, and are used as prebiotics to stimulate the growth of bifidobacteria in intestinal flora which potentiates mineral ion absorption *(e.g.* calcium and magnesium)(3). FOS are industrially produced from sucrose by microbial enzymes with transfructosylating activity (fructosyltransferase; EC 2.4.1.9.; FTase), mainly found in fungi such as *Aspergillus, Aureobasidum, Penicillium*and *Fusarium*(4).

On the other hand, spray drying process is the most commonly used method in industries to produce fruit juice powders in large scale. Fruit juice powders have many benefits and economic potentials over their liquid counterparts such as reduction of volume or weight, easier handling and transportation, and imply an effective method of prolonging shelf life. Since, banana is a tropical fruit with a pleasant flavour and nutritional value it may be able to compete in the market of fruit juice. In addition, banana fruit is an important source of carbohydrates as the result of starch-reserve degradation and subsequent conversion to soluble sugars: mainly sucrose, but alsoglucose and fructose (5). Nevertheless, drying of sugar-rich juices to powder is difficult because of the high content of low molecular weight (MW) sugars such as fructose, glucose, sucrose (6) and organic acids, which can cause problems during spray drying (7).Spray drying of fruit juices is possible by adding high MWcomponents to the fruit juices or by modifying the dryer chamber. Addition of some additives such as the starch, arabic gum, and maltodextrins are commonly used as the support materials to increase the glass transition temperature (T_g) of the products during the spray-drying. The major limitations of the use of drying aids are the subsequent change in the product properties and the cost. Furthermore, large amounts (often 35-50%) of drying aid (*e.g.* maltodextrin) are required to convert sugar-rich fruit juices into a powder form (8). Since FOS have MWsgreater than simple sugars like glucose or fructose, may be used as drying aids. Many researchers have reported the $T_g$values for individual sugars (9,10,11). However in literature, to the best of our knowledge**,** there are not data of T_g for anhydrous pure 1-kestose, nystose or 1^F-fructofuranosylnystose, which are the main short chain FOS.

Thus, the aim of this research was to synthesize FOS by using an FTase enzyme from *Aspergillusaculeatus* and banana juice as substrate. The existence of fructosyltransferase activity in the commercial preparation Pectinase (Pectinex Ultra SP-L) from *A. aculeatus* has been reported by others authors (12,13). Additionally, in the present study an enzyme with FTase activity was semipurified by

using sequential steps of a thermal procedure and gel filtration chromatography. Finally, the T_g value of the main commercial saccharides and short chain FOS was determined; likewise, the influence of FOS synthesized from a sucrose solution on the $T_g$also was evaluated.

Materials and methods

Materials

Pectinase from *A.aculeatus*, a commercial enzyme preparation, was purchased from Sigma (St. Louis, MO). FOS standards (1-kestose, nystose and 1^F-fructofuranosylnystose) were obtained from Wako Pure Chemical Industries (Osaka, Japan).Sucrose, D-glucose, and D-fructose were purchased from Sigma (St. Louis, MO). HPTLC(aluminum) plates coated with silica 60 were from Merck (Darmstadt, Germany). All other chemicals were of the highest purity commercially available.

Banana juice preparation

Banana juice was prepared by a method adapted from Ibarra-Junquera*et al.* (14). Ripe bananas (yellow with green tip) were bought at a local supermarket. After separation from the peel, the pulp (100 g) was homogenized to puree by using an electrical blender. Five hundred microliters of pectinase from *A.niger* (Macerex, PM; Enmex, Mexico) per kilogram of pulp was added during milling. The juice was separated from the residue by centrifugation at 20,000 x *g* for 20 min. The supernatant was heated in boiling water for 10 min to inactivate the enzymes.The juice was cryoconcentrated according to the Virgen-Ortíz*et al.* (15) method.

Partial purification of afructosyltransferase (FTase)from A.aculeatus commercial enzyme preparation

The partial purification of FTase from the commercial enzyme preparation was performed by using sequential steps of a thermal procedure and gel filtration chromatography. The enzyme preparation was subjected to preliminary thermal treatment to remove thermosensitive materials. Thermal treatments were carried out in a temperature range of 65-80 °C for 10-90 min and 300 rpm in a thermomixer (Eppendorf, USA). Materials were separated by centrifugation at 20,000 x *g* for 15 min. FTase specific activity of each set temperature-time was determined and used as response variable in an optimization procedure. For further purification of FTase, thermotolerant proteins were dialyzed overnight at 4 °C against deionized water. The dialyzed enzyme was concentrated by cryoconcentration (15). The concentrate was loaded onto Sephacryl S-200 column (2.5 cm x 55 cm) previously equilibrated with 50 mM sodium acetate buffer (pH 5.5). The fractions with similar FTase activity were combined and used for the synthesis of FOS. The activity of FTase was measured by following the release of reducing sugar from sucrose by using DNS method. The standard reaction mixture contained 100 µL of enzyme solution and 1.0 mL of 60% sucrose in 50 mM sodium acetate buffer (pH 5.5). The reaction was conducted at 60 °C for 24 h and then stopped in boiling water bath for 10 min.

Enzymatic synthesis of FOS

Enzymatic synthesis of FOS from banana juice using partially purified FTase preparation from Pectinex was carried out at pH of 4.7. This valuecorresponds to the natural pH of banana juice. Reaction mixture contained 100 µL of enzyme solution (20 µg) and 1 mL of concentrate banana juice. Reactions were performed in microtubes incubated in athermomixerat 60 °C and 300 rpm. Aliquots of 100 µL were withdrawn from the reaction mixture at different times (3, 6, 12, 24, 48, 72, and 120 h) and immediately immersed in boiling water for 10 min to inactivate the enzyme. The samples were centrifuged a 20, 000 x *g* and the supernatant was stored at -20 °C for subsequent analysis. Experiments were carried out in duplicate.

Analysis of reaction mixtures

Thin layer chromatography (TLC) was used for qualitative analysis of carbohydrates. TLC was carried out using five ascents of solvent system of propanol/butanol/water (12:3:4, v/v/v) on silica thin-layer plates

(15). FOS quantitative analysis was carried out by HPLC. Samples were manually injected into an HPLC system (Varian Inc., Walnut Creek, CA) composed by a ternary pump (Solvent Delivery System Model 9012) and a Refractive Index detector (Model Star 9040). The HPLC system was equipped with a YMC-Pack Polyamine II column (4.6 mm x 250 mm) with a spherical particle size of 5 µm (YMC Inc., Wilmington, NC), which was kept at 26 °C. The mobile phase was acetonitrile/water 70/30 (v/v) and was pumped at a flow rate of 1 mL/min.

Glass transition temperature

The T_g of the reaction products was determined using a differential scanning calorimeter (Q1000; TA Instruments, New Castle, DE, USA) equipped with an autosampler and refrigerated cooling unit (TA Instruments, New Castle, DE, USA). Prior to each measurement, the DSC was calibrated to obtain a baseline using an empty cell. The temperature and melting enthalpy of the phase changes were calibrated using indium (melting temperature of 156.6°C and ΔH = 28.45 J/g). Empty aluminum pans were used as reference. The dehydrated materials were stored at room temperature (25°C) in hermetically sealed jars, containing anhydrous P_2O_5 as desiccant, until analysis. Ten-milligram samples of the dehydrated juices were crimped in hermetic aluminum pans, and immediately placed in the DSC cell. Thermal scanning was carried out as follows: sample was maintained isothermal at -30°C for 1 min, heated at 10°C/min up to 120°C and maintained for 1 min. Thereafter, samples were cooled at 20 °C/min up to -30 °C. T_g values were determined after three heating/cooling cycles to improve reproducibility in determinations. Calculation of T_g was done by DSC software (Universal Analysis 2000 Ver. 4.2C; TA Instruments-Waters LLC) after manual setting a range of temperature. All samples were analyzed in duplicate.

Statistical analysis

Results are expressed as mean ± standard deviations of triplicate analyses for each sample unless otherwise stated. Statistical significance was assessed with one-way analysis of variance (ANOVA) using the MatLab software (MathWorks, USA).Treatmentmeans were considered significantly different at $P < 0.05$.

Results and discussion

An important task of bioengineers is to search and obtain proteins with optimized properties for specific industrial applications. In order to do this, bioengineers must be able to isolate and/or purify proteins of interest so their substrate specificities and specific activities can be studied. Each protein purification step usually results in some degree of product loss. Therefore, an ideal protein purification or semipurificationstrategy is one in which the highest level of purification is reached in the fewest steps. Thus, here it was investigated the thermostability of the FTase enzyme and this property was used to design a straightforward approach to semipurifying it by denaturing the other proteins in the Pectinexmixture, then cooling the solution and the denatured proteins removed by centrifugation. The commercial enzyme preparationfrom *A. aculeatus* was treated by heating it under different conditions of time and temperature. Fig. 1shows the effect of temperature and time on the specific activity in the range which variables were studied. As illustrated in the 3D surface plot and 2D contour plot (Fig. 1), maximal specific activity was obtained by using a thermal treatment at 75.4 °C for 46 min, where the specific activity of FTase increased by 5.3-fold. This improvement of specific activity may be attributed to either sedimentation or inactivation of enzymatic inhibitors.The FTaseenzyme was further semipurifiedby using gel filtration chromatography. The collected fractions from Sephacryl S-200 chromatographywere assayed for FTase activity and analyzed by TLC. The Sephacryl S-200 fractions showing high activity, as shown in lanes 50-53 of Fig. 2, were pooled and were used as semipurifiedFTasefor all studies reported herein.

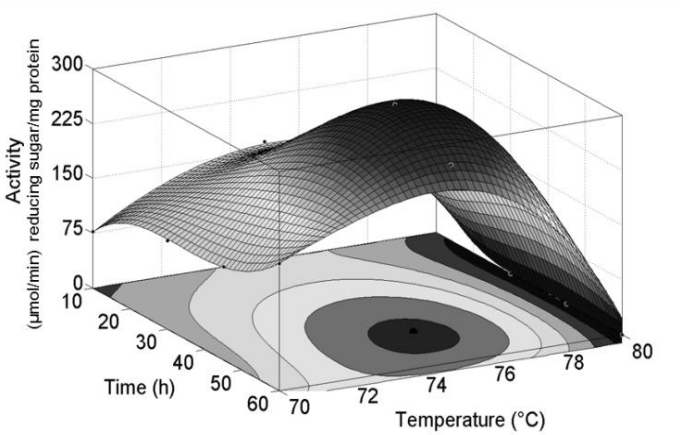

Figure 1. Optimization of thermal treatment applied in the first partial purification step of a fructosyltransferase enzyme from *A. aculeatus*.

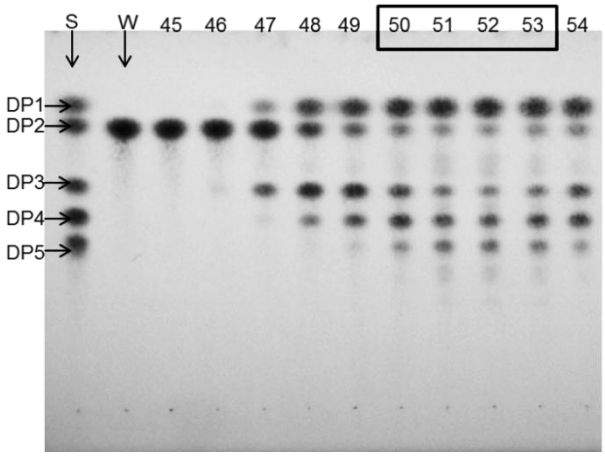

Figure 2. Thin layer chromatography analysis of semipurifiedFTase activity of fractions from Sephacryl S-200 chromatography with sucrose as substrate.Lane S corresponds to the standard of carbohydrates, DP1: glucose, DP2: sucrose, DP3: kestose, DP4: nystose, DP5: fructosylnystose. Lane w is the reaction mixture without enzyme and was used as blank. Lanes of 45 until 54 are reaction mixture corresponding tochromatographic fraction.

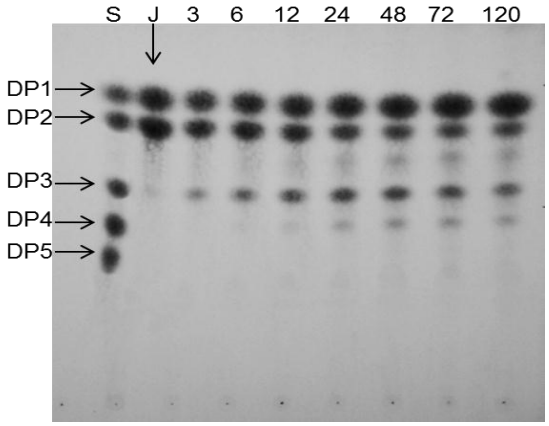

Figure 3. Thin layer chromatography analysis of enzymatic synthesis of FOS from banana juice using partially purified FTase enzyme from *A. aculeatus*. Lane S corresponds to the standard of carbohydrates (cfr. Fig. 2). Lane J is banana juice. Lanes of 3 until 120 are reaction mixture at different times (3-120 h).

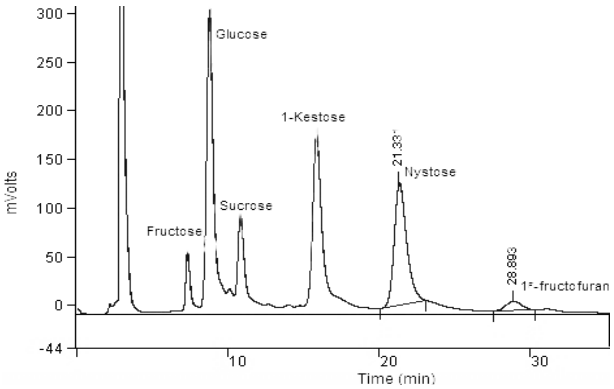

Figure 4.Synthesis of FOS by using a solution of sucrose as substrate for the semipurifiedFTase enzyme from *A. aculeatus* and their HPLC analysis.

The semipurifiedFTase was assayed for the production of FOS. One militer of 60% (w/w) sucrose solution containing semipurified enzyme (20 µL) was incubated at 60 °C for 24 h and then analyzed by HPLC. The analysis of results indicated a FOS production of 56.2% (w/w), referred to total carbohydrates in the reaction mixture. In particular, 23.3 % (w/w) was 1-kestose, 30.7 % (w/w) nystose and 2.2 % (w/w) 1^{F}-fructofuranosylnystose. The rest of carbohydrates in the mixture were fructose (8.5%, w/w), glucose(31.1%, w/w) and remaining sucrose (4.1%, w/w). ThisHPLC profile of sugars is shown in Fig. 4.On the other hand, the concentration of total soluble solids of fresh banana juice was 19 °Brix, which after cryoconcentration procedure increasedto 57 °Brix. This concentrate banana juice was used as substrate for synthesis of FOS. Fig. 3 shows the TLC chromatogram of reaction mixture with respect to reaction time, 1-kestose and nystose were mainly the FOS obtained after 24 h of reaction. HPLC analysis

of banana juice cryoconcentraterevealed a FOS content of 1.5% (w/w) referred to total carbohydrates. After biosynthesis reaction, FOS content increased to 30.6% (w/w) of total carbohydrates, corresponding 77.9% of 1-kestose, 21.3 % of nystose, and 0.6% of 1^F-fructofuranosylnystose. Fig. 5 shows the thermogram obtained from DSC of anhydrous FOS. The corresponding midpoint values of T_g(°C) were for fructose9.27 ± 2.51, for glucose 36.65 ± 0.56, and for sucrose 65.18 ± 0.08, which concurred well with the literature values for these saccharides (9). These results support the accuracy of the method usedhere. Therefore, it is possible to consider as reliable the midpoint values of T_g(°C)of the short chain FOS which are given in Table I. With respect to the reaction mixture containing FOS, its T_g was found to be 9.35 ± 0.29. This value is very low in relation to the expected, this may be due to the reaction mixture was not entirely dehydrated.

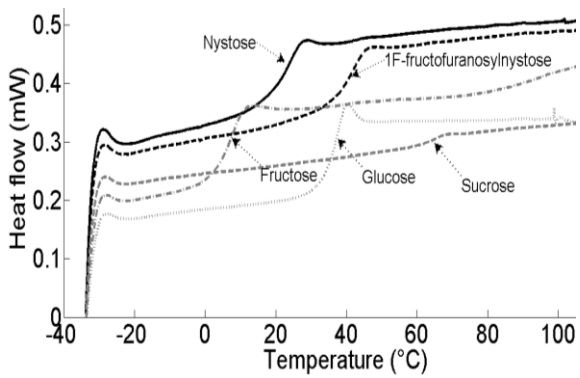

Figure 5. Scanning DSC thermograms for the glasstransition temperature determination in pure amorphoussimple sugars (fructose, glucose, and sucrose) and FOS(nystose, and 1^F-fructofuranosylnystose). Dotted arrow indicates the region of T_g

Table I. Glass transition temperature onset and midpoint of some simple sugar and FOS like as nystose,and 1^F-fructofuranosylnystose.

Carbohydrate	Glass transition temperature (°C)			
	Onset		Midpoint	
	Experimental	[1]Literature	Experimental	[1]Literature
Fructose	2.8 ± 0.23	5	9.27 ± 2.51	10
Glucose	32.88 ± 0.51	31	36.65 ± 0.56	36
Sucrose	61.44 ±0.04	62	65.18 ± 0.08	67
Nystose	18.46 ±0.47	[2]NPR	24.36 ± 0.15	[2]NPR
1^F-fructofuranosylnystose	36.65 ±0.18	[2]NPR	42.88 ± 0.89	[2]NPR

[1]Literature: T_g value given by Roos (9)
[2]Not previously reported

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Effect Of Heat Treatment On The Degradation Of Nanocapsules Of β-Carotene In A Scraped Surface Heat Exchanger [SSHE]

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Introduction

β-carotene is one of the most important food additives and it is widely used as a colorant in foods and beverages, it is also a most effective vitamin A precursor, and it is a preventive agent against certain types of cancer and cardiovascular diseases.Thermal processing is a common process to preserve food products. Unfortunately, sensory properties including nutrient content, color and texture deteriorate during the process [2, 4].Specifically, scraped-surface heat exchangers (SSHEs) are widely used in the food industry to cook, chill, crystallize or sterilize certain foodstuffs quickly and efficiently without causing unwanted changes to the constitution, texture and appearance of the final product [1].The Emulsification–Diffusion Method (EDM) allows the preparation of nanocapsules (NCs) which architecture enhances to encapsulate lipophilic active-substances. The method involves the formation of an oil-in-water emulsion formed by an oily phase of a partially water-miscible solvent (previously saturated) which contains a polymer and an oil and an aqueous phase (previously saturated) which contains a stabilizer. The dilution of this emulsion (diffusion process) provokes the aggregation of the oil an polymer in nanocapsules [4]. The EDM is a agood option to prepare harmless and safe food nanosystems in particular biodegradable NCs considering the low residual amounts of stabilizer and solvent after conventional purification [5, 6].

Materials and methods

Materials

Poly-ε-caprolactone (PCL) (Mw ~80 kDa, ρ = 1.147 s/cm^3 at 25°C) was obtained from Sigma-Aldrich® (USA). The stabilizing agent was Pluronic®, F-127 (Poloxamer 407, PF-127) (BASF, México). Sunflower oil (ρ=0.921 g/cm^3 at 25 °C), β-carotene was used as oily core from Sigma-Aldrich® (USA). The partially water-miscible solvents was ethyl acetate (EA) HPLC grade supplied Fermont (México). The solvent was selected due to their low toxicity (ICH class: 3). Distilled water was of Milli-Q quality (Millipore®, USA-Bedfore, MD). All other reagents were of analytical grade and were used without further purification.

Nanocapsules Preparations

NCs were produced by the Emulsification-Diffusion Method (EDM) described in detail elsewhere (Quintanar-Guerrero et al., 1998) according to optimization performance proposed by Zambrano-Zaragoza et at., (2011).

Particle Size (PS) and Polydispersion Index (PDI)

PS distribution and PDI were determined by the laser light scattering technique at 273° fixed angle and 25ºC, using a Z-sizer 4 (Zetasizer Nano Series Malvern Ltd, France). The dispersionwas diluted in Milli-Q® water, according to the volume frequency histogram. Measurements were made at 25°C in triplicate.

Zeta Potential (ζ)

ζ was evaluated for the system prepared using a Z-sizer 4 (Zetasizer Nano Series, Malvern Ltd, France) after appropriate dilution in distiller water Milli-Q®. ζ values were normalized with polystyrene standard dispersion (ζ = -55 mV). Measurements were made at 25°C in triplicate.

Morphological studies

NCs were purified by three ultracentrifugations (30,000 rpm for 30 min at 5°C) in order to eliminate the excess stabilizer. A droplet of this concentrated suspension was spread on a glass surface and dried. Finally, the dried sample were mounted on stubs and shadowed in a cathodicevaporator with a gold layer (~ 20 nm) using a JFC-1100 Sputter Coater (JEOL, Tokyo, Japan), and were then observed under a low vacuum scanning electron microscope (LV-SEM, JSM 5600 LV) with a resolution of 5 nm. The NCs micrographs were obtained with equipment settings of 20kV electron acceleration voltages and a pressure of 12 - 20Pa in the specimen chamber.

Residence Time Distribution (RTD) Measurement Technique

Fluid RTD within the SSHE was measured using a tracerresponse technique. The experiments were conducted with a fluid pulse injection of 1% of the dead volume in each section of the process (heating, heating + maintenance and total residence),a blue dye number 5 with a concentration of 300 µg/ml using a model fluid a dispersion of CMC 0.5 with NCs. Tracer was injected immediately before the SSHE inlet using a spring loaded piston which introduced the tracer. The light absorbance of the outlet stream was measured continuously at λ = 630 nm using a spectrophotometer (Cintra 10 UV – Visible, GBC Scientific Equipment, Australia).

β-carotene Degradation Kinetic Modeling

The β–carotene residual was determined by spectrophotometryCintra 10 UV-Visible at λ = 480 nm to the output of exchanger, all measurements were done in triplicate.β-caroteneconcentrations were obtained from the calibration curve previously made. The kinetics of degradation of β-carotene was described by a kinetic first order reaction using the Arrhenius equation (Ea) in addition decimal reduction time (D).

Experimental Design

The experimental design was a central composite routable design, with 8 factorials points, 6 central points and 6 axial points with α = 1.68719.It was performed in order to obtain the coefficients of the response surface equation. The ANOVA was performed to evaluate any significant differences between the independent variables using MINITAB release 16 (Minitab Inc., PA, US).

Results and Discussion

Particle size, PDI and zeta potential (ζ).

Nanocapsuleshad anaverage particlesizeof300nmwith a PDIof0.16, indicating a submicronic size with narrow particledistribution. The ζ was of -36.45 mVsuggesting a physical stabledispersion with low probability of aggregation [6].

Nanocapsules Morphology

Figure 1 shows the micrographs corresponding to the nanocapsules of β-carotene. They are the best evidence the capsular structure consisting of an oily nanodroplet with surrounded by a thin envelope.

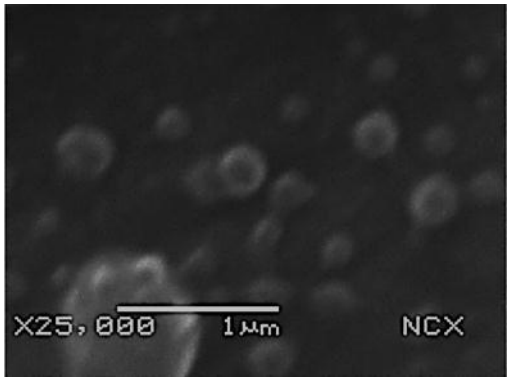

Figure 1 Micrographs of nanocapsules of β-carotene.

Residence Time Distribution (RTD)

Figure 2 shows the effect of flow rate (26-93 %) and the steam pressure (0.5 - 1.5bar) on residence time for determination of degradation rates in NCs according to the Arrhenius law.

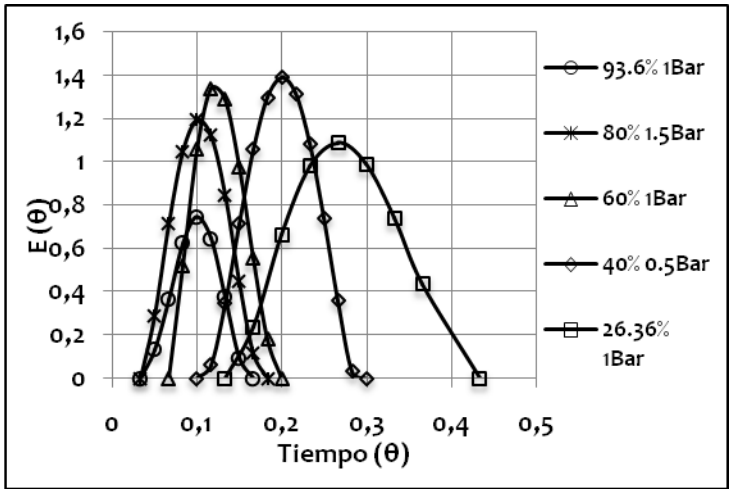

Figure 2 Characterization of flow behavior with NCs of β-carotene dissolved in CMC 0.5

The distribution of residence times followed normal behavior. The greater influence was of flow velocity, reflected by the changes in the amplitude of the distribution which had the highest age distribution when the flow was of 26 %. The stream pressure has an effect on rheological properties that contribute to increased age frequency and the narrow distribution when steam pressure was of 1.5bar minimize the resistance effect of rotor speed.

Analysis of response surfaces

To visualise the effect of the independent variables on the dependent ones, surface response and contour plots of the quadric polynomial models were generated by varying two of the independent variables within the experimental range while holding the other constant at the high factorial points.

Activation Energy (Ea)

Figure 3a-b shows the response surface of the energy activation of NCs. Figure 3a reveals that there is a significant curvature around the central point of the model (R^2) 0.9925, being the most significant factors all linear terms and interactions ($p \geq 0.000$) with a maximum Ea of 186.79 kJ/mol when rotor speed is 31.82 rpm and the steam pressure is around 1 bar. Figure 3b shows a similar behavior around the central points with a maximum of 175 kJ/mol.

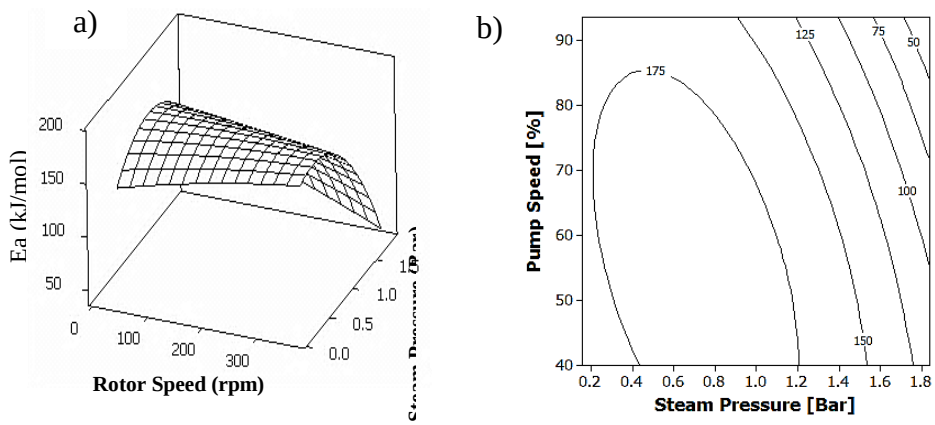

Figure 3 Response surface (a) and contour (b) plots of the activation energy as a function of (a) rotor speed and steam pressure (b) steam pressure and pump speed of 80% and 300 rpm, respectively.

Equation (1) is the second order model for Ea (Y_1) in significant terms:

$$\mathbf{Ea = 2.81X_1 + 0.22X_2 + 206.24X_3 - 0.02X_{11} - 68.32X_{33} - 1.06X_{13} - 0.17X_{23}} \quad (1)$$

a) Loss of β – carotene Nanoencapsulated

The maximum loss of β-carotene nanocapsuleswas at pump speed 60%, rotor speed 200 rpm and steam pressure 1.84bar with a value around 30%. The minimum losswas atpump speed 94.64%, rotor speed 200 rpm and steam pressure 1 bar with a value of 7%.

b) Decimal Reduction Time (D)

The effects of the independent variables on decimal reduction time are shown in Figure 4a-b. Figure 4a was generated by varying the pump speed and rotor speed while holding steam pressure 1.5 bar andit shows the response surface of the independent variablesbeing the most significant factors all linear terms and interactions ($p \geq 0.000$) with a minimum D = 2.49 min when rotor speed is 200 rpm and the pump speed is 93.64%. Figure 4b was generated by varying the rotor speed and steam pressure while maintaining pump speed at 80 %. The expression evaluated for D (Y_2) of unencapsulated β-carotene is:

$$\mathbf{D = 14.86 - 1.6E^{-3}X_{11} + 4E^{-4}X_{12} + 3.63E^{-2}X_{13} - 2.25E^{-2}X_{23}} \quad (2)$$

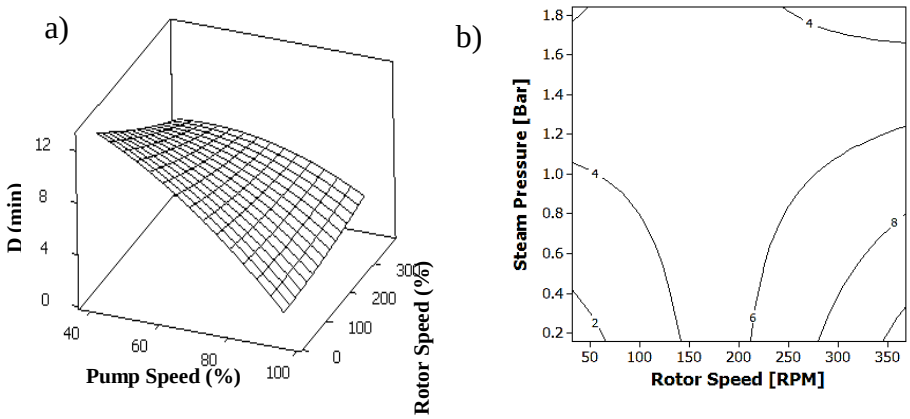

Figure 3 Response surface (a) and contour (b) plots of the decimal reduction time as a function of (a) pump speed and rotor speed (b) rotor speed and steam pressure of 1.5 bar and 80%, respectively.

Optimization

The optimal process conditions obtained for the thermal degradation of β-carotene nanocapsuleswere 82.8 % of pump speed, rotor speed of 283 rpm and steam pressure of 1.24 Bar, with optimum values of Ea = 140 kJ/mol, D = 5 min. with a maximum loss of β-carotene nanoencapsulated of 16%.

Conclusions

The current study showed that the second-order polynomial model fits to describe and to predict the responses of the activation energy and loss of β-carotene nanocapsules. The independent parameters and the quadrics had a significant effect on the loss and activation energy of nanocapsules of β-carotene. Nanoencapsulation provides an additional protector effect that avoids the degradation ofthermolabile compounds in a thermal process such as pasteurization; this effect is attributed to the presence of the membrane film formed around the oily core.

Acknowledgements

The authors acknowledge the financial support for this work from PAPIIT: IT231511 and IN222411-3. We would like to thanks M en C. Alicia del Real for taking the micrographs of nanocapsules.

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Mass Transfer Modeling During Osmotic Dehydration Of Foods Shaped As Annular Cylinders

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Abstract

Unsteady-state mass transfer equation based on Fick's second law of diffusion is proposed to mathematically describe water loss and solute gain curves during osmotic dehydration (OD) of longitudinal sections of annular cylinder-shaped food products, with mass transfer occurring along radial, angular and axial directions. Proposed model was validated through water and solute diffusivity estimation using cucumber cuts as food system. Fresh, well-graded cucumber fruits were washed, peeled, deseeded and cut into annular halves (outer radius = 0.05 m, inner radius = 0.025 m, length = 0.025 m). The resulting product was subjected to OD in sodium chloride solutions (15 g/100 g) at 40, 60 and 80°C. Under the described experimental conditions, effective water and solute diffusivities were in the ranges of 3.70-4.94 × 10^{-9} and 2.31-6.36 × 10^{-9} m^2/s, respectively. Corresponding dehydration and impregnation levels of cucumber at equilibrium were estimated between 0.26-0.0.40 g water/g fresh product and 0.08-0.11 g solute/g fresh product, respectively. Results showed that the proposed model may be accurately fitted to the experimental osmotic dehydration kinetics (R^2 > 0.98) allowing for the reliable estimation of diffusion coefficients in products with the proposed geometry.

(Keywords: Cucumber; Diffusion; Mass Transfer; Modeling; Osmotic Dehydration)

Introduction

OD is a mass transfer operation where water is partially removed from foods by immersing them in hypertonic aqueous solutions such as syrups or brines.[1] Product dewatering is accompanied by a simultaneous solute gain from the solution into the product because of the semi-permeable nature of cell membranes.[2] A good understanding of mass transfer rates for both solute gain and water loss as well as the final impregnation-dehydration levels of a given product is required for an adequate design of this operation. Several mass transport phenomena can coexist during OD; however, most authors have considered that water and solute movement within the product do not depend on each other and both can be described by a diffusion mechanism based on Fick’s second law.[1-4] Depending on modeling assumptions, unsteady-state mass transfer equation may be solved under analytical or numerical techniques. However, the use of simple analytical models for water diffusivity prediction is preferred due to numerous practical advantages such as easier implementation and lower computational effort. Most studies dealing with the estimation of mass transfer properties have focused on product geometries where water loss or solute gain occurs along a single direction (for example, spheres, infinite cylinders or flat slabs).[2,4] Nevertheless, not every product could be cut in such simple shapes. The aim of this study was to develop a simple analytical OD model for 3D mass transfer in foods shaped as sections of annular cylinders. Proposed model was then further used to investigate mass transfer properties during OD of cucumber cuts.

Methods and materials

Osmotic dehydration curves. Fresh well-graded cucumber fruits were washed, peeled, deseeded and cut into annular halves (outer radius = 0.05 m, inner radius = 0.025 m, length = 0.025 m). The resulting product was subjected to OD in sodium chloride solutions (15 g/100 g) at 40, 60 and 80°C. A brine-to-vegetable mass ratio of 20:1 was used in all experiments to avoid a significant change in brine concentration. Magnetic stirring at about 120 rpm was used to allow for negligible external (convective) resistance to mass transfer. Cucumber cuts were numbered with a marker and their weight was individually registered before each experiment. Samples were withdrawn from the solution (without replacement) at predefined immersion times (10, 20, 30, 45, 60, 80, 100, 120, 150 and 180 min, respectively), quickly rinsed with distilled water to remove the excess of solute adhered to the product surface, gently blotted dry with a paper towel and then analyzed for their water/dry solids contents. The amount of solids leaching out of cucumber cuts during osmosis was considered negligible. Therefore, gained NaCl was estimated as the difference in dry solids contents between fresh and processed samples. Fresh and osmotically-dehydrated samples were oven-dried until constant mass weight to determine their moisture and solid contents. These data were further used to evaluate the solute gain and water loss according to the following mass balances,

$$\Delta_{wt} = \frac{m_{w0} - m_{wt}}{m_{p0}} = \frac{m_{p0} X_{w0} - m_{pt} X_{wt}}{m_{p0}} \tag{1}$$

$$\Delta_{st} = \frac{m_{st} - m_{s0}}{m_{p0}} = \frac{m_{pt} X_{st} - m_{p0} X_{s0}}{m_{p0}} = \frac{m_{pt}\left(1 - X_{wt}\right) - m_{p0}\left(1 - X_{w0}\right)}{m_{p0}} \tag{2}$$

Mathematical modeling of osmotic dehydration curves. Unsteady-state mass transfer equation based on Fick's second law of diffusion is frequently used to describe water loss and solute gain curves during OD of food products. Water and solute diffusion along radial, angular and axial directions within an homogeneous and isotropic product shaped as a longitudinal section of an annular cylinder (Figure 1) is given by:

$$\frac{\partial C_k}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left(rD\frac{\partial C_k}{\partial r}\right) + \frac{1}{r^2}\frac{\partial}{\partial \theta}\left(D\frac{\partial C_k}{\partial \theta}\right) + \frac{\partial}{\partial z}\left(D\frac{\partial C_k}{\partial z}\right) \tag{3}$$

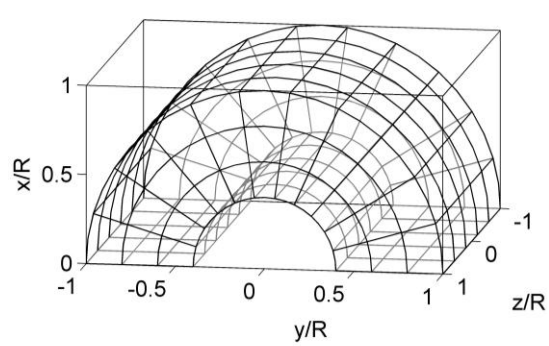

Figure 1. Longitudinal section of annular cylinder.

If the internal resistance to mass transfer by diffusion is accepted as the only mechanism controlling both water loss and solute gain rates, then concentration of diffusing substances at product surface can be

considered to instantaneously reach its equilibrium value. Under this assumption, Eq. (3) has the following boundary condition,

$$C_{ki} = C_{ke} \text{ for } r = \varepsilon, R;\ \theta = \pm\theta_{\max};\ z = \pm L \tag{4}$$

Analytical o numerical solution to Eq. (3) is very often expressed in terms of the dimensionless concentration:

$$\Psi = \frac{C_k - C_{ke}}{C_{k0} - C_{ke}} = 1 - \frac{\Delta_k}{\Delta_{ke}} \tag{5}$$

The solution for the unsteady-state 3D mass transfer problem can be separated in their radial, angular and axial components as $\Psi_{r\theta z} = \Psi_r \Psi_\theta \Psi_z$. The axial component Ψ_z is calculated from the well-known analytical solution

$$\Psi_{kz} = \frac{8}{\pi^2} \sum_{i=1}^{\infty} \frac{1}{(2i-1)^2} \exp\left[-\frac{(2i-1)^2 \pi^2 D_k}{4L^2} t \right] \tag{6}$$

The radial-angular component $\Psi_{r\theta}$ can be calculated from the solution to the unsteady-state mass transfer problem in an infinite longitudinal section of the proposed geometry, and exhibits the general form,

$$\Psi_{kr\theta} = \sum_{i=1}^{\infty} \sum_{j=1}^{\infty} A_{ij} \exp\left(-\frac{\lambda_{ij}^2 D_k}{\delta^2} t \right) \tag{7}$$

where $\delta = (R - \varepsilon)/2$. Eq. (8) may be approximated with the following simplified expression:

$$\Psi_{kr\theta} = \frac{8}{\pi^2} \sum_{i=1}^{\infty} \frac{1}{(2i-1)^2} \exp\left[-\frac{(2i-1)^2 \lambda_{11}^2 D_k}{\delta^2} t \right] \tag{8}$$

where λ_{11}^2 is the asymptotic slope of the $\ln \Psi_{r\theta}$ *vs* $D_k t / \delta^2$ plot (obtained from the numerical solution to the reduced 2D mass transfer problem) and depends on the inner-to-outer radius ratio and the cutting angle of the product ($\lambda_{11}^2 = 2.5545$ for $\varepsilon/R = 0.5$ and $\theta_{\max} = \pi/2$). By combining Eqs. (5), (6) and (9) the following model is obtained,

$$\Delta_k = 1 - \frac{64\Delta_{ke}}{\pi^4} \left\{ \sum_{i=1}^{\infty} \frac{1}{(2i-1)^2} \exp\left[-\frac{(2i-1)^2 \pi^2 D_k}{4L^2} t \right] \right\} \left\{ \sum_{i=1}^{\infty} \frac{1}{(2i-1)^2} \exp\left[-\frac{(2i-1)^2 \lambda_{11}^2 D_k}{\delta^2} t \right] \right\} \tag{9}$$

Model fitting. Proposed model was fitted to water loss or solute gain curves in order to obtain parameters Δ_{ke} and D_k. Quality of fitness of the identified model was quantified by the determination coefficient (R^2). Nonlinear regression (based on ordinary least squares) and statistical analyses were performed with the Matlab Statistics Toolbox 8.0 (MathWorks Inc., Natick, MA, USA).

Results and discussion

Fresh cucumber had a moisture content of 0.966±0.004 g water/g wet product (mean ± s.d.). Representative experimental and predicted water loss data obtained at 40°C are plotted in Figure 2. A good agreement was found between experimental and predicted data for both water loss and solute gain ($R^2 > 0.98$).

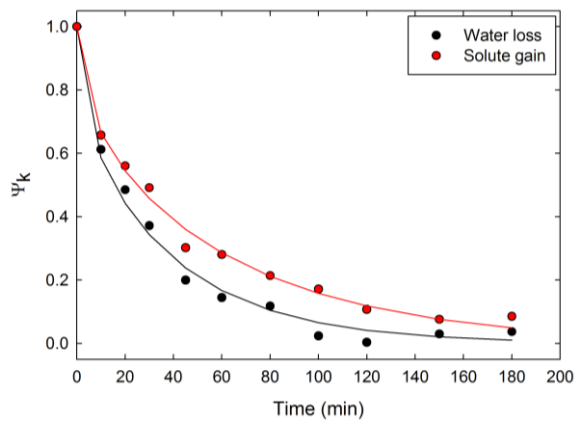

Figure 2. Experimental and predicted water loss and solute gain during OD of cucumber cuts at 40°C.

Summarized statistics for the regression analysis of water loss and solute gain curves are presented in Tables 1 and 2. The use of higher processing temperatures was found to increase diffusion coefficients as well as water loss at equilibrium, while the opposite trend was observed for solute gain ($p < 0.05$).

Table 1. Estimated water loss and solute gain at equilibrium during OD of cucumber cuts

Temperature (°C)	Δ_{we} (kg water/kg fresh product)	Δ_{se} (kg solute/kg fresh product)
40	0.258	0.108
60	0.276	0.101
80	0.400	0.080

Effective water and solute diffusivities were in the ranges of 3.70-4.94 × 10^{-9} and 2.31-6.36 × 10^{-9} m^2/s, respectively. These values are comparable to literature data for other food products such as potato (D_w = 0.87-1.23×10^{-9} m^2/s, D_s = 0.82-1.22×10^{-9} m^2/s, 22-55°C, 0.10-0.18 g solute/g solution) and cherry tomato (D_w = 1.21-1.77×10^{-9} m^2/s, D_s = 0.17-0.54×10^{-9} m^2/s, 25°C, 0.10-0.25 g solute/g solution) osmotically dehydrated in NaCl solutions.[2,3]

Table 2. Estimated diffusivities during OD of cucumber cuts

Temperature (°C)	$D_w \times 10^9$ (m^2/s)	$D_s \times 10^9$ (m^2/s)
40	3.70	2.31
60	4.83	3.18
80	4.94	6.36

Conclusions

A new model has been developed to describe the OD process of food products shaped as longitudinal sections of annular cylinders. The method was validated during OD of cucumber cuts and allowed the reliable estimation of water and solute diffusivities, as well as dehydration and impregnation

characteristics of product at equilibrium. While applied to a diffusion-controlled OD process, current results may be generalized to consider external resistance to water removal or solute uptake.

Nomenclature

C	mass concentration (kg/m^3)
D	effective diffusivity (m^2/s)
L	half-the-cylinder height (m)
m	mass (kg)
r	radial coordinate (m)
R	outer radius (m)
t	time (s)
X	mass fraction (wet basis) of a given component in food product
z	axial coordinate perpendicular to xy-plane (m)
Greek letters	
δ	cylinder semi-thickness along radial direction
Δ	water loss (kg water/kg fresh product) or solute gain (kg solids/kg fresh product)
ε	inner radius (m)
λ_{11}^2	asymptotic slope for solution of $\Psi_{r\theta}$ component
θ	azimuthal angle in the xy -plane from the x-axis (rad)
Ψ	dimensionless concentration
Subscripts	
0	at the beginning of the OD process
e	at equilibrium
k	for either water ($k = w$) or solute ($k = s$)
p	for product
r	denotes the radial direction
s	for solute
t	during OD process
w	for water
z	denotes the axial direction
θ	denotes the angular direction

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Storage Stability Of Lemon Juice Microcapsules Obtained By Spray Drying

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Introduction

Lemon (*Citrus limon* L.) is the third most important cultivated citrus species after orange and mandarin. The countries with the most important lemon productions for processing are Argentina (1,000,000 tonnes), Italy (304,000 tonnes), Spain (300,000 tonnes), USA (230,000 tonnes), and Mexico (296,000 tonnes). Lemon fruit contains many important natural chemical components, including phenolic compounds (flavonoids) and vitamins (Vitamin C), which provides some benefits as prevention of diseases, such as obesity, diabetes, blood lipid lowering, cardiovascular diseases, and certain types of cancer [1]. In order to preserve natural bioactive compounds of lemon juice, microencapsulation by spray drying as a preservation method, may be an alternative for a better utilization, creating new varieties of products and make available throughout the years. Spray drying can be used to convert lemon juice into stable powder with new possibilities of industrial applications (i.e., beverages, ice cream, flavors), should have instant properties and served as a source of vitamin C for addition into food products. Biopolymers used as wall materials for food ingredients encapsulation by spray drying include mesquite gum, maltodextrins [2], and whey protein concentrate [3]. It is hoped that by doing this, the microcapsules can be potentially incorporated in dry form into the functional foods (instant beverages, cake mixes, gelatine desserts, chewing gums, breakfast cereal, etc.). The aim of this work was establish the most suitable storage conditions for lemon juice microcapsules establishing their minimum integral entropy.

Materials and Methods

Materials

Lemon fruits were purchased of a supermarket in Toluca City (Toluca, State of Mexico, Mexico), mesquite gum (MG) was hand collected in the form of tear drops from *Prosopis laevigata* trees in the Mexican State of San Luis Potosi and purified [4], maltodextrin DE-10 (MD) was purchased from Industria Ragar, S.A. de C.V. (Mexico City, Mexico) and whey protein concentrate (WPC) with 80 % protein on dry basis was purchased from Hilmar Ingredients (Hilmar™ 8000, Hilmar, CA, USA). All chemicals used in this study were reagent grade. All the water used in the experiments was bidistilled.

Extraction of Lemon Juice (LJ)

The fruits were washed, cut into halves and were pressed (~25 °C) removing the seeds. The lemon juice (LJ) was filtered through a Tyler # 9 (2000 μm) sieves in order to eliminate solids in suspension.

Microencapsulation of spray-drying of the LJ filtered

Two different biopolymers blends were selected: MG17%-MD66%-WPC17% and MG17%-MD17%-WPC66% w/w to obtain LJ microcapsules. Both biopolymer blends were dissolved into the LJ to a total solids content of 30% w/w and stirred to homogeneity with an Ultra-Turrax T50 basic homogenizer (IKA-

WERKE Works Inc., Wilmington, NC, USA) at a speed of 5200 r.p.m. for 8 min. The solutions were then fed at a rate of 40 mL/min to a Nichols/Niro spray-drier (Turbo Spray PLA, NY, USA) operated with inlet temperature of 135 ± 5 °C, outlet temperature of 80 ± 5 °C and injecting compressed air at 4 bar. The spray-dried powders (MG17%-MD66%-WPC17% and MG17%-MD17%-WPC66%) were collected, kept in plastic bags wrapped with aluminum foil. Spray drying of each formulation was done in triplicate.

Sorption isotherms, thermodynamic properties of microcapsules and determination of Vitamin C
The adsorption isotherms were determined by the gravimetric method described by Pérez-Alonso et al. [5]. The differential enthalpy and integral entropy were calculated by the method described by Pérez-Alonso et al. [5]. Vitamin C was determined by using 2, 6-dichloro-indophenol (Sigma Aldrich, Toluca, State of Mexico, Mexico) according to Official Methods of Analysis, AOAC [6]

Statistical analyses
Data were analyzed using a one way analysis of variance (ANOVA) and a Tukey test for a statistical significance $P \leq 0.05$, using the SPSS Statistics 19.0 (IBM Corporation, NY, USA). All experiments were done in triplicate.

Results and discussion

Sorption isotherms and thermodynamic properties of the microcapsules
The experimental sorption isotherms at 20, 35 and 40 °C for MG17%-MD66%-WPC17% and MG17%-MD17%-WPC66% are shown in Figure 1 a and b, respectively. The adsorption isotherms of the microcapsules had a typical sigmoid shape. Generally, the equilibrium moisture content is expected to decrease with increasing temperature at constant water activity. This trend was observed for both microcapsules, where moisture content increased with the temperature.

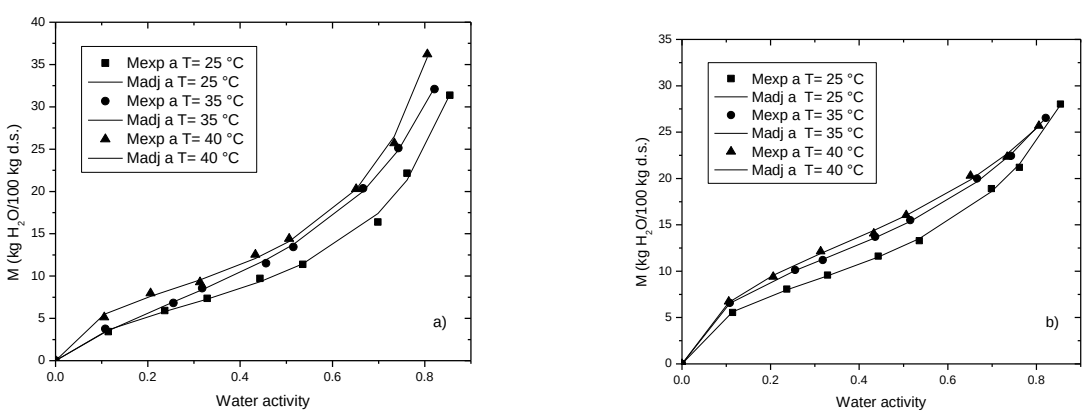


Figure 1. Sorption isotherms of the microcapsules: a) MG17%-MD66%-WPC17%; (b) MG17%-MD17%-WPC66% at 20, 35 and 40 °C

The experimental sorption isotherms data at 20, 35 and 40 °C for both microcapsules fitted very well with the GAB model. The obtained GAB model parameters are given in Table 1. The mean relative deviation modulus value was less than 5% at 20, 35 and 40 °C and the coefficient of determination r^2 was over 0.99 for both samples. The value of the monolayer (M_0) fell within the range of 6.29 to 11.60 kg H_2O/100 kg d.s. and decreased as temperature increased from 20 to 40 °C for both microcapsules. M_0 values are of particular interest, as it indicates the amount of water that is strongly adsorbed to specific sites and is considered as the optimum value at which a food is more stable against microbial spoilage. The values of C and K for both microcapsules (Table 1) fell within the range of $4.59 \leq C < \infty\infty$ and of $0.708 < K \leq 1$.

Table 1. Estimated parameters of the GAB equation for microencapsulated lemon juice

T (°C)	M_0 (kg H_2O/100 kg d.s.)	*C*	*K*	r^2	*E* (%)
MG17%-MD66%-WPC17%					
20	6.29	8.85	0.942	0.997	2.99
35	9.32	4.59	0.892	0.999	2.84
40	7.49	16.48	0.986	0.998	3.06
MG17%-MD17%-WPC66%					
20	7.92	16.73	0.846	0.999	1.22
35	10.19	15.81	0.767	0.999	0.74
40	11.60	13.92	0.708	0.999	1.41

The differential enthalpies (ΔH_{dif}) of the microcapsules plotted as function of water content are presented in Figure 2. All ΔH_{dif} values were negative within the entire water content and temperature range considered. Negative enthalpy values confirmed that strong attractive forces existed between the microcapsules surface and water. A maximum in the -ΔH_{dif} values was presented at 36.0 kJ/mol for a water content of 3.98 kg H_2O/100 kg d.s. for MG17%-MD66%-WPC17% and at 25.20 kJ/mol for a water content of 10.05 kg H_2O/100 kg d.s. for MG17%-MD17%-WPC-66%, respectively.

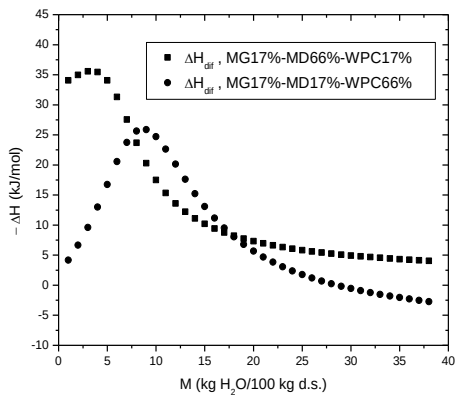
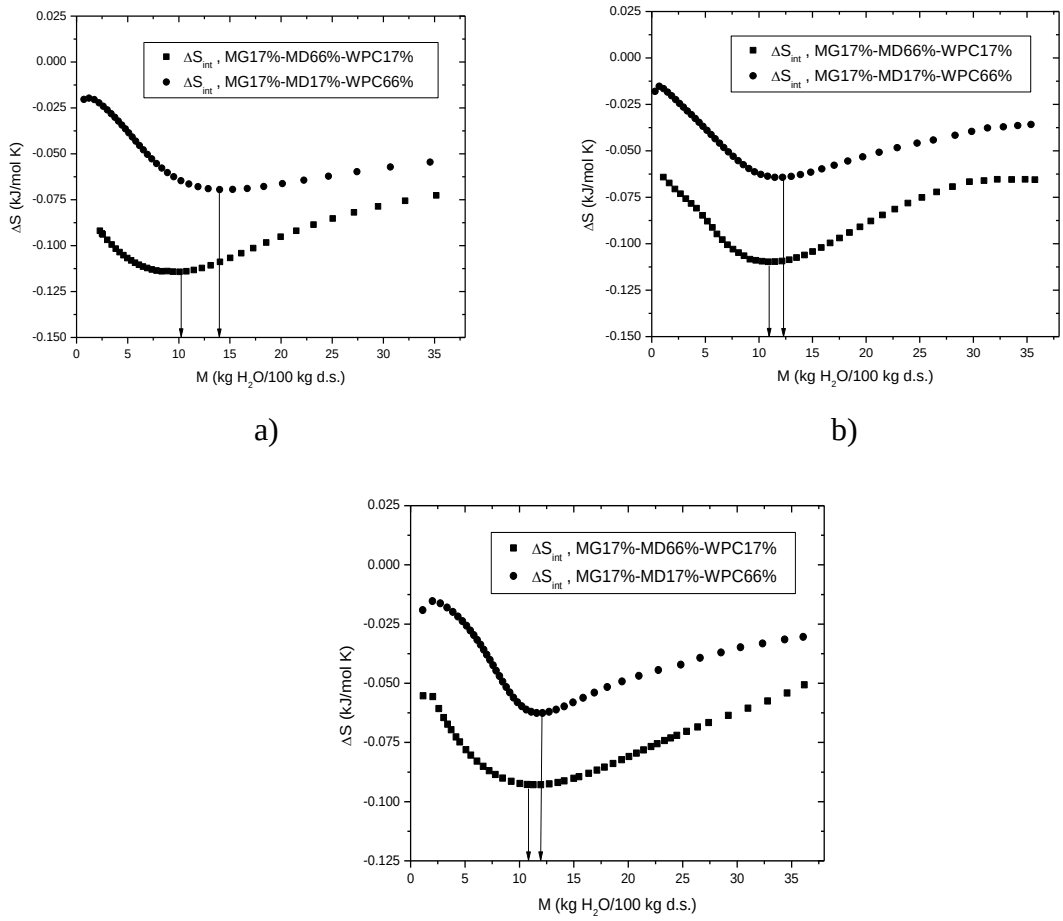


Figure 2. Differential enthalpy as a function of moisture content for microcapsules

Microcapsules may exhibit active sites with different binding energies on their surface, but water molecules are sorbed preferentially onto active sites with the forces producing the most negative ΔH_{dif} values. The maximum enthalpy value indicates the covering of the strongest binding sites and greater water-solid interactions.

Figure 3 shows the variation in the integral entropy, with moisture content at 20, 35 and 40 °C, for the microcapsules. As the powders adsorbed moisture the entropy diminished to a minimum point that is considered as that of maximum stability, because it is where the water molecules achieve a more ordered arrangement within the solid. Values of minimal integral entropy were founded: a) MG17%-MD66%-WPC17% in -0.07 kJ/mol at 25 °C (14 kg H_2O/100 kg d.s.), -0.06 kJ/mol at 35 °C (12.3 kg H_2O/100 kg s. s.) and -0.06 kJ/mol at 40 °C (12 kg H_2O/100 kg d.s.); for b) MG17%-MD17%-WPC66% in -0.115 kJ/mol at 25 °C (10.3 kg H_2O/100 kg d.s.), -0.11 kJ/mol at 35 °C (11.5 kg H_2O/100 kg d.s.) and -0.09 kJ/mol at 40 °C (11.1 kg H_2O/100 kg d.s.).



a) b) c)

Figure 3. Integral entropy as a function of moisture content for microcapsules: (a) 25 °C; (b) 35 °C; (c) 40 °C.

The conditions for maximum storage stability of both microcapsules derived from the thermodynamic analysis are shown in Table 2. As can be seen, as the temperature increases, the water activity increases, but this does not affect in a noticeable way the moisture content adsorbed by the microcapsules of Lemon Juice.

Table 2. Maximum stability conditions for the microencapsulated Lemon Juice

Blend	**T (°C)**	***M* (kg H_2O/100 kg d.s.)**	**a_W**	**Retention of vitamin C (%)**
MG17%-MD66%-WPC17%	25	10.07 ± 0.20	0.482 ± 0.009	65.28 ± 1.31
	35	10.89 ± 0.22	0.421 ± 0.008	56.47 ± 1.13
	40	11.28 ± 0.23	0.406 ± 0.008	50.26 ± 1.00
MG17%-MD17%-WPC66%	25	13.99 ± 0.35	0.553 ± 0.014	51.32 ± 1.28
	35	12.21 ± 0.30	0.377 ± 0.009	38.73 ± 0.97
	40	12.12 ± 0.30	0.328 ± 0.008	29.55 ± 0.74

The best level of vitamin C was at 25° C, a_w = 0.482 to MG17%-MD66%-WPC17% and a_w = 0.553 to MG17%-MD17%-WPC66%. Thus its recommendable storage the microcapsules in water activities above

of 0.5 and temperatures lower than 35 °C. With an increase in temperature, water activity and vitamin C in microcapsules decrease to both microcapsules. Water activity and a_w influence retention of Vitamin C, which is correlated with wall material, porosity and glassy of wall materials.

Conclusions

The stability of the vitamin C contained in the lemon juice extract microencapsulated by biopolymer blends were influenced by the water activity and temperature at which they are stored. Therefore, the microcapsules described in this study represents and interesting food additive as flavor.

Acknowledgements

The authors wish to thank the financing of this research to the Universidad Autónoma del Estado de México through grant 3118/2011.

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Stability And Antioxidant Activity Of Seed-Coat Black Beans (*Phaseolus vulgaris* L.) Microencapsulated By Spray Drying

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Introduction

Recently, research and application of polyphenols have been areas of great interest in the functional foods, nutraceutical and pharmaceutical industries (1). Polyphenols constitute one of the most numerous and ubiquitous groups of plant metabolites, and are an integral part of both human and animal diets which possess a high spectrum of biological activities, including antioxidant, anti-inflammatory, antibacterial, and antiviral functions (2). A large body of preclinical research and epidemiological data suggests that plant polyphenols can slow the progression of certain cancers, reduce the risks of cardiovascular disease, neurodegenerative diseases, diabetes, or osteoporosis, suggesting that plant polyphenols might act as potential chemopreventive and anti-cancer agents in humans (2).

Black beans (*Phaseolus vulgaris* L.) are the most important grain legumes for human consumption. Black beans have been cultivated for thousands of years, and have played (and still do) an important role in the traditional diets of many regions throughout the world. Black beans are widely known for their fiber, mineral and protein contents; however, its nutraceutical value is yet to gain as much attention in the prevention of chronic diseases. The protective effects of black beans in disease prevention such as cancer may not be entirely associated to dietary fiber, but to phenolic and other non-nutritive compounds. Polyphenols from black beans may possibly act as antioxidants, hindering the formation of free radicals that eventually lead to the deterioration of biological molecules. These naturally occurring phenolic compounds are predominantly present in the seed coat and possess antimutagenic and antioxidant activities (3).

Unfortunately, the concentrations of polyphenols that appear effective *in vitro* are often of an order of magnitude higher than the levels measured *in vivo*. The effectiveness of nutraceutical products in preventing diseases depends on preserving the bioavailability of the active ingredients. This is a big challenge, as only a small proportion of the molecules remain available following oral administration, due to insufficient gastric residence time, low permeability and/or solubility within the gut, as well as their instability under conditions encountered in food processing and storage (temperature, oxygen, light), or in the gastrointestinal tract (pH, enzymes, presence of other nutrients), all of which limit the activity and potential health benefits of the nutraceutical components, including polyphenols (2).

The utilization of encapsulated polyphenols instead of free compounds can overcome the drawbacks of their instability, alleviate unpleasant tastes or flavors, as well as improve the bioavailability and half-life of the compound *in vivo* and *in vitro* (2).

The aim of this work was to microencapsulate aqueous extract of seed-coat black beans in two different biopolymers by spray-drying, to determine the most suitable storage conditions of the dried powders and antioxidant activity of powders rehydrated.

Materials and Methods

Materials

Mesquite gum (MG) hand collected in the form of tear drops from *Prosopis laevigata* trees in the Mexican State of San Luis Potosi and purified as indicated by Vernon-Carter *et al*. (4) and Gum Arabic (*Acacia senegal*) (GA) was purchased from Industria Ragar, S.A. de C.V. (Mexico City, Mexico). The black beans were purchased from regional supermarket in Toluca City (Toluca, State of Mexico, Mexico). 2,2-Diphenyl-1-picryhydrazyl radical (DPPH•) and reagents were purchased from Sigma Aldrich (Toluca, State of Mexico, Mexico). All the water used in the experiments was bidistilled.

Black bean aqueous extract (BBAE) preparation

The beans were cleaned and 5kg of selected raw beans were ground with a mortar and pestle to remove the coated seed. The coated seed beans were thoroughly rinsed in bidistilled water and 1 kg were soaked in 2 L of water overnight (approximately 15 h) at room temperature (~20 °C). The black bean aqueous extract (BBAE) obtained was separated by decantation and stored in amber jars at 4°C. Finally BBAE was lyophilized using a freeze-dry system (LABCONCO freeze dry system 18, Kansas City, MO, USA) and collected in plastic bags wrapped with aluminum foil and stored in desiccators containing silica gel at room temperature (~20 °C).

Microencapsulation of BBAE

The two biopolymers (GA and MG) were added independently with the BBAE to a total solids content of 15 % w/w and stirred to homogeneity with an Ultra-Turrax T50 basic homogenizer (IKA-WERKE Works Inc., Wilmington, NC, USA) at a speed of 5800 r.p.m. during 5 min. The solutions were then fed at a rate of 40 mL/min to a Nichols/Niro 40 mL/min spray-drier (Turbo Spray PLA, NY, USA) operated with inlet temperature of 135 ± 5 °C, outlet temperature of 80 ± 5 °C and injecting compressed air at 4 bar. Microcapsules M_{GA} and M_{MG} were obtained. The spray-dried powders were collected, kept in plastic bags wrapped with aluminum foil and stored in desiccators containing silica gel at room temperature. Spray drying of each formulation was done in triplicate (5).

Storage stability of BBAE in the microcpasules

Microcapsules M_{GA} and M_{MG} were stored at two temperatures (25 and 35°C), and four water activities (0.108, 0.115, 0.515 and 0.536) and its analyzed each 8 days, during 56 days in order to verify the effect of these conditions on antioxidant activity reduction of the BBAE microencapsulated.

Determination of antioxidant activities in BBAE free and microencapsulated

Antioxidant activity of BBAE free and microencapsulated were determined by the method of Brand-Williams *et al*. (6). 1,1-diphenyl-2-picrylhydrazyl (DPPH•) radical was dissolved in aqueous ethanol (ethanol: water, 80:20 v/v) to give a final concentration of 200 mmol/l. A series of simple concentrations of 0.1 ml for each sample (10:90, 20:80, 30:70, 40:60 and 50:50) (BBAE: aqueous ethanol) was added to 2.9 ml of DPPH• radical solution. For the control, 0.1 ml aqueous methanol (methanol: water, 80:20 v/v) was added to 2.9 ml of DPPH• radical solution. The mixture was shaken and allowed to stand in the dark at room temperature for 30 min. The absorbance of the mixture was then read at 517 nm. The percent inhibition was calculated as follows:

$$\text{Inhibition} = [A_{517\ \text{control}} - A_{517\ \text{sample}}] / A_{517\ \text{control}} \times 100$$

where $A_{517\ \text{control}}$ is the absorbance of the control (DPPH· solution without test sample) and $A_{517\ \text{sample}}$ is the absorbance of the test sample (DPPH·, solution plus antioxidant). A standard curve was constructed with

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma Chemical Company, St. Louis, MO, USA). The results were presented as µmol Trolox Equivalents/g d.s.

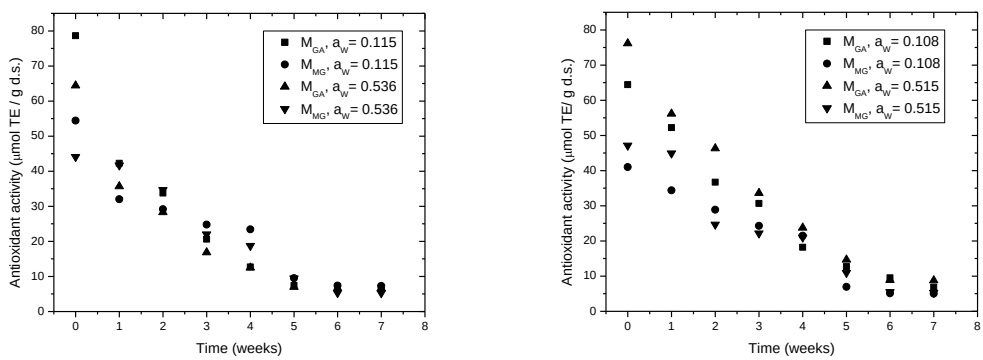
Statistical analyses

Data were analyzed using a one way analysis of variance (ANOVA) and a Tukey test for a statistical significance $P \leq 0.05$, using the SPSS Statistics 19.0 (IBM Corporation, N.Y., U.S.A.). All experiments were done in triplicate.

Results and discussion

Antioxidant activity

Antioxidant activity in the BBAE before spray drying was 222 µmol TE/g dried matter. Figure 1 shows the antioxidant activity of powders produced with different carrier agents and storage at different conditions of temperature and water activity. Microcapsules produced with gum Arabic did not significantly differ between them with respect to antioxidant activity at every temperature in the zero time, while the microcapsules produced with mesquite gum showed the lowest antioxidant activity in the same period of time.



(a) (b)

Figure 1. Antioxidant activity of the microcapsules: a) T = 25 °C; b) T= 35 °C.

Antioxidant activity determined by the DPPH free radical scavenging method was measured throughout the 56 days of storage. The results obtained are presented in Fig. 1, antioxidant activity exhibited higher decrease in the first 21– 28 days of storage with all carriers agents and presented two first-order kinetics: the first one, with higher reaction rate constant, up to 21–28 days of storage, and the second one, after of 28 days, with lower degradation rate (tables 1 and 2).

Table 1. Parameters kinetics of antioxidant activity of BBAE microencapsulated at 25 °C

Microcapsule	Water activity (a_w)	Storage stability (weeks)	Coefficient of determination (R^2)	Reaction rate constant (k)
M_{GA}	0.536	*W0-W5*	1	0.033
		W5-W7	0.983	0.021
M_{GA}	0.115	*W0-W5*	1	0.033
		W5-W7	0.985	0.024
M_{MG}	0.536	*W0-W5*	1	0.034
		W5-W7	0.966	0.021
M_{MG}	0.115	*W0-W5*	1	0.036
		W5-W7	0.980	0.024

Table 2. Parameters kinetics of antioxidant activity of BBAE microencapsulated at 35 °C

Microcapsule	Water activity (a_w)	Storage stability (weeks)	Coefficient of determination (R^2)	Reaction rate constant (k)
M_{GA}	0.515	*W0-W5*	1	0.037
		W5-W7	0.983	0.026
M_{GA}	0.108	*W0-W5*	1	0.036
		W5-W7	0.983	0.022
M_{MG}	0.515	*W0-W5*	1	0.034
		W5-W7	0.966	0.021
M_{MG}	0.108	*W0-W5*	1	0.038
		W5-W7	0.980	0.024

The presence of two first-order kinetics periods was also observed by Tonon et al. (7), in microencapsulated anthocyanins. The period with higher reaction rate corresponds to the degradation of the superficial polyphenolic compounds (non-encapsulated) or to the polyphenolic compounds in contact with the oxygen present in pores or entrapped in bubbles, which leads to oxidation. Moreover, at times greater than 28 days, the matrix density and distance to the entrapped material would limit oxygen transfer, which explains the lower degradation rate at this period.

The influence of temperature on the degradation rates was higher for the samples stored at the higher relative humidity. This indicates that water activity also plays an important role on polyphenolic compounds degradation of powdered BBAE. The higher the water content, the higher is the molecular mobility inside the food, which facilitates the physicochemical reactions of degradation.

Conclusions

The stability of the antioxidant activity contained in the BBAE microencapsulated by gum Arabic and mesquite gum blends were influenced by the water activity and temperature at which they are stored. Therefore, the microcapsules described in this study represents and interesting nutraceutical food additive.

Acknowledgements

The authors wish to thank the financing of this research to the Universidad Autónoma del Estado de México through grant 3118/2011.

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Modeling The Apparent Density And Shrinkage Characteristics Of Fruits And Vegetables During Convective Drying

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Abstract

Convective drying of apple, chayote, jicama and potato cuts has been carried out to both evaluate and model the effect of moisture content and temperature on their apparent density (AD), dry solids concentration (SC), thickness (TS) and volume shrinkage (VS). Fruits and vegetables were washed, peeled, and cut into circular slices (diameter = 0.06 m, thickness = 0.01 m). The resulting product was subjected to convective drying at 40, 60 and 80°C with an air velocity of 1.5 m/s. Under the described experimental conditions, AD was in the range of 0.70-0.77 kg/m^3, while SC range was between 0.05-0.75 kg/m3. Drying of product cuts resulted in samples with about 17-41% of their original thicknesses, while final volume represented about 14-30% of the initial value. As expected, the use of higher drying temperatures increased effective water diffusivities ($p < 0.05$), with mean values in the range of 2.68-7.43 $\times 10^{-10}$ m^2/s. However, no drying temperature effect was observed on AD, SC, LS and VS behaviors of selected products ($p < 0.05$).

(Keywords: Apparent Density; Drying; Shrinkage; Modeling)

Introduction

Convective drying is one of the most important preserving technologies in food industry. While this operation involves several simultaneous heat and mass transfer mechanisms, water diffusion within the product is frequently considered the governing factor. Numerous studies have recognized dimensional changes of product as the main factor to consider in order to achieve both an accurate simulation of drying process and the reliable estimation of mass transfer properties.[1,2] At high moisture contents, product shrinkage is directly proportional to the evaporated water volume. However, as drying proceeds, non-straight shrinking behavior can occur caused by both product deformation and cellular structure collapse. Water removal from food is not only related with size reduction, but also with changes in dry solids concentration and apparent density, both properties having a direct impact on the visual attributes and rehydration characteristics of final product.[3] The aim of this study was to mathematically describe changes in apparent density (AD), dry solids concentration (SC), thickness (TS) and volume shrinkage (VS) during air drying of four different fruits and vegetables. Besides, experimental drying data were further used to evaluate water diffusivity corrected for shrinkage.

Methods and materials

Drying experiments. Fruits and vegetables were washed, peeled, and cut into circular slices (diameter = 0.06 m, thickness = 0.01 m). The resulting product was subjected to convective drying at 40, 60 and 80°C with an air velocity of 1.5 m/s. A high air velocity was chosen to allow for a diffusion-controlled process (negligible external resistance to mass transfer). Moisture evolution was calculated by continuously weighing the product, while volume and thickness changes were determined by using a volume displacement method with glass beads and direct measurement of sample thickness with a micrometer,

respectively. Apparent density and dry solids concentration were calculated according to the following formulas:

$$\rho = \frac{m_p}{V} \tag{1}$$

$$c = \frac{m_s}{V} = \frac{m_p(1-Y)}{V} \tag{2}$$

Experimental moisture and shrinkage data were further used to estimate water diffusivity in dried products by using the slopes method.[1]

Mathematical modeling of shrinkage data. The following relationships were proposed in this study to relate dimensional changes of product with moisture content:

$$\frac{L}{L_0} = \lambda + (1-\lambda)\Psi^m \tag{1}$$

$$\frac{V}{V_0} = \upsilon + (1-\upsilon)\Psi^n \tag{2}$$

$$\rho = \frac{\frac{1}{X_0} + \Psi}{\frac{\Psi}{\delta_0} + \frac{1}{\delta_1 X_0}} \tag{3}$$

$$c = \frac{\frac{1}{X_0}}{\frac{\Psi}{\gamma_0} + \frac{1}{\gamma_1 X_0}} \tag{4}$$

Above formulas have been successfully applied to mathematically describe shrinkage data of several fruits and vegetables.[1-3]

Model fitting. Experimental data were fitted to proposed models in order to estimate their constants. Significance of the regression parameters was tested by constructing their 95% confidence intervals, while quality of fitness of the identified models was quantified by the determination coefficient (R^2). Nonlinear regression (based on ordinary least squares) and statistical analyses were performed with the Matlab Statistics Toolbox 8.0 (MathWorks Inc., Natick, MA, USA).

Results and discussion

Initial moisture contents of apple, chayote, jicama and potato were determined as 85.47, 94.89, 89.32 and 81.80 g water/100 g wet product, respectively. Representative experimental and predicted shrinkage data for chayote are plotted in Figures 1 and 2.

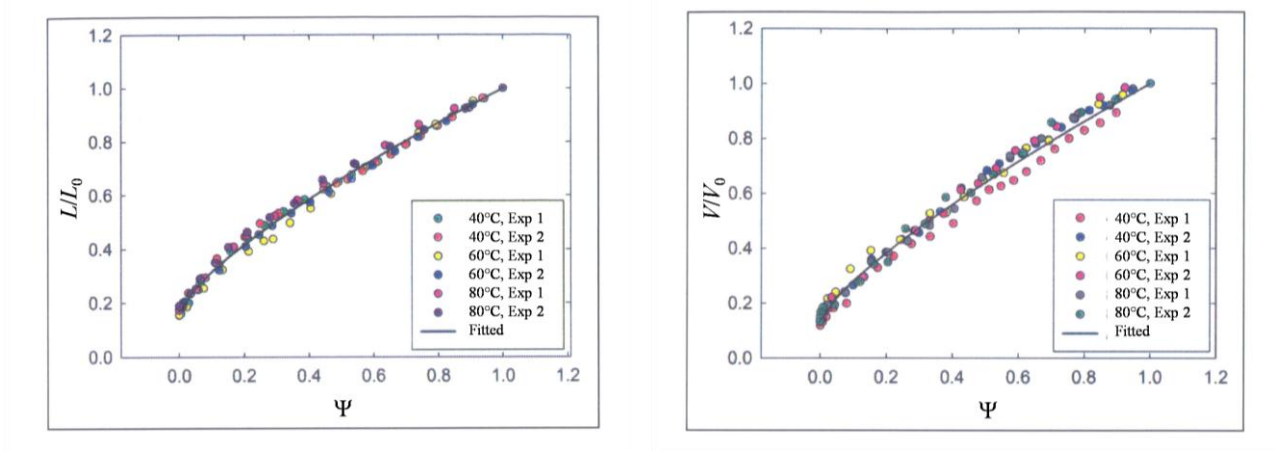

Figure 1. Experimental and predicted thickness (left) and volume (right) shrinkage during convective drying of chayote slices.

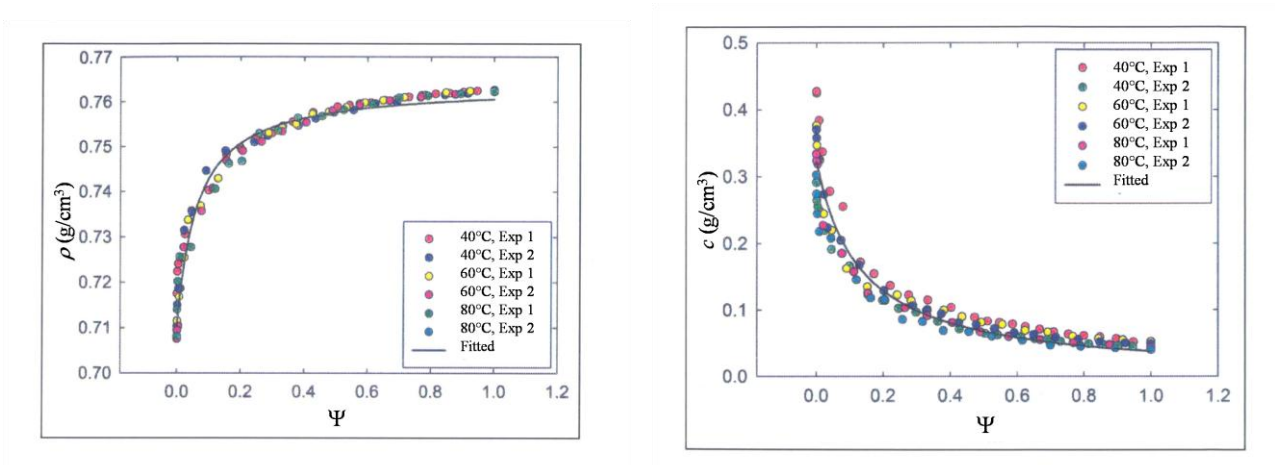

Figure 2. Experimental and predicted apparent density (left) and dry solids concentration (right) during convective drying of chayote slices.

Summarized statistics for the regression analysis of shrinkage-related properties for all studied products are presented in Tables 1-4. Overall, a good agreement was found between experimental and predicted results ($R^2 > 0.84$). No significant temperature effect on studied properties was observed ($p < 0.05$). With the exception of apple TS, all products exhibited a non-straight behavior of TS and VS data when plotted versus moisture content, which can be explained by both product deformation and cellular structure collapse.

Table 1. Estimated parameters for thickness shrinkage model*

Product	λ	m	R^2
Apple	0.409 (0.400, 0.417)	0.912 (0.880, 0.945)	0.990
Chayote	0.171 (0.160, 0.182)	0.754 (0.731, 0.777)	0.994
Jicama	0.287 (0.265, 0.309)	0.668 (0.624, 0.713)	0.974
Potato	0.267 (0.249, 0.285)	0.784 (0.740, 0.828)	0.977

*Values in parentheses indicate the 95% CI.

Table 2. Estimated parameters for volume shrinkage model*			
Product	υ	n	R^2
Apple	0.296 (0.276, 0.317)	1.009 (0.925, 1.094)	0.955
Chayote	0.137 (0.125, 0.150)	0.779 (0.750, 0.809)	0.991
Jicama	0.200 (0.183, 0.217)	0.826 (0.780, 0.872)	0.975
Potato	0.234 (0.214, 0.254)	0.835 (0.779, 0.891)	0.963

*Values in parentheses indicate the 95% CI.

Table 3. Estimated parameters for apparent density model*			
Product	δ_0	δ_1	R^2
Apple	0.766 (0.765,0.768)	0.734 (0.728, 0.731)	0.840
Chayote	0.773 (0.771, 0.774)	0.716 (0.714, 0.717)	0.946
Jicama	0.773 (0.771, 0.774)	0.729 (0.727, 0.731)	0.867
Potato	0.766 (0.765, 0.768)	0.732 (0.731, 0.734)	0.872

*Values in parentheses indicate the 95% CI.

Table 4. Estimated parameters for dry solids concentration model*			
Product	γ_0	γ_1	R^2
Apple	0.789 (0.707, 0.872)	0.324 (0.312, 0.336)	0.868
Chayote	0.714 (0.644, 0.784)	0.322 (0.311, 0.334)	0.928
Jicama	0.817 (0.713, 0.920)	0.375 (0.357, 0.393)	0.844
Potato	0.824 (0.733, 0.915)	0.515 (0.493, 0.538)	0.861

*Values in parentheses indicate the 95% CI.

Mean water diffusivities were in the range of 2.68-7.43 × 10^{-10} m^2/s. As expected, the use of higher drying temperatures promoted a higher water mobility in the food system ($p < 0.05$). Moisture diffusivities, corrected for product shrinkage, are comparable to literature data for other food products such as litchi fruit (4.83×10^{-10} to 1.53×10^{-9} m^2/s, 50-80°C) and mango (2.61×10^{-10} to 1.30×10^{-9} m^2/s, 40-70°C).[4,5]

Table 5. Estimated water diffusivities during convective drying of studied foods*			
Product	40°C	60°C	80°C
Apple	3.618	5.748	7.435
Chayote	2.683	3.959	4.979
Jicama	3.544	4.937	7.263
Potato	2.713	4.230	4.956

*Values×10^{10} (m^2/s).

Conclusions

Four models have been proposed to describe TS, VS, AD and SC evolution during convective drying of fruits and vegetables. Models were validated during air drying of apple, chayote, jicama and potato slices, providing a good description of experimental behavior in all cases. Identified models may be applied for the accurate drying simulation of proposed products.

Nomenclature

c	dry solids concentration (kg/m^3)
L	product thickness (m)
m	power constant in thickness shrinkage equation

m_p	product mass (kg)
m_s	dry solids mass (kg)
n	power constant in volume shrinkage equation
V	product volume (m^3)
X	moisture content (dry basis) (kg water/kg dry solids)
Y	moisture content (wet basis) (kg water/kg product)
Greek letters	
γ_0, γ_1	parameters for dry solids concentration model
δ_0, δ_1	parameters for apparent density model
λ	resulting fraction of the sample thickness at the end of the drying process
ρ	apparent density (kg/m^3)
υ	resulting fraction of the sample volume at the end of the drying process
Ψ	free moisture fraction
Subscripts	
0	at the beginning of the drying process

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Study On Diafiltration For Purifying Carbohydrates Of High And Low Molecular Weight

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Abstract

Purification of carbohydrates by nanofiltration technology under different operation modes was studied in this essay which describes an investigation of total recirculation mode and Continuous Diafiltration (CD) mode of a sugary model solution.
The model solution of carbohydrates of different molecular weight was 6 % (w/v) inulin as fructo-oligosaccharide and 3% (w/v) sucrose as disaccharide. The mixture has been studied with a cross-flow filtration, different pressure and a retentive flow and constant concentration with the aim of investigating the significance of the operating parameters and evaluating a possible purification process.
The results indicate that trans-membrane pressure was the most important parameter in the separation of different molecular weight carbohydrates. Trans-membrane pressure and retentive flow were set for a maximum retention of inulin and lower retention of sucrose about 6.5 bar and 80 L/h respectively. The comparison between the total recirculation mode and diafiltration mode for the purification of sugars was maintained until 85% of retention of inulin for both operations. Furthermore; a removal rate of sucrose around 75% with the dilution process by diafiltration.
The study shows the potential use of diafiltration in the purification of carbohydrates from mixtures of high and low molecular weight.

Introduction

Oligosaccharides are polymers formed by monosaccharides with a polymeration degree between 2 and 10 units, which are found in a row form in various foods. They are non-digestible food ingredients, which favor the selective stimulation of beneficial to-the-health bacteria. Besides, due to their physicochemical and functional properties they are widely used in food processing.
However, oligosaccharide commercial products often contain low molecular weight sugar (monosaccharides), which do not contribute to the beneficial properties of oligosaccharides [1,2].

That is why alternatives of separation methods are required such as membranous processes which consist on the usage of a pressure gradient with the purpose of forcing the way of the components of a solution through a porous semi-permeable membrane and to obtain separations over the size and/or molecular charge. These processes are generally used to accomplish with two objectives: a) to separate solutes from a liquor of a process and b) to concentrate a purified solution thus obtaining a final product. An example of a membranous operation, which has been very well established in food industry and biotechnology as a mean to purify enzimes, polysaccharides and oligosaccharids of a high molecular weight is the nanofiltration.[1,5]

A variation of this process of nanofiltration is diafiltration, which is, at an industrial scale, a potential method for the purification and concentration of carbohydrates mixtures. Recent studies have showed the feasibility of fractionation of such mixtures up to an 85% of oligosaccharides through the diafiltration mean [6].

Diafiltration mode consists on a washing process through the membrane by using dilution water at a constant volume (CVD), with the purpose of improving the recovering of filtered material and the purity of retained material

Experimental section

Nanofiltration Equipment. Experiments were carried out in a pilot scale nanofiltration equipment (Polymem, Francia). It is constituted by a feeding tank, a spiral wounded membrane, two outflow streams and a cooling jacket.

The feeding is contained in the feeding tank with continuous agitation. It is pumped by two pumps, (centrifugal pump and a high pressure pump.) These are connected in series to a pressure module that contains one spiral wounded membranous element with a membrane surface of $2.5m^2$. The module has two outflow streams, permeative and retentive; the permeative stream flows freely while the retentive stream is controlled by a poppet valve. A cooling jacket is connected in the retentive stream which was fitted to allow temperature control of the system.

The unit is instrumented with pressure indicators in the inlet and outlet of the membrane module and between the pumps, flow indicators in the retentive and permeative streams, a digital temperature indicator in the feeding stream and a low level indicator in the feeding tank connected to an emergency stop alarm.

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Membrane module

The nanofiltration membrane was HPA-600 (MWCO = 600 Da, hidrophilized polyamide material, Permionics, India). It has a maximum operating pressure of 27.5 bars, and maximum operating temperature of 40°C and operating pH range 2-11.

Permeability measurement of the membrane. The HPA 600 membrane was conditioned with demineralized water, likewise the permeability of the membrane at different pressures was carried out within the pressure range of the membrane scope, in the range of temperature between 20°C and 30°C. Measurement was carried out before and after every experiment.

Nanofiltration: Total recirculation mode. Inulin/sucrose mixtures were studied with a 2:1proportion respectively, in a pilot-scale nanofiltration equipment, with a total concentration of solids of the 9%. Temperature of the system was kept at 30^oC. The system was kept in an equilibrium state through the recirculation of the retentate and permeate to the feeding tank and, when reaching the permanent regime, samples of the concentration of the solution in the feeding (Co), permeate (Cp) and retentate (Cp) lines were taken.

Nanofiltration: diafiltration mode. In the diafiltration mode the permeative stream is collected in a different tank to separate the streams, meanwhile, the feeding tank is provided with dilution water, where the flow is controlled by a peristaltic pump, and the water inlet flow is equal to the permeative outflow stream. Mixtures were studied at pressures between 3 and 14 bar, at a retentive flow of 80 L/h and 380 L/h. The system was kept in a permanent regime through a total recirculation until reaching the equilibrium. Permeate flow was completely eliminated of the system and it was simultaneously fed with demineralized water as dilution water, keeping the relationship between the volume of entrance of the dilution water and the permeative flow constant. Throughout the process, samples of the feeding and permeative line were taken with time intervals of 10 min. Finally, samples of the final feeding and permeative tank in the total recirculation mode were taken.

Analysis of samples. The samples were analyzed by using a Hi-Plex Ca,

300 x 7,7 mm column and an HPLC equipment adapted to a Evaporative Detector of Light Dispersion (DEDL). The column was kept at 85°C, bidistilled water as a mobile phase and a flow of 0.6 ml/min.

Results and discusion

The operation of nanofiltration in the total recirculation mode allowed to establish that the permeative flow (Jv) raises with the transmembranous pressure (TMP). This lineal relationship Jv and TMP at a retentive flow (Qr) of 80 L/h, determines that, under these conditions, it has not reached the limit flow of the process yet, where the polarization concentration layer is shown and it reaches a high degree, avoiding the permeability of the solution, indeed [7]. However, for a retentive flow of 380 L/h starting at a PTM of 9 bar, the permeative flow is independent from the pressure thus being constant afterwards. The fact that the flow is permeate is an important factor because it determines the productivity of the process, i.e. to a higher permeative flow the recovering tends to be higher as well; therefore, it is more convenient to use higher permeative flows. Besides, results show that the difference of pressures in the process influences in a direct way on the retentive percentage (%TR) of sacrose, as shown in the figure 1. However, the differences on the retentive percentage of inulin when increasing the transmembranous pressure are relatively constant within a range between 85-95%. On the other hand, the retentive flow has an influence over the %TR of the sacrose, determining that the lowest flows allow a lower retention during the process withing a range of 10 to 55%.

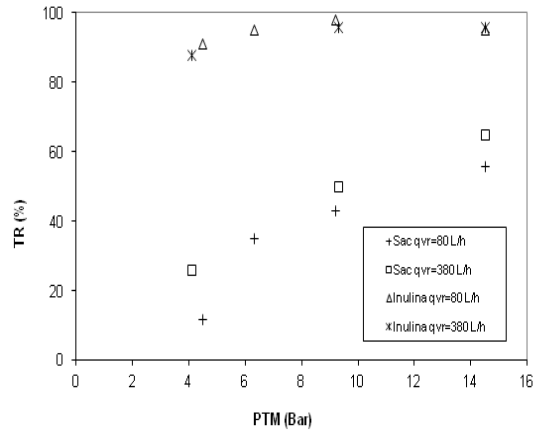

Figura 1. Retentive rate in function of the Transmembranous Pressure. (PTM)

During the continuous diafiltration mode at a constant volume (CVD), a transmembranous pressure of about 6.5 bar was chosen and the lowest retentive flow of 80 L/h, with the purpose of achieving a constant retention of approximately 95% and 40% for inulin-sucrose respectively, throughout the process. The relationship between the concentration of inulin-sacrose and the opeation time are showed on the figure 2. The concentration of sucrose decreases constantly as time increases, while the diminishing rate of inulin was almost nule, being constant throughout the process. The difference of the starting and final concentration during the process for both carbohydrates allowed to determine the sucrose elimination rate at about 75%. The purity degree of inulin raised from 66% to 89% with a performance of about 85% for inulin. These results are comparable to the ones obtained by other authors in the purification of fructooligosaccharides and purities are obtained in the range of 80-90% with performance levels close to 80% [1,3,6].

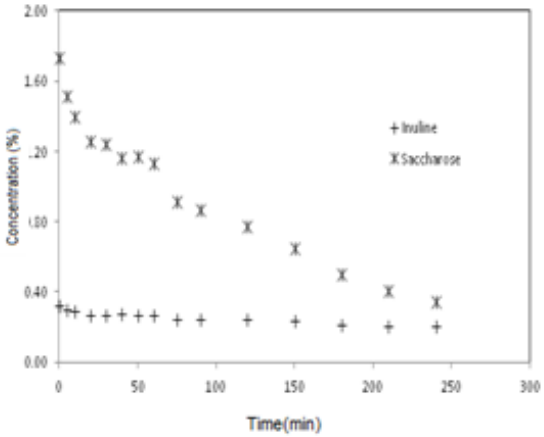

Figure 2. Solutes Concentration in the permeate in time PTM= 6.5 y Qr= 80 L/h

Conclusions

In this work the potential application of the process of nanofiltration in diafiltration mode has been proven, for the recovering of inulin-like oligosaccharides in a mixture with sucrose (disaccharide). The recirculation mode in nanofiltration has allowed to establish the operation parameters which affect throughout the separation process such as transmembranous pressure and the retentive flow likewise it allows to determine the existence of a limit process flow which is influenced by the concentration of polarization. The purification of oligosaccharides through diafiltration provides satisfactory performances of around 85% for inulin and 22% for sucrose. Sucrose elimination rates are better at retentive flows and low pressures (3-6 bar), meanwhile for inulin they may be kept constant in these conditions.
This work allows to establish the basis to establish models that allow to understand and control the operation parameters, thus propose a strategy of extrapolation with the most appropriate conditions of process.

Aknowledgements

This investigation is a cooperative effort between the UASLP - Université de Orléans, especially to the IUT d'Orléans work team.

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Physical Properties Of Pitaya Juice Encapsulated By Spray Drying

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Abstract

The Pitaya is the fruit from the Pitayo cactus and it is juicy and sweet, this fruit can be considered as a functional food due to its high capacity to reduce the spread of free radicals in the body because of its content of total phenols and ascorbic acid that offers to those who consume it protection from chronic illnesses. This study reports the effects of the inlet drying air temperature (125 and 130°C) and encapsulating agents concentrations (maltodextrin and pectin) on the physical properties of the pitaya juice powder. The physical properties include bulk and true density, water activity and color.
(Keywords: pitaya juice, spray drying, physical properties)

Introduction

The pitayo cactus measured between 6-9 m, this cactus produces a fruit called pitaya. The genus pitaya *Stenocereus pruinosus* has an ovoid shape with equatorial diameter (transverse) between 5-12 cm, this fruit weighs between 150-500 g, her shell is smooth green or red, depending on the type and degree of maturation. The pulp of the fruit can be red, yellow, white or magenta. This fruit has a sweet taste. (1)

Spray drying is the most common technology used in the food industry for the microencapsulation of heat sensitive food ingredients by entrapping the ingredient in a coating material (2). Spray driying is a process that results in products with good quality, low water activity an easier transport and storage. (3) Some coating materials used for microencapsulation are maltodextrin and pectin. Maltodextrin is a hydrolyzed starch, it offers advantages such as low cost, neutral aroma and taste, low viscosity at high solids concentrations (4). Pectin is a polysaccharide consisting mainly of galacturonic acid, it is soluble in water but insoluble in most organic solvent, the solutions have low viscosity compared to other plant gums (5).

In spray drying, the operating conditions depend of the characteristics of the material to be dried and the desired powder specifications. Product characteristics are important as they have bearing on transport costs, packaging considerations, and achieving precise quality standards (6).

Studying the effect of operating parameters on the physical properties of powder helps to identify the optimum operating conditions of spray dryers and their effect on powder characteristics (6).

In this study reports the effect of varying pectin and maltodextrin concentrations and spray drying temperatures on the physical properties of pitaya juice powders. These include bulk and true density, water activity and color.

Materials and Methods

2.1 Material preparation

The pitaya fruits were washed carefully with water to eliminate spines from the peel. Then, the fruits were cut into pieces and the peel was removed from the fruit pulp manually. This pulp was crushed manually. Then this pulp was filtered using a metallic sieve (No. 100) to obtain the pitaya juice.

2.2 Sample preparation

After the sieving process, two solutions were prepared, for the first solution the pitaya juice and water distilled were mixed at a ratio of 1:1 with maltodextrin (7 % w/v) and pectin (6 % w/v) to give a final solution of 7 °Bx , for the second solution the pitaya juice and water distilled were mixed at a ratio of 1:1.4 with maltodextrina (14 % w/v) and pectin (11 % w/v) to give a final solution of 5.4 °Bx.

2.3 Spray drying of pitaya juice

A Mobile Minor concurrent flow spray dryer (Niro, Copenhagen, Denmark), equipped with a rotary atomizer (TS-Minor, M02/A) was used to dry the pitaya juice. The diameter of the drying chamber was 800 mm × 620 mm/cone 60◦. The pitaya juice was fed into the drying chamber using a peristaltic pump (Watson-Marlow 505S/RL). The Drying conditions of the first suspension were as follows inlet air temperature (130 °C), feed flow rate (0.64 L/h) and atomizer speed (26,000 rpm), of the second suspension were as follows inlet air temperature (125 °C), feed flow rate (0.58 L/h) and atomizer speed (19,000 rpm).

2.4 Analysis of physical properties

2.4.1 True density

True density of the pitaya juice powder was determined by a Stereopycnometer (Quanta Chrome, Boynton Beach FL, USA). Triplicate samples were analyzed.

2.4.2 Bulk density

A standard volume of measuring cylinder (10-mL) was used in the bulk density measurement. The weight of the powder taken inside the measuring cylinder was recorded and the bulk density of the powder was determined by following formula

$$\text{Bulk density} = \frac{\textbf{Power mass}}{\textbf{Volumen of the power}} \quad (1)$$

2.4.3 Water activity

Water activity of the pitaya juice powder was determined by a Vapor Sorption Analyzer VTI-SA^{+} (TA Instruments, New Castle, DE, USA) with temperature of 25 °C and 50°C with relative humidity values of 10 to 85%.

The experimental data were fitted to the BET (Brunauer-Emmett-Teller) equation (2) and to the GAB (Guggenheim-Anderson-DeBoer) equation (3) using non-linear regression.

$$X_e = \frac{X_m C_{BET} a_w}{(1 - a_w)(1 - a_w + C_{BET} a_w)} \quad (2)$$

$$X_e = \frac{X_m C_{GAB} K a_w}{(1 - K a_w)(1 - K a_w + C_{GAB} K a_w)} \quad (3)$$

where X_e is the equilibrium moisture content (g H_2O/g dry matter), X_m is the monolayer moisture content (g H_2O/g dry matter), a_w is water activity, C_{BET} constant of eq. (2), C_{GAB} constant of eq. (3) and K constant of eq. (3) factor correcting properties of the multilayer molecules relative to the bulk liquid.

2.4.4 Color

The color of the pitaya juice powder was determine by a Spectrophotometer (Shimadzu, Columbia, MD, USA) at a wavelength range of 485 and 535 nm. Both powder samples were prepared so that have the same total solids concentration that the fresh juice.

Results and Discussion

3.1 True density

The densities of the pitaya juice powders are shown in Table 1. In this study, the density was significantly affected by the drying temperature, with decreasing density observed with increase drying temperature. The powders with higher density have a small particle size. This is because of the arrangement of the particle. The particles are in close form an there are fewer spaces between the particles.

Table 1.
Densities of the pitaya juice powder

Temperatura °C	True density (g/cm^3)	Bulk density (g/cm^3)
130	1.468	0.361
125	1.520	0.374

3.2 Water activity

Figures 1and 2 shows the adsorption isotherms of pitaya juice powder, to two temperatures 25°C y 50 °C with relative humidity values of 10 to 85%. The isotherms demonstrate an increase in equilibrium moisture content with increasing water activity, at constant temperature. The adsorption parameters for the BET model and GAB model are present in the Table 2.

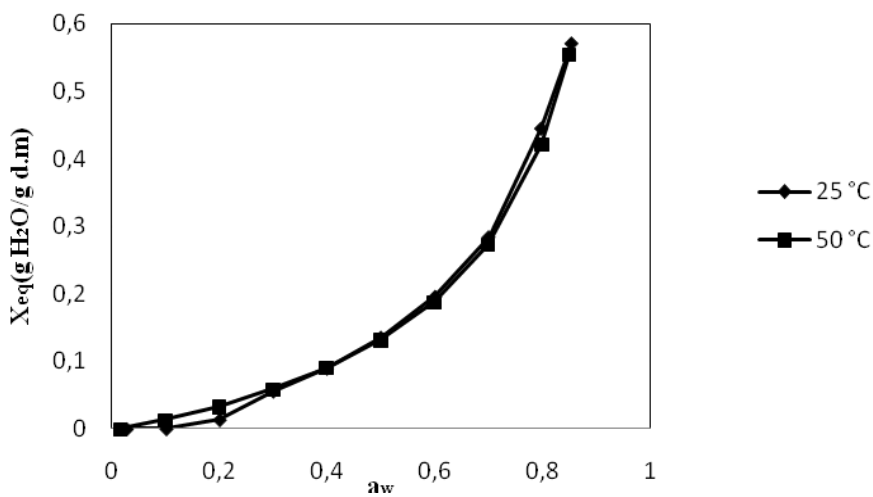

Fig 1. Adsorption isotherms of pitaya juice powder (temperature of dried 130 °C)

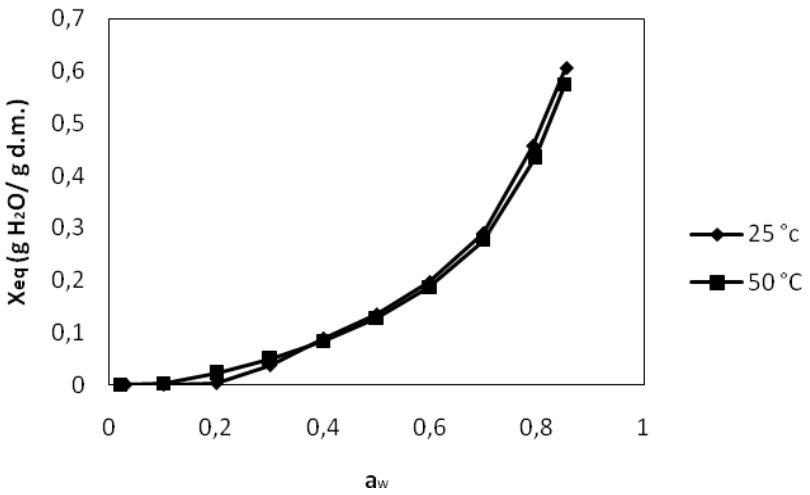

Fig 2. Adsorption isotherms of pitaya juice powder (temperature of dried 125 °C)

Table 2
The adsorption parameters for the BET and GAB models

Model	Parameters	Sample dried at 130 ° C		Sample dried at 125 ° C	
		25°C	50°C	25 °C	50 °C
BET	X_m	0.1013	0.095	0.095	0.091
	C_{BET}	1.722	1.867	2.177	2.399
	R^2	0.987	0.995	0.990	0.997
GAB	X_m	4.5	0.224	0.599	0.134
	C_{GAB}	0.031	0.555	0.238	1.176
	K	0.737	0.883	0.778	0.941
	R^2	0.997	0.999	0.998	0.999

3.3 Color

Table 3 show a decrease of the absorbance in the spray dried samples compared with the fresh pitaya juice which indicates a decrease of color by effect of drying temperature and a decrease of the absorbance between the spray dried samples to 130 and 125 °C by effect of maltodextrin and pectin concentrations.

Table 3
Absorbences of samples dried at 130 and 125 °C

Absorbance of fresh juice		Absorbance of sample dried at 130 ° C		Absorbance of sample dried at 125 ° C	
485 nm	535 nm	485 nm	535 nm	485 nm	535 nm
0.610	0.557	0.587	0.427	0.407	0.284

Acknowledgments

The authors wish to thank the COFFA-IPN and CONACyT by the support granted.

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Thermodynamic Analysis Of Coffee Extract Spray Drying

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Introduction

Spray dryers are widely used in a number of industries to convert liquid feedstocks into dry solid products. Like most other dryers, spray dryers consume large quantities of energy [(3)]. In practical applications drying is a process that requires high energy input because of latent heat of water evaporation and relatively low energy efficiency of industrial dryers[(11)]. This is because the dryers are frequently operated inefficiently. Drying accounts for 10 % of all energy consumption in the food industry. Utilization of high amounts of energy in the industrial drying makes it as one of the most energy-intensive operations with great industrial significance[(7)]. Thus, one of the most important challenges of the industrial drying is to reduce the cost of energy sources for good quality dried product. In this regard, it is essential to perform an effective thermal analysis of spray drying process to provide energy savings and optimum processing conditions.

Exergy analysis has been widely used for assessing energy status of a thermal process from thermodynamic point of view [(1)]. Exergy analysis leads to a better understanding of the influence of the thermodynamic phenomena on the process efficiency, comparison of the importance of different thermodynamic factors, and the determination of the most efficient ways of improving the process under consideration[(7)]. Therefore, the interest of the convective driers thermal efficiency has been incremented recently [(2,4)].

However, the prediction of thermal efficiency needs the mathematical representation of the process. Mathematical modeling of convective drying for foods has been widely described [(6, 8, 9)]. The model proposed by Palencia et al. (2002) is particularly useful because is completely mechanistic and represents the behavior of a spray dryer.This model is represented by

$$\frac{dX_{\gamma j}}{dt}=\frac{k_{c\gamma}a\left(X_{\gamma ji}-X_{\gamma j}\right)}{\varepsilon_{\gamma}}-\frac{G_{\gamma}\left(X_{\gamma j}-X_{\gamma j-1}\right)}{V_j\rho_{\gamma}\varepsilon_{\gamma}}$$

$$\frac{dT_{\beta j}}{dt}=\frac{h_{\beta}a\left(T_{ji}-T_{\beta j}\right)}{\left(Cp_{\beta}+Cp_{w}X_{\beta j}\right)\rho_{\beta}\left(1-\varepsilon_{\gamma}\right)}-\frac{T_{\beta j}Cp_{w}}{\left(Cp_{\beta}+Cp_{w}X_{\beta j}\right)}\frac{dX_{\beta j}}{dt}-G_{\beta}$$

For j=1, 2,…, N

$$X_{\gamma}-X_{\gamma i}-\frac{k_{c\beta}\rho_{\beta}}{k_{c\gamma}\rho_{\gamma}}\left(X_{\beta i}-X_{\beta}\right)=0 \qquad (5)$$

$$-h_{\gamma}\left(T_i-T_{\gamma}\right)-h_{\beta}\left(T_i-T_{\beta}\right)+k_{c\beta}\rho_{\beta}\left(X_{\beta i}-X_{\beta}\right)\lambda=0 \qquad (6)$$

$$X_{\gamma\beta} = \frac{a_w p_w^0/p}{1 - a_w p_w^0/p} \frac{M_w}{M_{air}} \text{ where } a_w = f_1\left(X_{\beta i}, T_i\right) \quad (7)$$

Therefore, the aim of this work is to evaluate the energy consumption and first and second law thermal efficiency of coffee extract spray drying process using different air flows and temperatures.

ThermodynamicConsiderations

Akpinar et al., (2006) evaluated the drying second law efficiency based in the classically defined exergy of air,

$$\dot{E}x_\gamma = G_\gamma\left(\Delta H_\gamma - T_{out}\Delta S_\gamma + \Delta v^2/2 + \Delta p_f/\rho_\gamma\right) \quad (8)$$

It is important to note that eq (8) defines the free energy or exergy of a close system that can be converted to work. However the drying air is not a close system. Drying air interchange mass (water vapor) with the surrounding. Therefore eq (8) it is not adequate for the description of an air mass free energy available for water evaporation into itself[10]. The water vapor-air psychometric is well understood, and it is known that is an adiabatic phenomenon in which,

$$Cp_\gamma\left(T_\gamma - T_{\gamma sat}\right) \approx \lambda\left(X_{\gamma sat} - X_\gamma\right) \quad (9)$$

In this case of drying the system is an air mass that must be able to receipt a given amount of water vapor and transfer the heat required for the evaporation. Therefore,the maximal heat flow that the dryer input air can transfer, called drying air free energy is,

$$E_{max} = \dot{E}x_\gamma = G_\gamma\left(X_{\gamma sat} - X_{\gamma 0}\right)\lambda \quad (10)$$

The amountof energy that mustbe added to thedrying air,assuming there is noentropy productionis,

$$E_a = G_\gamma\left(H_{\gamma 0} - H_{\gamma out}\right) \quad (11)$$

The energy used toheat the productand evaporate thewater, can beexpressed as,

$$E_u = G_\beta\left(X_{\beta 0} - X_{\beta 1}\right)\lambda \quad (12)$$

Then, the maximal theoretic efficiency of a convective drying process is,

$$\eta_{max} = \frac{E_{max}}{E_a} \quad (13)$$

The first law efficiency of the dryer is the fraction of the total energy applied that is used for evaporate the water is,

$$\eta_1 = \frac{E_u}{E_a} \quad (14)$$

The second law efficiency of the dryer is defined as the fraction of the maximal efficiency reached,

$$\eta_2 = \frac{\eta_1}{\eta_{max}} \quad (15)$$

Another important index for thermal efficiency is the amount of the energy added per kg of obtained product,

$$E = \frac{E_a}{G_\beta(1 + X)} \quad (16)$$

In this study the ambient temperature and environment air relative humidity were taken as, T_{out} = 20 ºC and φ = 75 %, respectively.

Results

Energy consumption and first and second law thermal efficiency of coffee extract spray drying were evaluated at different air flows and temperatures. The analysis was developed by simulation using the model proposed by Palencia et al., (2002). This model is represented by four differential equation, representing temperature and moisture of product and air, joint with three algebraic equations, representing interface moistures and temperature.The dryer operation was simulated by solving eqs (1) to

(7) with four order Runge-Kutta method. The simulation was carried out considering the nominal state from an industrial coffee extract spray drier.

Table1 and 2 show theoperation variablesof industrialcoffee extract spray dryerand properties used for model solution, respectively.

Table 1. Operation variables of industrial coffee extract spray dryer

Variables	Value
G_β	575 kg h^{-1}
G_γ	14397 kg h^{-1}
$T_{\beta 0}$	30 °C
$T_{\gamma 0}$	212 °C
$X_{\beta 0}$	1 kg kg^{-1}

Table 2. Properties used for model solution

Property	Value or Equation
Cp_γ	1000 J kg^{-1} °C^{-1}
Cp_β	1657 J kg^{-1} °C^{-1}
Cp_{wv}	1800 J kg^{-1} °C^{-1}
Cp_w	4185 J kg^{-1} °C^{-1}
$D_{w\beta}$	6.67 x 10^{-10} m^2 s^{-1}
H_{wv}	2501.4 x 10^3 J kg^{-1}
k_β	0.1418 + 0.00493 $X_\beta/(1+X_\beta)$Wm^{-1}°C^{-1}
k_γ	8.4044 x 10^{-5} (T+273.15)+4.63 x 10^{-5} W m^{-1} °C^{-1}
μ_γ	4.25 x 10^{-8} (T+273.15)+5.87 x 10^{-6} kg $m^{-1}s^{-1}$
ρ_β	800 kg m^{-3}
ε	0.96

The resultsobtained bysimulationshow thatoutletproductmoisture(0.0054 kgkg^{-1})and outletair temperature (107°C) are close to the obtained inthe actual drying process.Noting thattheoutlet airtemperatureis very high,causingthe drying processisinefficientbecause thedrying airstillhas thepotential to continuedryinghoweveris discharged to theenvironment, which causes agreat wasteof energy.

Therefore, withthe purpose of reducingenergy consumptionand increasethermal efficiencyofcoffee extractspraydrying processwerecarried outseveral simulationsof the drying processat different air flows (8700–14397 kg h^{-1}) and temperatures (140-212 °C). The feed flow of coffee extract was constant (575 kg h^{-1}) with a 50% of solids content. The spray drying process was considered ideally isolated.

Figure 1 shows that outlet moisture of the product was increased at lower air flow and temperature. However the air outlet moisture tended to saturation. Therefore was fixeda limitof0.01 kgkg^{-1}in dried product and 90% of saturation in air outlet moisture.Under theserestrictions,theminimum value of energy consumption was obtained at inlet air flow of 13000 kg h^{-1} and air temperature of 160 °C,achieving savings of 34% over the energy consumption of the actual process (4781 kJkg^{-1} at inlet air flow of 14397 kg h^{-1} and air temperature of 212 °C), as shownin Figure2.

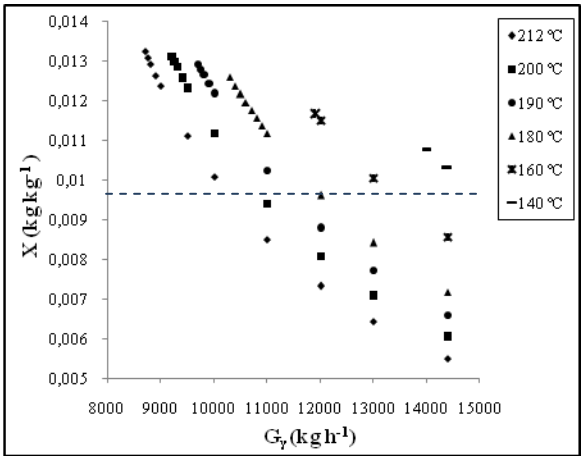

Fig. 1. Effect of air flow and temperature over the final moisture of product.

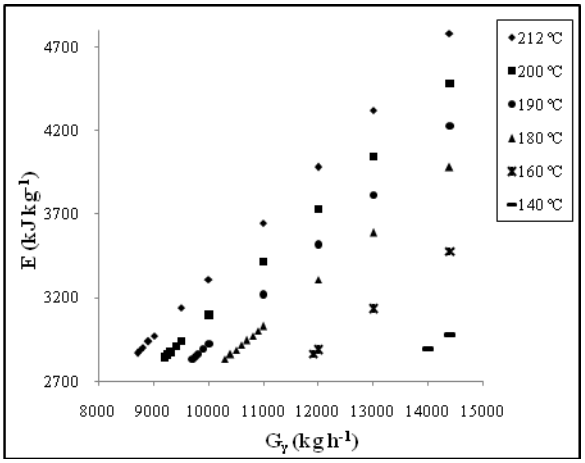

Fig. 2. Effect of air flow and temperature over the energy consumption per kg of obtained product.

Figure 3 shows that the first law thermal efficiency increased from 0.46 (first law thermal efficiency of actual drying process) to 0.74at inlet air flow of 13000 kg h^{-1} and air temperature of 160 °C, so the coffee extract spray drying process was38 % more efficient. Figure 4 shows that the second law thermal efficiency increased from 0.56 (second law thermal efficiency of actual drying process) to 0.87 at inlet air flow of 13000 kg h^{-1} and air temperature of 160 °C, so the coffee extract spray drying process was 36 % more efficient.

As can be seen from Figures 3 and 4, the drying process was more efficient both by first and second law at lower air flow andtemperature, this isbecause the energy usedin thespray dryingprocess was higher at inlet air flow of 13000 kg h^{-1} and air temperature of 160 °C than that usedat the operating conditions ofactualdrying process (air flow of 14397 kg h^{-1} and air temperature of 212 °C), which represents agreater utilizationof the energyadded to thedrying air and therefore less energy loss. In addition to theenergy consumption perkgof product obtainedwas lower than the energy consumed in the actual coffee extractspraydrying process.

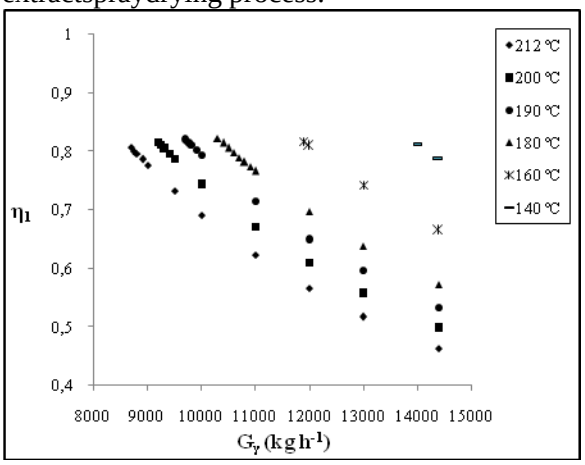

Fig. 3. Effect of air flow and temperature over the first law thermal efficiency of coffee extract spray drying.

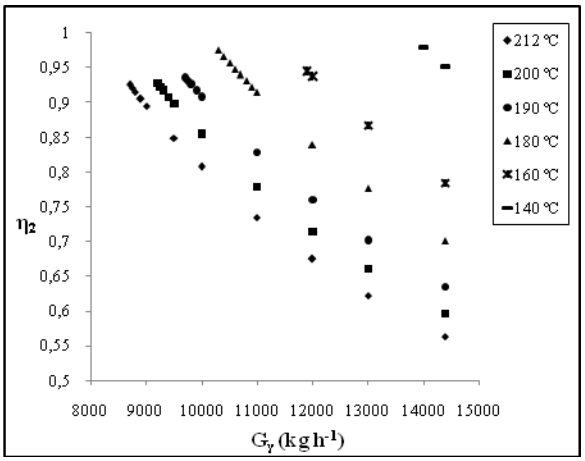

Fig. 4. Effect of air flow and temperature over the second law thermal efficiency of coffee extract spray drying.

Conclusions

The effects of temperature and air flow on energy consumption and first and second law thermal efficiency of industrial coffee extract spray drying processwere investigated.The results showedthat,at inlet air flow of 13000 kg h^{-1} and air temperature of 160 °C, the energy consumption was lower and the

thermal efficiency of industrial drying process was improved significantly because there was greater use of the energy added to the drying air.

Nomenclature

a	specific surface area	m^2m^{-3}
a_w	water activity	-
A	area	m^2
Cp	specific heat	$Jkg^{-1}{}^oC^{-1}$
D	mass diffusivity	m^2s^{-1}
E	total energy added	Jkg^{-1}
$\dot{E}x$	exergy flow	Js^{-1}
G	mass flow	Js^{-1}
h	heat transfer coefficient	$Wm^{-2}{}^oC^{-1}$
H	enthalpy	Jkg^{-1}
k	thermal conductivity	$Wm^{-1}{}^oC^{-1}$
k_c	mass transfer coefficient	ms^{-1}
M	molecular mass	$kgkmol^{-1}$
N	number of ideal steps	-
p	pressure	Pa
q	heat flow	Js^{-1}
T	temperature	^oC
t	time	s
X	moisture content (dry basis)	$kgkg^{-1}$
V	volume	m^3
Greek letters		
ε	bed porosity	-
η	thermal efficiency	-
λ	latent heat of vaporization	Jkg^{-1}
ρ	density	kgm^{-3}
Subscripts		
0	initial or inlet condition	
1	first law	
2	second law	
air	air	
f	friction losses	
i	solid-gas interface	
j	A mixed stage	
out	Environmental conditions	
s	dry matter in solid	
w	water	
wv	for water vapor	
β	solid phase	
γ	gas phase	
Superscripts		
0	At reference	

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Development And Implementation Of A Sterilization System With Compensated Pressure, In Order To Study Heat Penetration In A Retort Pouch Product.

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Abstract

The demand of efficient, economic and quality processes in food micro-industries has lead to the use of new tools in order to generate technological innovation. One of the needs identified, is the implementation of a sterilization system with compensated pressure for retort pouches; therefore, the objective of the present work was to design a prototype for commercial sterilization, coupled to a compensated pressure system, to process retort pouch food products. For this, a vertical autoclave (pressure cooker) was modified with a pneumatic pressure system based on compressed air, in order to facilitate heat treatment in the retort pouches. The modifications made in the prototype were of pneumatic type, coupling a valves system, so that compressed air could enter the autoclave, but would not let water vapor exit the system. Pressure compensation was necessary to avoid package from collapsing. It was necessary to assure a hermetic process, because any leak could affect the system pressure. Bags of nylon and pp with 15 x 110 x 150 mm were sterilized retort pouches containing foods. Heating media for retort pouches were selected through heat distribution tests at 121º C in a full retort with times of 30 min. Results of heat penetration curves were obtained using the graphical method of Bigelow, using tables generated in Microsoft Excel, to monitor that F0 value achieved was 2.4, in order to assure the abscense of *C. botulinum.*

Keywords: Retort pouch, pressure compensated, autoclave

Introduction

A retort pouches consist of 3 or 4 laminated thin layers of polyester, aluminum and a mixture of polyethylene used to form a flexible packaging material for food. Food is placed inside the package and sealed and sterilized at temperatures of 115-132° C. The packaged food can be heated in the same packaging system in boiling water or in a microwave oven [1].

The retort pouches are actually considered as a reinvention and are an actual trend on food packing. Basically this packaging mechanism is associated with thermal sterilization of foods of high water activity (greater than 0.85) and low acidity (pH greater than 4.5). Foods packed in these containers may be sold at room temperature, which are considered separately for its ability to decrease handling time and better sterilization packaging than if glass or tin is used[2].

The retort pouches offer potential advantages over other packaging materials of food products. As for the protection, preservation and quality, these are superior to frozen like cans and jars of preserves. They also provide greater shelf life, take up less volume and weigh less than canned food, resulting in savings of money to store and transport (empty storage volume of these is from 1 to 25 in relation to cans).

Among its main characteristics are heat resistant and have high tensile strength and impermeability to gases and steam water. More over, sterilization processes supported above 121 ° C for times ranging between 30 and 60 minutes in an autoclave with pressure compensated. Being a flat gasket has a greater surface area for heat transfer, where by the heating and cooling are reduced for faster sterilization times (30% to 40%, no products overcooked),which results in improved end product quality and energy savings of the process [3].

Heat sterilization is one of the most effective methods to preserve food for long periods of time. The aim of this is to extend the life of food products and make sure the food for the consumer by destroying food borne pathogens [4]. Jiménez Islas [5] mentioned that to ensure safety in the production of canned food, it is important to know the dynamics of heating container cold spot. If the heat treatment is excessive, the

food loses nutritional value and sensory characteristics. Conversely, if food is not properly sterilized, the danger of growth of anaerobic microorganisms that develop such as *Clostridium botulinum* is increased.
To provide a suitable heat treatment, it is important to consider pressure conditions, since pressure and temperature are directly proportional; therefore, if you can control pressure, temperature can be controlled as well. The pressure compensation occurs by the interaction isobaric balance between the inner chamber of the container and the flexible packaging system, namely pressures are kept constant. Upon completion of the sterilization cycle, normal change in pressure is shown and is discharged through the pressure compensated pneumatic system, reflecting the decrease in temperature at a constant pressure. Therefore, collapse is prevented by retort pouches generation compensated pressure effect. The aim of this study was to modify a pressure cooker, to suit a sterilization system in retort pouches.

Materials and methods

Changed the pneumatic system of an autoclave for sterilizing food adapt modifications consisted of two connections of stainless steel type T where the pressure gauge is placed, compressed air connection for the inlet and thermocouple, addition and safety valve gauge existing in the system. In one thermocouple was placed connections and the manometer, sealing with high temperature silicone thermocouple input. The thermocouple was connected to a temperature gauge Datalogic DL QD Series. The other connection "T" adapted to the compressed air inlet, which consisted of a check valve and a pressure regulating valve. With the change in the pressure cooker ran several tests in order to determine the heat penetration in the package.

Results and discussion

Modified pressure cooker to ensure sterilization of different foods within a retort pouches, is shown in Figure 1-A.
The pneumatic system provides favorable conditions of operation, the sterilization chamber is easily adapted to the adjustments done; pressure regulation was as expected, decreasing the temperature inside the packingand holding the pressure there in. Figure 1-B shows the actual prototype tire adapted pneumatic system.
The data obtained from the tests performed demonstrate that the sterilization process is adequate, as shown and explained in Figure 2 below.
Figure 2 shows the behavior of the temperature within the package, where there is a rapid temperature rise in the first minutes. This is because the characteristics of the packaging, which has a larger heat transfer area to be flat, and a lesser distance from the center of the outer package [5].

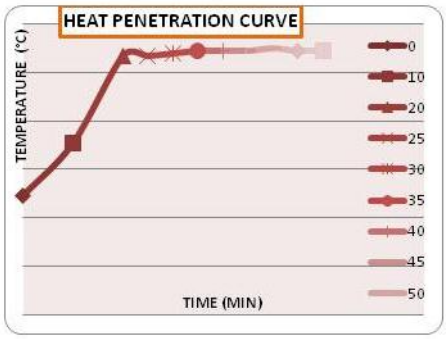

Figure 2. Heat Penetration curve.

a) Temperature gauge: Is used to measure the temperature transmitted by the thermocouple, which indicates when the sterilization temperature is reached.
b) Manometer: It is used to measure the pressure inside the chamber.
c) Pressure regulator: Device on which is varied section of a hole to control spending regulated agent [6]. In this case, it helped to keep the vapor pressure of water and compressed air during the experiment.

d) Checkvalve: the operation is given by the flow of retention in the opposite direction that opens only when connecting an external valve and this acts as overflow valve [7]. The check system retained the steam out let while allowing the compressed air inlet.
e) Pressure control valve with filter and manometer: The control valve allows the flow of compressed air, which is monitored by the pressure gauge. The filter prevents the entry of foreign material into the system.
f) Thermocouple: was adapted a thermocouple type "J", which is used as a temperature sensor within the retort pouches.

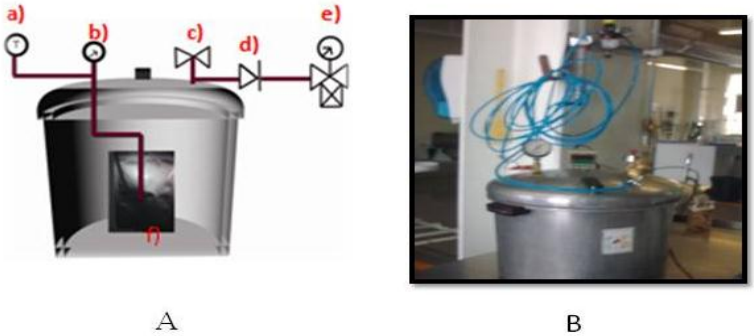

Figure 1: A. Pneumatic system; B. Scale prototype pneumatic system.

Conclusion

The modifications done in the pressure cooker provided a good system for retort pouches sterilization system. Computer adjustments to the technical specifications of the food product, and the dimensions of pan and food paced material, help on fulfilling the specifications required by the consumers.

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Changes In Properties And Physical Quality Of The Drying Corn

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Abstract

The effect of temperature on the grain characteristics was evaluated. Batches of 1 kilogram of each corn at 25-29% of moisture content were heat at 40, 60, 80 and 100ºC in a bean. After the temperature process was completed the corn batches had moisture content averaging between 11 and 12%. The grains maize after and before of thermal process was analyzed for chemical and physical properties. Parameters evaluated were; size, length weight of one thousand kernels, weight per hectoliter, percent floaters, germination, and hardness. The temperature was significant ($p \leq 0.05$) on their physical characteristics such as weight per hectoliter, specific gravity and hardness principally. Chemical composition was unchanged by drying temperature. Increasing drying temperature decreased test weight, germination, and it increased kernel breakage susceptibility and percentage of floating kernels. Because breakage susceptibility increased upon high-temperature drying, some chemical or physical change in the kernel cell-wall matrix or in the starch granules may have affected breakage susceptibility. Thus corn density breakage susceptibility and germination may change upon drying. These relationships may provide methods for identifying grain that is damaged or of lower quality due to high temperature drying

Keywords: Corn, (Zea mays) thermal process, physical properties

Introduction

Low temperature drying usually has little effect on corn quality factors such as stress-cracking, density, breakage susceptibility or germination. The quality of corn dried with heated air can, however, vary greatly. Excessive drying-air temperatures and high initial grain moisture percentages increase the degree of damage to corn kernels in the normal one-pass high-temperature grain dryer (Watson 1960). Corn with a high percentage of viable kernels is used for industrial. Drying grain at elevated temperatures may be lowers flaking grit yield in dry milling makes starch-protein separation more difficult in wet milling and increases kernel breakage during handling in local and export markets (Hoseney 1986). Kernel stress cracks caused by abusive drying techniques increase the amount of breakage that occurs when grain is handled, causing a loss of material. Several quality criteria can be applied to commercial shelled corn to estimate the degree of grain damage caused by artificial drying. The test weight of grain is routinely used for grade designation and quality assessment. Stress-crack analysis is indicative of the amount of breakage to be expected in handling artificially dried corn and may also reflect internal or chemical grain damage. The over dried or heat damage grain lower quality. This effect show why it may provide better methods for detecting grain-quality damage

Materials and methods

Grain of commercial corn hybrids, from 20 % (wet basis) moisture content, was used for the study. A bin dryer was used for batch drying treatments. Temperatures of 40, 60, 80 and 100°C were used for each of the batch. Drying time ranged from 1 to 24hr for the different grain lots. Samples of each were taken from the grain of each treatments was determined for quality analysis once drying was complete, and the

moisture content was calculated from the weight loss in drying in an air oven. Final moisture content for all treatments was 12 % (wet basis)

Chemical Composition.-Nitrogen (Kjeldahl.), ash, starch content of whole Corn were determined using approved methods of the American Association of Cereal Chemists (AACC 1983). Moisture was determined in oven.

Physical Properties.-Test weights were measured. Kernel weights were determined for 100 whole, kernels. The percentage of floating kernels was determined on l00-g samples using a sodium nitrate solution (of 1.107 the specific gravity). Viability of the samples was determined of sprouted kernels alter incubating 20 –kernel subsamples at 27 °C for five days. The degree of stress-crack formation was assayed individual kernels were examined visually and categorized as 1) kernels with no stress caracks, 2) kernels displaying a single crack, and 3) kernels with multiple cracks. The number of cracked kernels in each category was reported as percentage of 100-kernel subsamples. Chemical analyses and physical tests were replicated two for each sample. General linear models statistical analysis was used to determine least significant difference for all test values (Peplinski et al 1994).

Results and discussion

Corn drying time varied inversely with the drying temperature (Brown et al 1979). It took from 1 hr at 100°C to 24 hr at 40°C to reduce each sublot to 12% moisture. Each sublot had reached 12% moisture, as measured by weight loss. Air-drying temperatures of 40—100°C had little or no statistically significant effect on kernel nitrogen, fat, starch, or ash. Chemical composition ranges were: 1.3—1.4% nitrogen, 3.8—4.0% fat, 74—78% starch, and 1.3—1.4% ash. Analysis of variance showed significant differences among the grain drying treatments and grain lost in viability of the dried grain table1 viability of grain was markedly reduced by any of the treatments involving drying temperatures above 60°C The rest of the drying treatment including low temperature drying did not differ significantly in their effect on viability. The differences among lots may have arisen from differences in the stage of maturity at harvest or from mechanical damage to kernels particularly at the higher moisture levels. As air-drying temperatures increased from 40 to 100°C, kernel test weight decreased, and percentage of floating kernels increased, and 100-kernel weight and stress-cracked kernels were unchanged. Drying treatment and grain lot were both significant in their effect on the test weight of dried grain. All of the corn dried had a test weight greater than the minimum value of 68Kg/hl required in the statutory definition of grade number 1. Previous studies examined the effect of heated-air drying on genotype response and protein changes related to reduce quality. Other investigators examined quality variation in corn harvested at various moisture levels High drying temperatures can decrease protein solubility protein moisture- binding capacity and enzymatic activity. Drying grain at elevated temperatures also lowers flaking grit yield in dry milling makes starch-protein separation more difficult in wet milling and increases kernel breakage during handling in local and export markets (Hoseney 1986). Test weight though low for a corn reached a maximum of 51 kg/hl at 40°C; this decreased to 48 kg/hl for corn dried at 100°C . Kernel weight (17-17.2g) per 100 kernels, stress- cracking (84-86%) breakage susceptibility (63-67%) and percentage of floating kernels (79-80%) were largest with 85-100°C drying temperatures. Corn dried at 60°C was only 29% viable; grain dried above 60°C did not germinate. The drying increased cracking to a maximum of only 86% at 85°C (Thompson, et al, 1963). This small increased in stress-cracking upon drying prevent us from showing any statistically significant relationship between stress-cracking and breakage susceptibility as shown previously (Peplinski et al, 1992). Some unknown chemical or physical change may occur in the endosperm call matrix or in the starch granules upon heated-air drying.

Conclusions

Drying temperatures increased from 40 to 100°C, kernel test weight and germination decreased, kernel breakage susceptibility and percentage of floating kernels increased, and 100-kernel weight and stress-cracked kernels were unchanged. The density, germination, decreases at elevated drying temperatures.

The flotation test differentiates density changes caused by drying better than do test weight measurements. Most changes in kernel physical properties became evident only in corn dried above 60°C. At 80°C, kernel breakage, percentage of floating kernels, and germination showed appreciable changes compared to those of corn dried at 40°C. Proximate analyses showed no major change in chemical composition of corn dried from 40 to 100°C. Kernel physical characteristics suggest that corn is damaged upon drying and decreases in quality only when dried at temperatures of 80°C of higher. Drying at temperatures of 40-60 °C produces little if any change in kernel chemical and physical properties.

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Microencapsulation Of Essential Oils Of Clove (*Eugenia caryophyllata*) And Mexican Oregano (*Lippiaberlandieri*) In β-cyclodextrins

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Introduction

The β-cyclodextrin (β-CD) molecule is made up of 7 D-glucose monomers linked by α(1,4) bonds, exhibiting the shape of a truncated hollow cone. The cavities of β-CD are hydrophobic, whereas the external faces are hydrophilic. Encapsulation in β-CD is considered a molecular complex formation, where the hydrophobic active constituents of essential oil (EO) interact within the hydrophobic cavity of β-CD, highlighting that when the capsule is formed the molecular exterior possesses a hydrophilic character. Considering the exterior properties of the capsule, its conformation could be affected by high relative humidity in the atmosphere, and the essential oil volatiles could be released to the environment. Microencapsulation in β-CD is one of the most effective methods for protecting active compounds against oxidation, heat degradation, and evaporation (1). Essential oils (EOs) of clove (*Eugenia caryophyllata)* and Mexican oregano *(Lippiaberlandieri),* are good antimicrobials agents, however, their high volatility and reactivity, limits their application as food preservatives (2). Hence, the main objective of this work was microencapsulation of these essential oils with β-CD.

Materials and methods

Clove (*Eugenia caryophyllata*) purchased in Commercial Cardona, Chihuahua, Mexico, and clove essential oil was obtained by hydrodistillation. Essentialoil of Mexicanoregano (*Lippiaberlandieri*) acquired in CIRENA (Centro de Investigaciones para los Recursos Naturales, Salaices, Mexico). Eugenol, eugenyl acetate, carvone, carvacrol, thymol, p-cymene and β-caryophyllene, GC standards were bought from Fluka Chemical Corp. β-CD (Cavamax W7 food grade) was kindly supplied by WackerBiochem, (Mexico). All other reagents used were analytical grade.

Microencapsulation of both essential oils with β-CD was studied at 4:96, 8:92, 12:88, and 16:84 (oil:β-CD) percent weight ratios.Microcapsules of clove EO:β-CD and Mexican oregano EO:β-CD were prepared separately according to the precipitation method (3). Microcapsule characterization included: optimization of the encapsulation process, gas chromatography coupled to mass spectrometry detector (GC-MS) , gas chromatography coupled to flame ionization detector (GC-FID) before and after encapsulation, morphological analysis by scanning electron microscopy (SEM), proton nuclear magnetic resonance (H^1RMN) and infrared (IR) spectroscopy.

Data collected were analysed by one-way ANOVA with a significance level of 0.95 and Tukey-Kramer multiple mean comparisons. Data were analyzed using the statistical package MINITAB Release16 (Minitab Inc., USA).

Results and discussions

Microencapsulation

The recovery of the oregano and clove EO microcapsules at the equilibrium state is presented in Table 1. With respect to microencapsulation yield (%) there was a significant increase ($P<0.05$) in the recovery of the 12:88 and 8:92 ratios compared to the 4:96 and 16:84 for both oil microcapsules. However in the case of microencapsulation efficiency, this represents percentage of essential oil encapsulated about essential oil initial. These data indicate that optimum ratios of EOs to β-CD during microencapsulation existed at around 8:92 for Mexican oregano EO and 4:96 for clove EO. At this concentration of essential oils in the starting solution, there is a major recovery of relative mayor volatile to oil components to form the microcapsules. The optimization of the process can be established with respect to the microencapsulation efficiency and relative mayor volatile which, for clove EOs microcapsules the optimal recovery ratio were 4:96 and for Mexican oregano EO was 8:92.

Identification of the volatile constituents of Mexican oregano and clove EOs before and after the microencapsulation process was accomplished by GC-FID analysis. Eugenol was the major constituent detected in the clove EO with eugenyl acetate and β-caryophyllene in fewer amounts. The main compound detected in Mexican oregano EO was carvacrol. Other minor constituents detected were p-cymene, β-caryophyllene and thymol. In summary, for the microencapsulated EOs, the more efficient ratios were8:92 with 71% of carvacrol and 4:96 with 97% of eugenol, respectively. These ratios were used to perform the ^{1}H NMR, IRspectroscopy and SEM.

Table 1. Recoveries of clove and Mexican oregano EOs microcapsules at various ratios of essential oil versus β-CD

Microcapsule ratio AE: β -CD	Microencapsulationyield (%)* (w/w)	Microencapsulationefficiency (%)**	Relative mayor volatile (%)of allvolatiles
ORÉGANO EO:β-CD			**Carvacrol**
4:96	89.22^{b}	54.95^{b}	66.13^{c}
8:92	93.69^{a}	71.40^{a}	71.22^{ab}
12:88	93.10^{a}	57.65^{b}	73.63^{a}
16:84	88.71^{b}	39.469^{c}	68.78^{bc}
CLOVE EO:β-CD			**Eugenol**
4:96	88.10^{c}	61.11^{a}	97.61^{a}
8:92	94.98^{a}	46.52^{b}	97.94^{a}
12:88	92.17^{b}	33.46^{c}	97.27^{a}
16:84	88.67^{c}	25.32^{d}	96.32^{b}

* (Final EO:β-CD ratio weight / Initial EO:β-CD ratio weight) * 100 (g, Dry weight)

**(mg encapsulatedEO/ mg initial EO)* 100

^{a,b,c}Superscriptswith the sameletters in the samecolumndidn’tpresentsignificantdifference $P>0.5$ (Tukey-Kramer multiple meancomparisons).

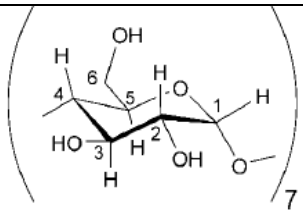
^{1}H NMR spectroscopy

In view of their various biotechnological applications, β-CD inclusion complexes have been extensively studied by several investigation methods. In particular, the NMR technique has been used successfully to gain information on the structural properties of β-CD complexes (4). In general, chemical-shift changes in the ^{1}H-NMR spectra are used to monitor the complex formation process, the most significant variations being usually observed for the H-atoms placed into the cyclodextrin cavity (5).

The ^{1}H chemical shifts of β-CD in the absence and presence of EO are shown in Table 2. As expected, only the H-3 and H-5 protons, located inside the cavity, and the H-6 proton, located on the cavity rim at the narrow end of the molecule, are appreciably shifted upfield. In fact, it is known that the inclusion of a

guest molecule in a β-CD ring shifts the ^{1}H NMR signals of the included guest downfield and those of the affected CD protons upfield, as a consequence of the hydrophobic interaction between the guest molecule and the interior of the host cavity (1). The upfield shifts of the protons located around the interior of the hydrophobic cavity (H-3, H-5 and H-6); also minimal variation of shifts from protons located at the exterior of the cavity (H-1, H-2 and H-4) provided clear evidence of an interaction between the guest molecule and the interior of the host cavity. In fact, it is known that the inclusion of a guest molecule in a b-CD ring shifts the 1H NMR signals of the included guest downfield and those of the affected CD protons upfield, as a consequence of the hydrophobic interaction between the guest molecule and the interior of the host cavity. Our results confirm the formation of complexes between β-CD and at least one EO component.

Table 2.^{1}H NMR Chemical (δ) of the free and complexed forms in D_2O solutions

	H-atom	δ /ppm	Clove: β-CD		Mexicanoregano: β-CD	
		β-CD free	δ /ppm	Δδ(complexed-free)/ppm	δ /ppm	Δδ(complexed-free)/ppm
	H-1	5.04	5.04	0	5.03	-0.01
	H-2	3.55	3.55	0	3.55	0.00
	H-3	3.93	3.9	**-0.03**	3.90	**-0.03**
	H-4	3.62	3.61	-0.01	3.61	-0.01
	H-5	3.82	3.78	**-0.04**	3.74	**-0.08**
	H-6	3.85	3.83	**-0.02**	3.80	**-0.05**

IR spectroscopy

The inclusion interactions involved in the formation and stability of the inclusion complex have been attributed to Van der Waals forces, hydrophobic interaction, hydrogen bonding and release of high-energy water molecules from the cavities of CDs. The complex formation may be confirmed by IR spectra because bands resulting from the included part of the guest molecular are generally shifted or their intensities are altered (6).

The IR spectra of β-CD (a), clove EO (b), their physical mixture (c), and clove microcapsules (d) are presented in figure 1. The 4:96 weight ratio was selected for this assay because of its high eugenol content. In the IR spectrum of β-CD (Fig. 1a.) the wide band is registered with the absorption maximum at 3411 cm-1, which is caused by the valence vibrations of the O–H bonds. Also, the absorption band with maximum at 2919 cm-1 belongs to the valence vibrations of the C–H bonds in the CH and CH_2 groups. In the region 1200–1030 cm-1 the absorption bands of the valence vibrations of the C–O bonds in the ether and hydroxyl groups of β-CD (1158, 1080 and 1028 cm-1) are registered. The absorption bands in the region 950–700 cm-1 belong to the deformation vibrations of the C–H bonds and the pulsation vibrations in glucopyranose cycle (7). Free clove EO showed the major IR absorptions at 3429 cm-1 in the OH vibrations region, which correspond to the hydroxyl group of eugenol, 3004–3077 cm-1 (C–H aromatic), 2853-2925 (CH, CH_2, CH_3 aliphatic), 1613-1638cm-1 (aromaticring C=C), 1711cm-1 (carbonyl group C=O) and 1234-1267cm-1 belongs to benzene-oxygenbond. Figure 1c. shows that the spectrum is essentially the combination of clove essential oil and β -CD spectra, which indicates that the physical mixture cannot lead to inclusion. In Fig.1d a new band appeared at 1516 cm-1 in the spectrum of the inclusion complex, which was absent from the β-CD spectrum and its assigned to the C=C stretching band of aromatic group for the eugenol included inside the β -CD cavity. This means that there were some interactions between the two components in the inclusion complex. So we can deduce that eugenol (mayor component of clove EO) was included into the cavity of β-CD(6).In the IR spectra of the microcapsules, clove EO bands are almost completely obscured by very intense and broad b-CD bands, which are hardly influenced by molecular complex formation (8).

The IR spectra of the β-CD (a), Mexican oregano EO (b), their physical mixture (c) and Mexican oregano EO microcapsules (d) are compared in Fig. 2. The 8:92 weight ratio was selected for this assay because of its carvacrol content. Free Mexican oregano EO showed the major IR absorptions at 3400 cm^{-1} (OH stretch) which correspond to the hydroxyl group of carvacrol and timol; 3020 cm-1 (C–H aromatic), 2850-2950cm^{-1}(CH, CH_2, CH_3 aliphatic). IR spectral analysis shows differences in some band positions of the microcapsules with respect to the free compounds and their physical mixture. First, the microcapsule's band centroid in the spectrum (3391 cm^{-1}) is shifted in the range of valence OH by about 20 cm-1 towards lower frequencies vibrations compared to the position of the similar band in the spectrum of β -CD (3411cm^{-1}). This indicates that an interaction has occurred between the oils, separately and β -CD, via hydrogen bonding and that the complexes are not just physical mixtures (8).

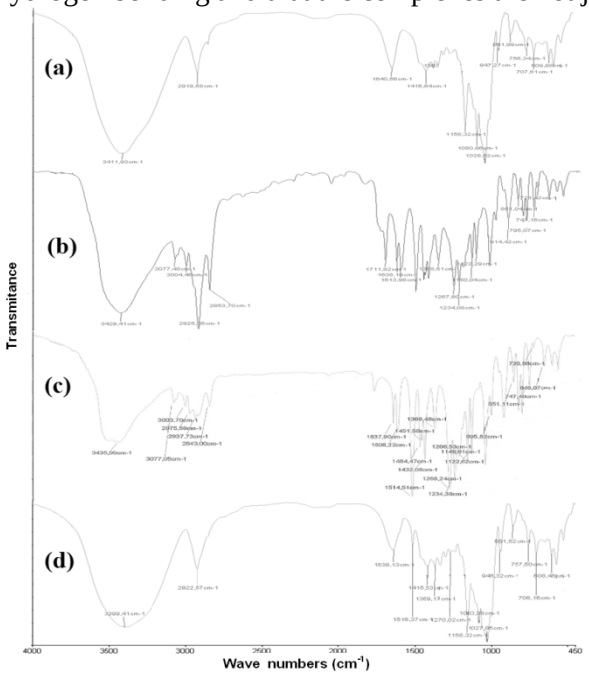


Figure 1. IR spectra of a) β-CD, b) clove EO, c) clove EO and β-CD physical mixture, d) clove EO: β-CD (4:96% w/w) microcapsules

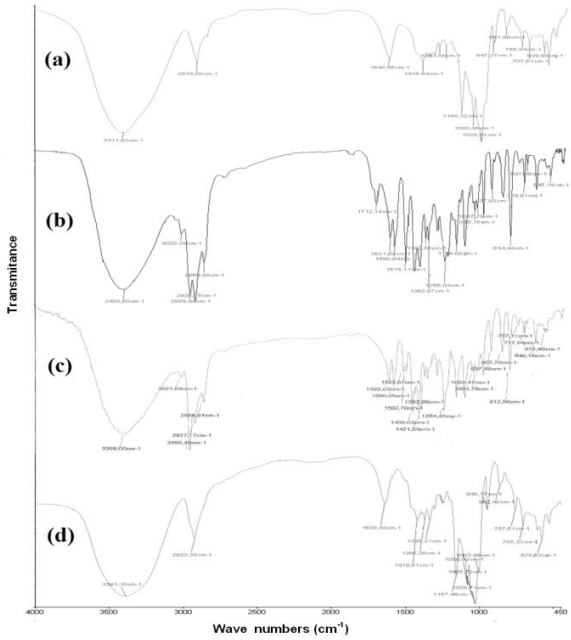


Figure 2. IR spectra of a) β-CD, b) Mexican oregano EO, c) Mexican oregano EO and β-CD physical mixture, d) Mexican oregano EO: β-CD (8:92% w/w) microcapsules.

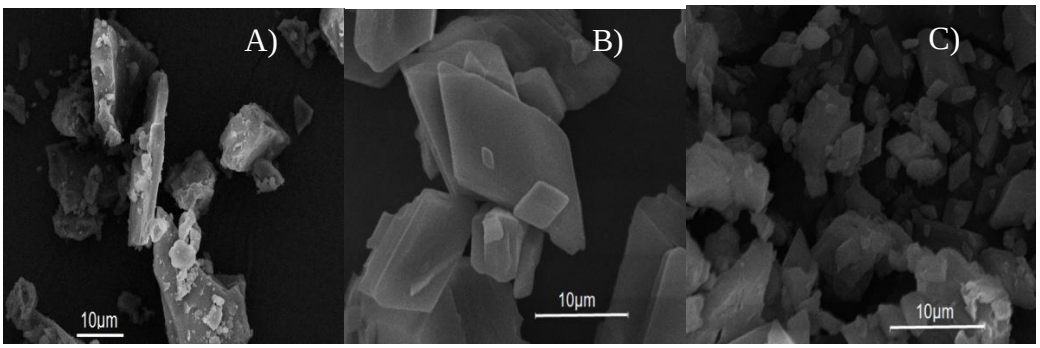


Figure3. Scanning electron micrographs of A) β-CD, magnification x1500; B) microcapsules of clove EO: β-CD, magnification x5000; and C) microcapsules of Mexican oregano EO: β-CD, magnification x5000

Morphology

The surface morphologies of the products were further investigated by means of SEM. Fig. 3a) shows the SEM images of pure β-CD. The β-CD was composed of different sizes of rectangular shaped crystals. In addition, there were small particles that adhered to the surfaces of the crystals, which is in agreement with the observations made by other authors (9).

As seen from Fig. 3 b) and c), there were drastic changes in particle shapes and original morphologies of the inclusion complex products. The complexation between EOs and β-CD at 12:88 ratio appeared as rhomboid shaped crystals. Also in theoregano EO capsulesare presentsmall aggregates ofamorphouspiecesof irregular size.Themicroencapsulated particlesshowed novisible fractures, cracks or pores, aspects indicative ofgood protection of thecore material.The results fortheparticlesize distributionindicatedifferent behavior forthe core materialsstudied.With lightvariations,the average sizesof the particles withclove essentialoilranged from7,128to16µmandsmallermean particle sizesfor the essential oilof oregano: 2.88to7.2µm.

Conclusion

Clove and Mexican oregano essential oils microcapsules can be successfully produced using β-CD. The encapsulation of antimicrobial volatile compounds from such oils in β-CD could be an alternative to solve the problem of volatility, providing a molecular complex. Additionally, Mexican oregano and clove oils are from natural origin, being the main reason for their suitability.

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Scale-Up And Sustainability Evaluation In The Solvent Extraction Of Peanut Skin Antioxidants

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Abstract
The study covers the analysis of solvent extraction of antioxidants in peanut skin from an industrial scale up perspective. Solvent selection was first performed in order to obtain five solvent choices for extraction. Design of experiments was proposed for the group of solvents selected. For each solvent two factors (temperature and time) were studied at three levels (30, 45, 60 °C and 15, 30, 45 min). Total Phenolic Content (TPC) and antioxidant capacity ORAC were the response variables. The ResponseSurface Methodology (RSM) was used to analyze the influence of the factors in the response and find the optimal extraction conditions for each solvent. Ethyl glycol (EG) showed the highest contents of TPC (>400mgGAE/g skin) and antioxidant capacity (>2800 µM Trolox/ g skin) whereas solvents such as isopropyl alcohol (IPA) showed the lowest (<130mgGAE/g skin and <2000 µM Trolox/ g skin) for the optimal conditions. Process simulation was performed in order to evaluate the performance of the process in an industrial scale using the optimal laboratory conditions.
Keywords: extraction, polyphenols, solvent selection, sustainability, scale-up.

Introduction
Recently the use of agroindustrial wastes as raw material is gaining the attention of the industrial and academic community. Among these wastes we found peanut skin where considerable quantities of added value compounds have been reported (1-3). Peanut skin has a high content of polyphenol compounds, these compounds can be exploited especially in the pharmaceutical and food industries (4). Polyphenol extraction from peanut skin as a raw material has showed positive results at laboratory scale. Nepote et al. (5, 6) investigated the effects of particle size, concentration of ethanol in water, and a number of stages in the extraction. In this study optimization was performed usingTotal Polyphenol Content (TPC) as a response variable. Ballard et al. (4) evaluated different concentrations of ethanol and methanol in water as well as temperature and time of extraction, evaluating TPC and ORAC. The results in the optimal condition were for TPC 118 mgGAE/g Dry solids and for the ORAC assay 2149µmolTrolox/g Dry solids (4). The development and commercialization of a new product is a slow and takes large investments and cost (8). Process simulation is an alternative to evaluate a process and reduce the efforts during the laboratory and the pilot plant (8). As an example, Kammerer et al. (9) analyzed the effect of temperature, flow rates and solute concentration over recovery of polyphenol compounds.This research evaluates the efficiency of solvent selection extraction from a laboratory and industrial perspective; this with the intention of producing information that can lead to a successful sustainable development of the operation in the near future.

Material and methods
Materials: Peanut skin samples were supply by a peanut local company. The samples were stored in a freezer at 3°C in a plastic coat until process. Solvents were purchased from local distributor. FolinCiocalteu reagent, Gallic Acid, Sodium carbonate, 6-hydroxy- 2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox standard), 2-2′-azobis (2-amino-propane) dihydrochloride (AAPH) and Fluorescein (FL) all are Sigma-Aldrich Co.

Solvent Selection: An analytical hierarchy process (AHP) mathematical model was used for solvent selection: this in order to optimize solvent solubility parameters, and obtain well known industrial (easy to scale) solvents(10).

Deign of experiment (DOE): 3^2 full factorial design was proposed; the factorial design was performed in Minitab Version 16 program. All runs were made for duplicate and a DOE was performed for each solvent.

Extraction procedure: Extractions were run with 10ml of pure solvent and 0.5 g of peanut skin in an Erlenmeyer flask. The flask was introduced into a shake incubator at a speed of 150 rpm. Samples were heated at a temperature of 30, 45 and 60°C for 15, 30 and 45 minutes.

Phenolic Content Determination: Total phenolic content was determinate spectrophotometrically using FolinCiocateu´s technique. Gallic acid standards were prepared at 100, 200, 300 and 400 ppm. 10g of sodium carbonate (Na_2CO_3) was dilute in 50ml of distillate water. TPC was expressed as mg of gallic acid equivalents (GAE)/g of dry peanut skins.

Oxygen Radical Absorbance Capacity (ORAC) Assay:The technique was run according to the literature (7). 60 fluorescence readings were taken every minute using an excitation wavelength of 485 nm and an emission wavelength of 535 nm samples and standards were run in triplicate.

Statistical Analysis: RSM was first used for Box and Wilson (11) optimization method.RSM was performed using Minitab Version 16 and Statistica 10. Statistical significance of the model was determinate at 5% probability level (α=0.05). The software used a quadratic model equation to build response surface.

Scale-up Simulation: A unique extraction process simulation including three important steps (Extraction, separation and solvent recovery) was evaluated for two of the studied solvents, this in order to analyze potential scaling issues. Aspen Batch Plus software was used for the simulation of the process.

Results and discussion

Optimum extraction condition based on TPC:Table 1 shows the TPC measurements and the significance values (p-values) for the variables in the ANOVA.

Table 1.Process conditions and total phenolic content (TPC) of for each solvent.

Solvent	Process conditions and p-values				Response
	Temperatura	p-value	Tiempo	p-value	FOLIN
Methanol	60	**0.006**	35	0.376	170.24
Ethanol	35.45	**0.016**	30	**0.002**	175.205
EthyleneGlycol	60	0.965	45	0.543	363.305
Isoprolil Alcohol	30	0.108	15	**0.072**	64.285
Water	59.91	**0.035**	44.09	0.271	120.13

Model fitting: The analysis of variance (ANOVA) of the quadratic regression models for methanol and ethanol in ORAC determination showed that the model was significant ($p<.05$) and the other 3 solvents were not ($p>.05$) significant (Table 2).

Table 2. Significant values and regression coefficients for the quadratic model in ORAC determination

Solvent	Model Values				
	MULTIPLE R	MULTIPLE R^2	Adjusted R^2	F	p-Value
Methanol	0.7983	0.6372	0.5256	5.7082	**0.0071**
Ethanol	0.9128	0.8332	0.6850	5.6208	**0.0091**
EtylenGlycol	0.6979	0.4870	0.3292	3.0853	0.0544
IPA	0.4107	0.1686	-0.0872	0.6593	0.6310
Water	0.6580	0.4330	0.2585	2.4818	0.0955

Optimum extraction condition based on ORAC activity: Ethanol, methanol and water the results were similar to reported values (4). Optimum conditions and statistical significance (p-Value)for each solvent are presented in Table 3.

Table 3.Solvent characteristics, optimum process conditions, and ORAC response.

Solvent	Extraction conditions and statistical significance				Response
	Temperature (°C)	p-Value	Time (min)	p-Value	ORAC activity (µMTrolox/g)
Methanol	60	0.511	35	0.514	2545.73
Ethanol	35.45	0.462	30	0.346	1999.94
E.G.	60	0.398	45	0.67	2818.35
IPA	30	0.153	15	0.418	1604.04
Water	59.91	**0**	44.09	0.916	1999.38

Scale-Up Analysis: As Table 3 shows extraction whit E.G. has the highest value of antioxidant capacity and Isopropyl alcohol (IPA) has the lowest, as a result, the two solvents were selected for the scale-up analysis. Looking at the solvents properties we can anticipate potential solvent recovery issues. E.G boiling point is higher than IPA, hence, energy demand will be high if separation such as distillation are considered. Another property what has an important impact over industry process is viscosity. E.G. has a higher viscosity than IPA, this causes that the E.G. has to be separated by vacuum filtration whereas IPA can be filtered with simple gravity.Optimum conditions for E.G. were 60°C, 45minutes and 30°C, 15minutes for IPA. This information also gives us hint about the process conditions and processing times.Simulation was performed using the same equipments and steps for the two solvents in order to generate a general view of a discrepancy between laboratory results and the application in a process plant.Process time and production rate were analyzed in order to determinate the feasibility of the process (Table 4).

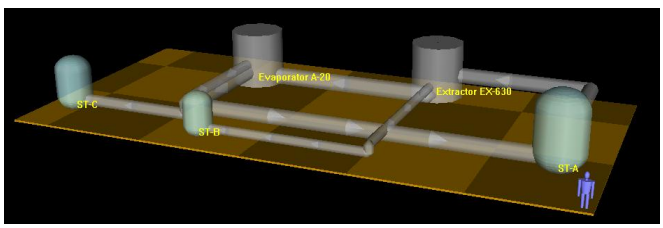

Figure 1. Process simulation, plan size and equipment distribution.

Table 4.Simulation summary.

Solvent	Total Material (kg_M)	Polyphenol extracted (kg_P)	Process Time (min)	Production Rate (kg_P/min)	Process Mass Intensity (kg_M/kg_P)	Global Warming Potencial ($kgCO_2$)
Isopropyl Alcohol	21000	41.69	218.51	0.2	503.72	2574.83
EthyleneGlycol	21000	231.59	277.1	0.87	90.68	323.93

The production rates in the Table 4 shows a big difference in the amount of polyphenols production. However it is necessary an energy balance to determinate the real “cost” of production. The Global warming potential (GWP) and the Process Mass Intensity (PMI) were calculated to measure the sustainability of the process. The GWP is obtained using the equation proposed for Jimenez-Gonzalez (12). Table 5 shows the energy consumption per gram of final product.

Table 5. Energy consumption in process simulation for each solvent

Solvent	Operation	Operation Time (min)	HeatDuty (kJ)	Total Heat Duty (kJ/min)	Total production Heat Duty (kJ/kg Polyphenol)
EthyleneGlycol	Heat	32.10	2.09E+06	2.14E+05	2.46E+05
	Concentrate	120.00	1.79E+07		
Isopropyl Alcohol	Heat	3.51	2.29E+05	1.29E+05	1.42E+05
	Concentrate	120.00	7.67E+06		

Table 5 shows the energy consumption for the production of the same amount of product is lower in the case of IPA even when in the laboratory study the ethylene glycol was for much the best option. These results indicate that it is necessary more research but whit the scale-up perspective and including a real application.

As a result from this research solvent selection is a critical aspect in the future development of sustainable process for the extraction of added value compounds from peanut skin. The experimental section shows how the effect of the variables can be different in the solvents. EG could considered as solvent for getting high yields, however, is necessary to consider scaling issues, such as separation (energy input), handling cost due to high density and viscosity. On the other hand, peanut skin can be considered as a potential source to obtain polyphenols with a high antioxidant capacity.

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Oily Phase Characterization Of A W/O Emulsion Stabilized With Carnauba Wax Crystals

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Introduction

Food colloids commonly contain dispersed particles with sizes ranging from just a few nanometers to tens of micrometers. In many of these systems the particles participate in the stabilization of the multi-phase structures by providing a physical barrier to emulsion droplet coalescence. This type of stabilization is commonly called "Pickering stabilization" (1). Pickering emulsions are attractive as new strategy for encapsulation, stabilization and transport, as the classical surfactant that stabilizes the oil droplets is replaced by solid particles, resulting in surfactant-free emulsions. Pickering emulsions show interesting properties that diverge from classical emulsions. The very high stabilization against coalescence allows keeping the droplet under severe conditions such as high concentrations of dispersed phase, presence of electrolytes and they can be safely dried and re-dispersed (2). A second point is the formation of a dense shell of solid particles around the droplets that act as a barrier against materials transfer (3). Binks (4) has discussed the preparation of such emulsions and how the particles affect the stability. Crystal morphology and especially crystal size are crucial for the effective stabilization of the emulsion. Understanding the conditions necessary for the formation of physically stable emulsions is an important area of research.

A wax may contain a wide variety of long chain alkanes, esters, polyesters and hydroxyl esters of long chain, alcohols and fatty acids. Waxes are insoluble in water and very hydrophobic, hence one of their uses is to waterproof fabrics. Particulate waxes are thought to be partly responsible for the extremely high stability to coalescence of water-in-crude oil emulsions, since such particles can adsorb to the oil-water interface providing a steric barrier to drop fusion (5). Both the flocculation and coalescence of the aqueous phase drops has been found to depend on whether wax crystals are present in the continuous oil phase prior to emulsification at room temperature or whether they are formed *in situ* by emulsification at high temperature followed by quench cooling.

The aim of this work were to investigate if wax crystals can act as sole emulsifiers of water in oil emulsions and to determine the effect of cooling rates on the properties of the oily phase and on emulsion stabilization. By this, it is hoped that insight is shed on the mechanisms that allow to obtain stable emulsions containing bioactive compounds such as carotenoids, flavonoids and phenolic compounds, among others, with enhanced functional properties.

Materials and Methods

Deionized water with a resistivity of 18.2 MΩ cm was used for the aqueous phase. Carnauba wax (CW) obtained from palm leaves *Copernicia cerifera* from Brazil, was purchased from Ceras Universales S.A de C.V. (Mexico City, México). Corn oil (CO) of the brand "La Gloria" was purchased from a local supermarket.

Preparation of crystal wax dispersions.

Dispersions carnauba wax crystals in corn oil (CWCD`s) were prepared as follows: CO was heated to 80 ºC, and added with grinded CW to different concentrations (0.5%, 1.0% y 2.0% w/w). Once homogeneous CW-CO dispersions were obtained, the mixtures were cooled down to 30 °C, employing different cooling rates (1, 6 and 12 ° C / min) to produce dispersion of CO containing carnauba wax crystals (CWC). All different CWCD`s were used as continuous phase in the formulations of emulsions.

Optical microscopy.

Brightfield and polarized light microscopy were used to examine the morphology of the CWCD`s dispersions. Samples were placed on viewing slides, upon which coverslips were gently placed. An Olympus BX45 microscope (Olympus Optical Co., Ltd., Tokyo, Japan) was used and images captured with Olympus E-620 digital camera were analyzed using Motic Images Advanced software version 3.2 (Motic Group Co., Ltd., Xiamen, China).

Emulsions preparation.

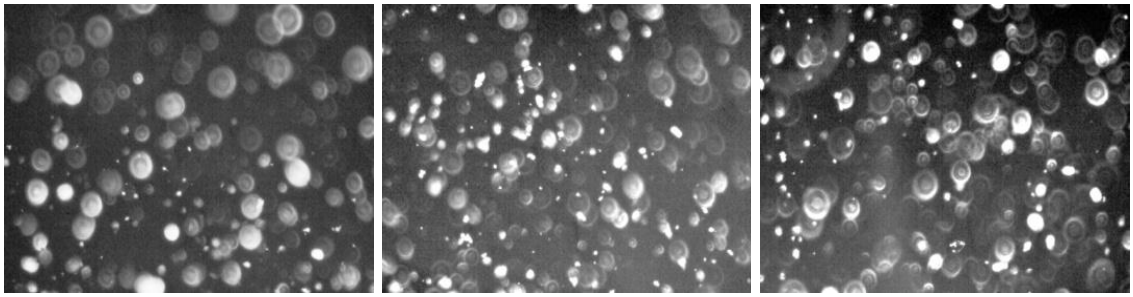
Water-in-oil emulsions with a disperse volume phase (ϕ) of 0.3 were prepared using the dispersions CWCD`s with cooling rates of 12 ° C / min and concentrations de 0.5, 1.0 and 2.0% (w/w) and deionized water. Emulsions were homogenized using an Ultra-Turrax T50 (IKA®-Works Inc., Wilmington, NC, USA) homogenizer, operating at 14000 rpm for 5 min keeping the temperature at 25 ºC with an ice bath. Each emulsion was prepared at least twice with good reproducibility, combined and coded $ECWC_x$ (Emulsion Carnauba Wax Crystals, x is concentration).

Calorimetric profiles.

Differential scanning calorimetry (DSC) technique was applied with a DSC Q1000 (TA Instruments, Newcastle, DE, USA) in order to characterize the stability of the emulsions. Dry nitrogen was used as the purge gas. Samples (~ 5 mg) were placed in sealed aluminum pans which were run alongside an empty pan. The samples were submitted to a regular cooling from 25 ºC to -80ºC and then heating back to 25 ºC using a heating rate of 5◦C/min.

Results and discussion

Binks and co-workers reported that stability of particle stabilized emulsions was dependent upon particle size (7), while Thompson et al. (8) found that the stability depended on the temperature history of the oil prior to emulsification, i.e., the crystals size and shape was determined by processing conditions. Oil that had been rapidly cooled resulted in the formation of small wax crystals, while the slow cooling of oil resulted in larger crystals (6). Different CWCD`S were characterized by optical microscopy (Figure 1).



(a) (b) (c)

Figure 1. Brightfield micrograph of carnauba wax crystals (CWC) as a function of cooling rate: (a) 1 ºC/min, (b) 6 ºC/min, and (c) 12 °C/min.

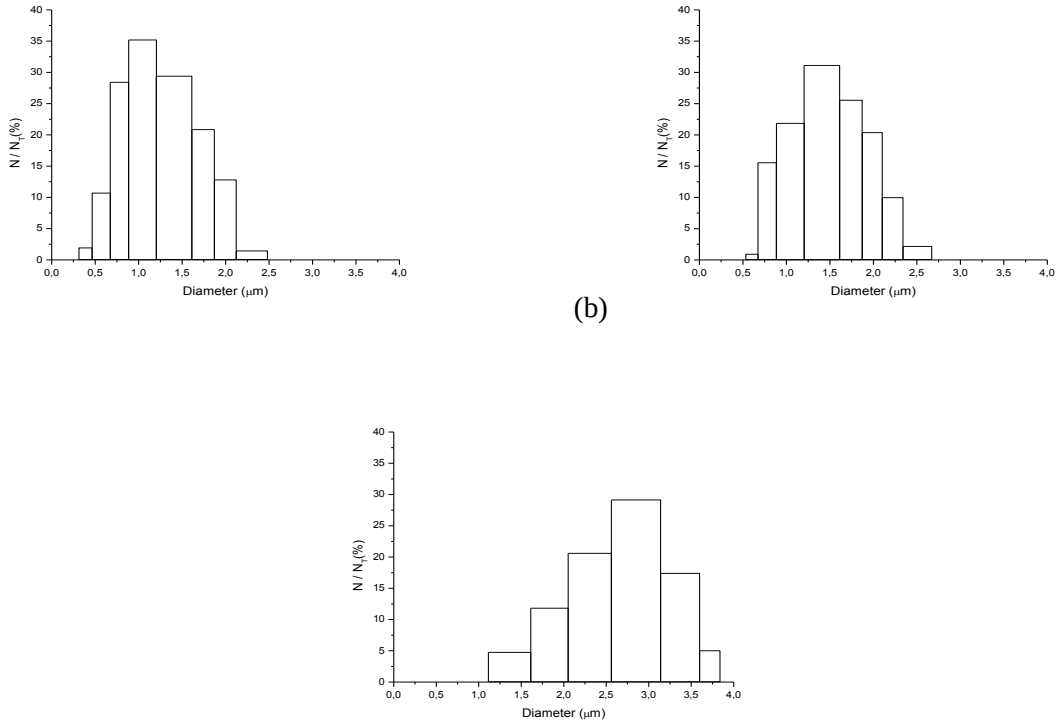


Figure 2. Distributions of carnauba wax crystals (CWC) as a function of cooling rate: (a) 12 ºC/min, (b) 6 ºC/min, and (c) 1° C/min.

CWC exhibited varied morphology with some crystals depicting spherical shapes and others irregular shapes. Smaller crystals were formed when cooling rates were faster and viceversa. Figure 2 shows that the average particle sizes were of 1 μm at a cooling rate of 12 °C/min, 1.5 μm at a cooling rate of 6 °C/min, and of 3 μm at a cooling rate of 1 °C/min.

One factor that could not be assessed with Brightfield and polarized light microscopy was the behavior of the CW crystallization and interactions with oil or wax crystal network properties developed. DSC was used for investigating the changes in wax crystals morphology and their interactions.

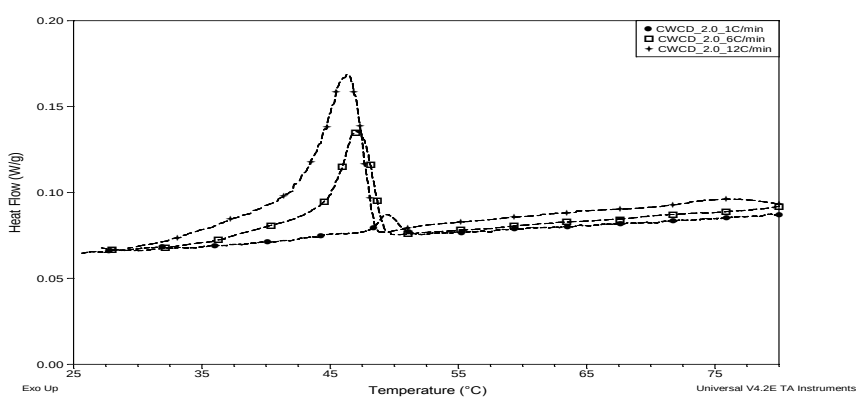
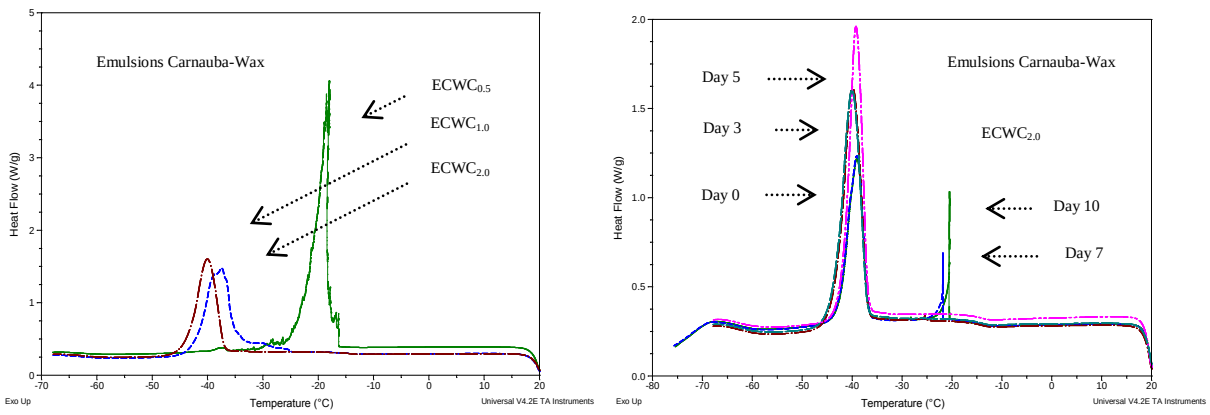


Figure 3 Evolution of DSC curves for CWCD as a function of cooling rate, maintaining a concentration of 2.0 %.

Figure 3 provides a graphical overview of how cooling rate affects the grade of crystallization. The lower the temperature of the exothermic peak, the smaller the crystal size formed. Differential scanning calorimetry (DSC) has emerged as an alternative to microscopy or light scattering techniques for characterizing materials or diluted emulsions (9).

Emulsions prepared with CWCD at concentrations of 0.5, 1.0 and 2.0 % (Figure 4a) were subjected heating-cooling cycles immediately after formation and at different time intervals (up to 10 days). Lower temperature exothermic peaks indicate that an emulsion has smaller droplet size. Thus $ECWC_{2.0}$ had smaller droplet size than $ECWC_{1.0,}$ and the latter than $ECWC_{0.5}$. Figure 4b shows heating cooling cycles for $ECWC_{2.0}$ immediately after formation and at 3, 5, 7 and 10 days. When the exothermal peak of an emulsion overlaps at different aging times, it is indicative of high stability, as droplet size remains constant. When the exothermal peaks shift towards higher temperatures the emulsion droplet size increases with aging time. This may be due to a destabilizing process (coalescence) or due to a reorganization of the solids particles at the interface. As can be observed from Figure 4b, $ECWC_{2.0}$ remained very stable up to 5 days, but the change in the profile of the curves by day 7 indicates that $ECWC_{2.0}$ evolved into an emulsion with larger droplet size, but remained almost the same after day 10.



(a) (b)

Figure 4. Evolution of DSC freezing curves for the Emulsion Carnauba Wax Crystals (ECWC): (a) different concentrations of Carnauba Wax Crystals (CWC); and (b) Solidification peaks thermograms obtained at different aging times (0 to 10 days) for emulsions with 2 % CWC.

It has been reported that in Pickering emulsions an evolution towards a dynamic equilibrium is created where the droplet size always adjusts to the thermodynamically stable state (9).

In conclusion, we found that very stable water in oil emulsions can be stabilized with CWCD without addition of surfactants, when using a sufficiently high CWC concentration (2.0 %) and a high cooling rate (12 °C/min). Although droplet changes were observed, these were due to restructuring of the solids at the water-oil interface. The consequence of this was the formation of highly structured three-D CWC solids barrier that rendered long-term stability to the emulsion.

Acknowledgements

The authors acknowledge the partial funding of this work the Institute of Science and Technology of the City (ICyTDF) through project PICSO-11-64.

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Microencapsulation Of *Bifidobacterium lactis* And *Bifidobacterium infantis* By Spray Dried Using β-Ciclodextrin-Gum Arabic And Skim Milk Like Encapsulante Agents

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Introduction

Bifidobacteria are gram positive-no mobile bacteria that naturally inhabit the guts of warm-blooded animals and mamals (Scardovi, 1986). These organisms are able to exert beneficial effects including improvement of intestinal microflora by preventing colonization of pathogens, amelioration of diarrhea or constipation, activation of the immune systems and increasing protein digestion (Ishibashi and Shimamura, 1993). Owing to these properties, bifidobacteria are now frequently used to prepare probiotic dietary adjuncts.

However, studies show that the addition of probiotic cultures, directly in food, presents undesirable organoleptic changes and it has not shown high viability of the bacteria from preparation to consumption of food, because these are subject to conditions of the gastrointestinal tract, resulting in the reduction of such bacteria, lowering the beneficial effect that might make, which is why, that microencapsulation presented as a solution that allows the bacteria to introduce a capsule, protecting them from the environmental conditions and release at the required time, so that the encapsulation is a viable alternative to prevent these phenomena (Rokka and Rantamaki, 2010).

Microencapsulation of probiotic bacteria is currently gaining attention as a method to improve the stability of probiotic organisms in functional food products (Kailasapathy,2002). Microencapsulation protects the cells from the adverse environment, and mild heat treatment from food processing thus potentially reducing cell injury and death. The development of a heat stable microcapsule may be suitable for heated functional food products such as cakes and sausages. There is a need for microencapsulation not only to help the probiotic bacteria to survive in the food product but also during the passage though the human stomach, where the pH can be as low as 2.0.

A crucial point in the encapsulation process is to maintain the concentration of probiotic cultures higher to 10^6UFC/g of product, where the latter is required to add it to a food, allowing their proliferation and provide the beneficial effect on the host. One of the methods used in the encapsulated bacteria is spray drying, due to the short times employed and the particle size obtained (~ micrometers) make it suitable for the formulation of food (Desmond *et al*., 2001).

Moreover, the selection of wall materials for spray drying is very vital for efficient encapsulation. They should have high solubility in water, low viscosity at high concentration, capability to form films and cheap cost (Re, 1998), and also ability to provide certain nutrients for the resting bacteria. For these reasons, gum arabic and maltodextrin, gelatin, β-cyclodextrin, carboxymethyl cellulose (CMC) and cornstarch had been selected as the wall material, obtained good efficiency of encapsulation of probiotic bacteria. This study evaluates the implementation of new encapsulante agents to produce dry micro-capsules of *Bifidobacterium lactis* y *Bifidobacterium infantis* with high viability. The study concentrated on determining the survival of the cells, encapsulated in matrix of β-ciclodextrin-gum arabic and skim milk by spray drying and the resistance of conditions gastro-intestinal simulated.

Materials and methods

Obtaining of cell paste. Probiotic cultures of *Bifidobacterium infantis* and *Bifidobacterium lactis* were activated in MRS broth supplemented with L-cysteine 0.05% (w/v) and incubated for 24 hours, at 35°C. The resulting cultures were transferred into 800 ml MRS broth with 0.05% L-cysteine (w/v) and incubated under same conditions grown. Cultures were harvested by centrifugation at 4000 x g at 4°C by 15 min and washed with phosphate buffer saline (pH 7.4) and collected by centrifugation as precipitate. The washed bacterial cells were mixed encapsulante solutions.

Encapsulation of probiotic bacteria of the genus *Bifidobacterium* by spray drying. Encapsulant solutions were prepared skim milk (15% w / v) sterilized at 70°C for 10 min, where the cell paste is added under sterile conditions. Using a spray dryer Mini Spray Dryer B-290 (Buchi, Switzerland) at temperature conditions of 170°C, a flow rate of 28 m^3 a pressure of 2.5 bar, the encapsulating solution is dried, obtaining as a final product a fine powder with probiotic bacteria. Taguchi L8 Design with 8 experiments was employed, analyzing 5 factors: bacteria type, encapsulante agents, encapsulante agent concentration (%), temperature and feed flow.

Viability study of gastrointestinal probiotic cultures and simulation. One gram of powder or product is subsequently hydrated to dilutions of 10^{-1} to 10^{-10}. The number of viable cells in the suspension was determined through serial decimal dilutions, 20 µl samples were seeded in Petri dishes MRS agar supplemented with L-cysteine using the Miles and Misra technique, incubated at 35°C in anaerobiosis for 48 hours for subsequent count of CFU per dilution.

Results and discussion

The encapsulation fo *Bifidobacterium lactis* and *Bifidobacterium infantis* by spray drying using b-ciclodextrins- gum Arabic and skim milk like encapsulante agents showed high survival percentage between at 74-100%, with an water activity of 0.190-0.417. Also, the encapsulated powder performances were between 9.7 to 56.8% (table 1).

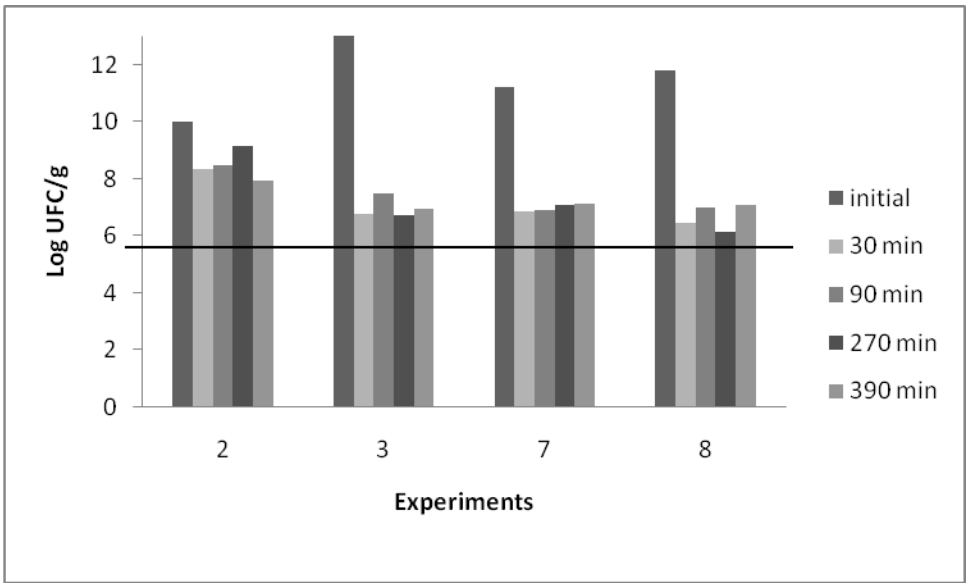
Exp.	Description TE(°C) FA ME %ME BAL	Log CFU/g of powder before encapsulation process	Log UFC/g of powder after encapsulation process	Survival percentage (%)	a_W	Performance of spray powder (%)
1	160 5 LD 15 BI	11	11.05	100.45	0.215	14.7
2	160 5 LD 20 BL	12.07	11.17	92.5	0.190	56.8
3	160 10 BA 15 BI	11.55	10.06	87.09	0.417	27.8
4	160 10 BA 20 BL	11.30	8.39	74.24	0.219	33.8
5	180 5 BA 15 BL	11.54	10.34	89.60	0.195	27.8
6	180 5 BA 20 BI	11.11	11	99.0	0.405	9.7
7	180 10 LD 15 BL	12.08	9.09	75.24	0.249	40.3
8	180 5 LD 15 BI	10.61	9.69	91.32	0.198	51.3

TE=temperature (°C), FA=feed flow (ml/min), ME= encapsulante material, %ME= encapsulante material percentage; LAB= lactic acid bacteria.

In the table 1 the experiments 1 and 6, presented the most high survival after of encapsulation process, these correspond of *Bifidobacterium infantis* with the different materials (β-cyclodextrin-gum Arabic and skim milk).

The water activity (aw) is a indirect measure of free water content in a product and it can be participate in different deteriorative reactions and it increases or discreases the microbial grown. The activities below at 0.25 guarantees the stability of probiotics bacteria encapsulated; whereby the experiments 2 and 5 (*Bifidobacterium lactis* employed an feed flow of 5ml/min and conditions of temperature, 160°C and 180°C, using β-ciclodextrin-gum Arabic and skim milk like encapsulante agents with different concentrations (15 and 20%), presented the most low water activities. These results were similar like the performance of spray powder.

The encapsulated bacteria by spray drying were submitted to gastro-intestinal simulation; in this part of study, just was analyzed experiments with performance higher that at 30%. The powders do not presented important evolutions at 270-390 min. Also, all experiments to the end of gastro-intestinal simulation showed a concentration same or mayor at the minimum concentration request by a probiotic food (6 Log CFU/g). (Graphic 1)



Graphic 1. Study of gastro-intestinal of probiotic powders

This resistance can be possible to the presence of biliar hidrolase salts, enzyme produced by lactic acid bacteria of *Bifidobacterium* genes, this can catalyses the hydrolysis of biliar salts, where his activity involved the production of huge amounts of organic acids to detect the presence low pH (Rondón et al., 2008).

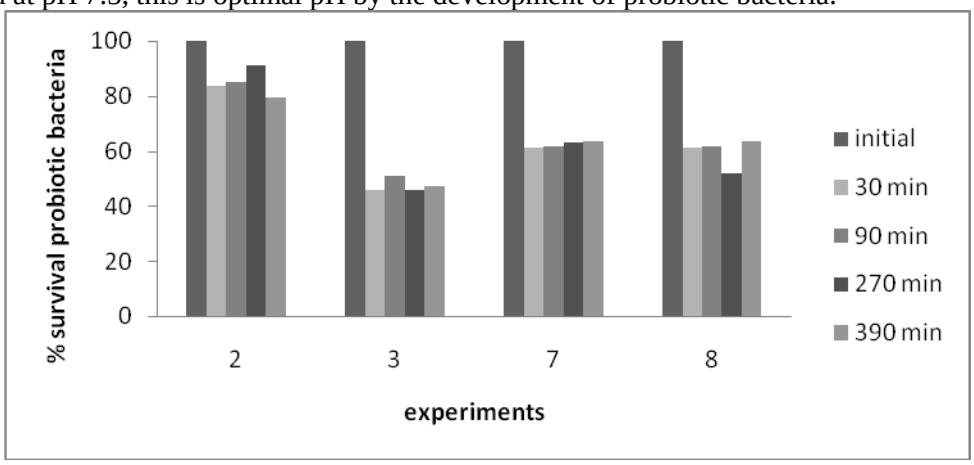
The table 2 shows the variance analysis and the Tukey test with a confidence level of 95%; the feed flow and the type of acid lactic bacteria showed significance effect on the encapsulation process by spray dried. The feed flow (5 ml/min) and *Bifidobacterium infanti*s promoted at 15% and 11% more at the viability of probiotic bacteria.

Table 2. Analysis of the different factors that interview at the encapsulation process of lactic acid bacteria

Factor	% de survival of lactic acid bacteria
Temperature	
160°C	87.553 a
180°C	89.806 a
DMS(p<0.05)	-2.252
Feed flow	
5 ml/min	**96.40 a**
10 ml/min	**80.95 b**
DMS(p<0.05)	15.447
Concentration encapsulante agent	
15%	86.64 a
20%	90.71 a
DMS(p<0.05)	-4.065
Encapsulante agent	
Skim milk	89.877 a
β-ciclodextrin- gum arábiga	87.482 a
DMS(p<0.05)	-2.395
Lactic acid bacteria	
B. lactis	**82.895 a**
B. infantis	**94.465 b**
DMS (p<0.05)	11.57

Values with the same letter do not be significance diferents between treatment, DMS = Different mínimum significance with a P<0.05

The graphic 2 presents the percentage of survival of probiotic bacteria submitted to conditions of gastro-intestinal simulation. The experiment 2 (*Bifidobacterium* lactis encapsulated with skim milk) showed major resistance to the acid conditions with a reduction of 20% of viability of probiotic bacteria in compare to other experiments; because these last showed dramatic reductions between to 30 -50%. Likewise, the experiments did not show important reductions, it can be possible because the simulation was realized at pH 7.5, this is optimal pH by the development of probiotic bacteria.



Graphic 2. Percentage of survival of probiotic bacteria front a gastro-intestinal simulation

Also, the experiment 2 and 7 (*Bifidobacterium lactis*) using skim milk, suggested that the encapsulante agents produced a protector effect during the dilution. The results showed that the encapsulante agents were not diluted totally and the capsule remained stable like during a period and submitted to extreme conditionals (high moisture and acid pH).

Conclusions

The β-ciclodextrins-gum Arabic and skim milk were shown be efficient to generate microcapsules with resistance of spray drying and conditions gastro-intestinal simulated. These can be introducing in food and produce benefic effect in human health.

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Correlation Among Polyphenol Oxidase Activity, Banana Peel Color, Fruit Puree Browning And Fruit Ripeness Stage, Based On Digital Image Analysis And Enzyme Kinetic Studies

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Introduction

Bananas are one of the world's most important food crops, consumed by millions as part of a daily diet and for nutrient enrichment since they are an important source of polyphenols, minerals and carbohydrates (1). Though commonly consumed as fresh fruit, bananas have an unfortunately short shelf-life due to softening and thus are often used in banana juice, puree or pulp processing as an alternative. A relevant issue during banana processing and storage is the browning; this can lead to deleterious changes in the appearance and organoleptic properties of fruit, causing quality loss and complete exclusion of the food product from certain markets.

The enzymatic browning reaction that occurs during handling and processing is caused by polyphenol oxidase (PPO) and peroxidase activities (2).Polyphenol oxidase is a copper-containing enzyme catalyses two distinct reactions in the presence of molecular oxygen: the hydroxylation of monophenol to o-diphenol (monophenolase activity) and o-diphenol is then oxidized to o-quinones (diphenolase activity) (3). This quinones formed are highly reactive substances, which normally react further with other quinines, proteins or amino acids to form brown pigments (melanins) of high molecular mass (4).

Traditionally, the polyphenol oxidase activity was used as browning indicators based on a biochemical index for quantify enzymatic browning, also used physical indicators such as color surface. Termination of browning in bananas using color information of surface of fruit by means of L*a*b* space or CIELab has been the most expensively used color mode (5).

The CIELab color space comprises of three color channels; L* is the luminance or lightness component, it ranges from 0 to 100, while a* (green to red) and b* (blue to yellow) are two chromatic components, with values varying from -120 to +120.

Colorimeters are of the instruments most used in the measurement of color; however they have the disadvantage that the surface to be measured must be uniform and rather small which makes the measurements obtained quite unrepresentative (6). For this reason, the determination of browning in bananas using L*a*b* space implemented by MatLab, represents a great advantage to the use of colorimeters, because this color channels can be determined independently of the shape of fruit, the size and conditions of maturity they are.

The aim of this work is to associate the PPO activity with the color changes both the peel and pulp of banana fruit at different ripening/maturity stages, through analysis of image, obtaining three color channels of CIELab system with the help of MatLab Software.

Materials and Methods

Biological Material

Bananas of the cultivar Enano gigante (Musa AAA, subgroup Cavendish) were acquired in the INIFAP experimental field of Tecomán, Colima, when they were green with a minimum length of 13 cm. The

banana bunches were ripened in laboratory conditions at 19°C without atmospheric control for different storage times.

Image Acquisition

The images used in this work were taken with digital camera; model DSC-W350 (Sony, USA), which was incorporated externally to system of Gel Doc XR+ of BioRad for independent manipulation of images. This equipment allows us to obtain a controlled geometry and brightness in all images captured. Images were obtained using white light illumination with white background. The camera was used with maximum resolution (1440 x 1080 pixels), zoom of 4x, no flash and storage in JPEG format. Additionally, all of the images were stored in RGB color space at 8 bits per channel. During monitoring of ripening fruit five images were captured.

Imaging processing of bananas

The analysis of the images obtained during the maturity were processed to obtain of three color channels L*a*b*, using an algorithms in the MATLAB 2010a (The Math-Works, Inc., USA). Defects like as spots on the surface was removed from the preprocessed of images. Color images were transformed from RGB space color to L*a*b* space color.

Preparation of Crude Enzyme

Crude PPO was extracted as follows. A portion of 50 g of banana pulp was homogenized in a blender for 30 s with 100 mL of a cold 0.1 M sodium phosphate buffer, pH 6.5 containing 1 %(w/v) polyvinylpyrrolidone and 0.5% Triton X-100. The homogenate was centrifuged at 11 000 g, 4 ºC for 30 min. (7). To fractionate the proteins recovered in the supernatant was added slowly solid ammonium sulphate to a saturation of 40, 60 and 80%. The extract was constantly stirred to avoid the local precipitation of protein. After stirring in an ice bath for 1 h, the precipitate was recovered by centrifugation at 11 000 g, 4ºC for 30 min. The precipitate was redissolved in a small volume of 1 mL of 0.1 M sodium phosphate buffer, pH 7.0, dialyzed against the same buffer for 6 h, with at least two changes of the dialyzing media. (8). The dialyzed solution was cryoconcentrated for method to concentrate protein solution based on dialysis freezing-centrifugation proposed by Virgen-Ortíz (9).

Preparation of puree of banana fruit

A small portion of 50 g of banana was processed in a blender for 15 s. The puree obtained was placed in a petri dish facilitating manipulation; this first step is performed in a manner fast, avoiding oxidation unexpectedly of the fruit. The browning of puree was monitored for 120 min using system of Gel Doc XR+ of BioRad. Images of puree were captured during time of zero (Taking as zero time the puree in the fotodocumentador) 15, 30, 60 and 120 min.

Purification of the Enzyme PPO for Gel Filtration Chromatography

To obtain a greater degree of purification, the enzyme PPO was purified by gel filtration chromatography. The cryoconcentrated solution was added to a Sephacryl S-200 column (54.5 x 2.6 cm) preequilibrated with 0.1 M sodium phosphate buffer, pH 7.0. The enzyme was eluted with the same buffer at a flow rate of 0.5 mL/min. Fractions were collected from 2.5 mL with a fraction collector (Frac-920, Amersham Biosciences) and it were measured absorbance at 280 nm with a spectrophotometer (BioPhotometer Plus, Eppendorf). The fractions were separated on a SDS-PAGE system to determine the relative concentration and purity of the enzyme PPO. The fractions that contain the enzyme were combined and concentrated by ultrafiltration (molecular weight cut-off of 2 kDa).

Determination of PPO activity

Polyphenol oxidase activity was determined spectrophotometrically by measuring the increase in absorbance at 410 nm at 30 ºC. The reaction mixture contained 200 µL of enzyme extract, 200 µL of catechol as the substrate and 1.5 mL of 0.1 M sodium phosphate buffer, pH 7.0. The enzyme activity was defined as the change of absorbance at 0.001 for 1 min for mL of the enzyme (10).

Determination of protein
The protein concentration of the crude extract and partially purified enzyme in each step were determined according to Bradford method (11), using a bovine serum albumin (BSA) as standard.

Polyacrylamide Gel Electrophoresis (PAGE)
The purity of the enzyme was checked using SDS-PAGE under denaturing conditions according to the method of Laemmli. The protein separation was effected at 80 V for 4 hours using the electrophoresis system Mini Protean Cell Electrophoretic System (Bio-Rad) polyacrylamide gels at 12%. We used a molecular weight marker (Precision Plus Protein Standards, Bio-Rad) that contains proteins of 10 to 250 kDa. After completion of the polyacrylamide gel electrophoresis, the gel was taken for protein staining with Coomassie Blue (G-250) 0.04% (w/v), acetic acid 10% (v/v), methanol 40% (v/v). Excess colorant was removed with discolored solution (same components except the Coomassie Blue). Identification of the band corresponding to the banana PPO enzyme was determined by its activity (zymogram). PPO activity was assessed on polyacrylamide gel under nondenaturing conditions; the procedure was same as for SDS-PAGE except that SDS and β-mercaptoethanol were not added. PPO activity in the non denaturing polyacrylamide gel was assayed by incubating the gel in phosphate buffer (0.1 M, pH 7.0) containing the catechol substrate (200 Mm) at 50 ºC for 20 min (8).

Statistical analysis
Results are expressed as mean ± standard deviations of triplicate analyses for each sample unless otherwise stated. Statistical significance was assessed with one-way analysis of variance (ANOVA) using the MatLab software (MathWorks, USA). Treatment means were considered significantly different at $P < 0.05$.

Results and discussion

A one-way ANOVA analysis was used to determine differences in the change of color during banana ripening. Color channel L*a*b of banana image were obtained by an algorithm implemented in MatLab. Five groups was found significant differences of color channel a* (green to red) of whole fruit. Fig. 1 shows the color channel a* behavior with respect to the days of monitoring.
Experimental data obtained by ANOVA of color channel a*, were adjusted by nonlinear regression using a sigmoidal function implemented in MatLab (Fig. 2).

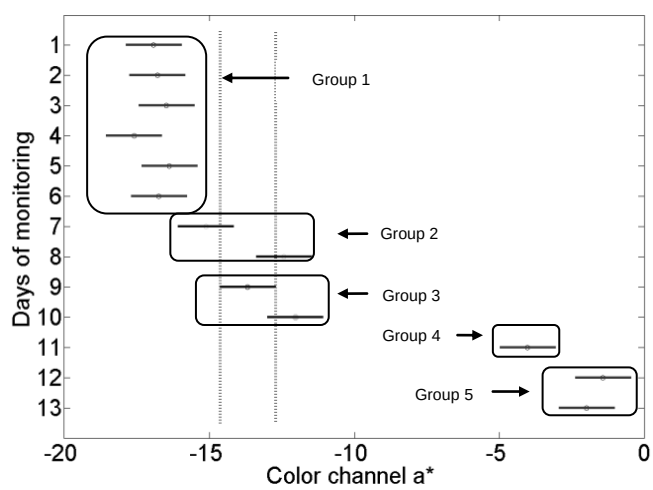

Figure 1. Identifying different groups depending on the ripening of banana. Analysis of variance of color channel a* representing the five groups with significant differences.

Data set corresponding to color channel a* were acceptably fitted (determination coefficient of 0.987) with the sigmoid equation following:

$$a^* = \frac{1.73x10^6 e^{-1.11t}}{1 + \frac{(1.18x10^{-1.11t})[(e]^{-1.11t})}{1.11}} - 0.11$$

The browning of banana puree depending on the ripening was determined by analysis of images. The captured images were processed for obtain L*a*b* color space.

Fig. 3 shows the browning banana puree with respect to time. Images of puree were captured at different intervals of time (freshly prepared, 15, 30, 60 and 120 min) after its preparation.

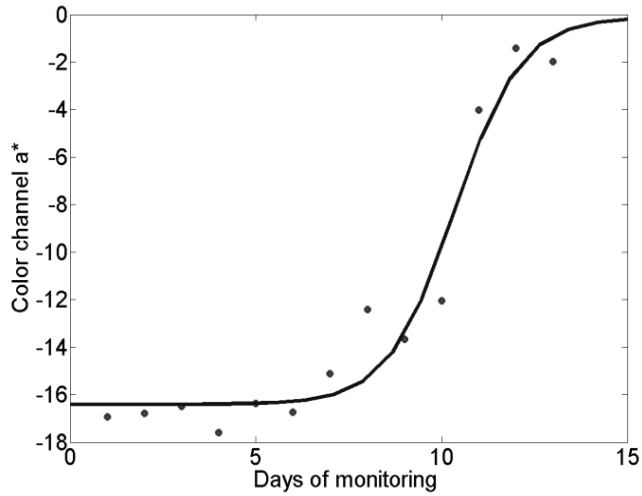

Figure 2. Dynamic behavior of color parameter a* during ripening of banana. The experimental data of the color parameter a* satisfactorily adjusted to a sigmoid function.

Freshly prepared 15 min 30 min 60 min 120 min

Figure 3. Browning banana puree. The images were captured according to the state of banana ripening.

The channel L* (luminance or lightness component) obtained from banana puree, was designated as an indicator of browning. During banana ripening, the lightness parameter of puree decreased (Fig. 4), indicating that the increased of ripeness stage of banana fruit, browning rate increases.

Obtaining greater browning of puree in group 5.

The protein content in the crude extract of each group of banana ripening was quantified by Bradford method.

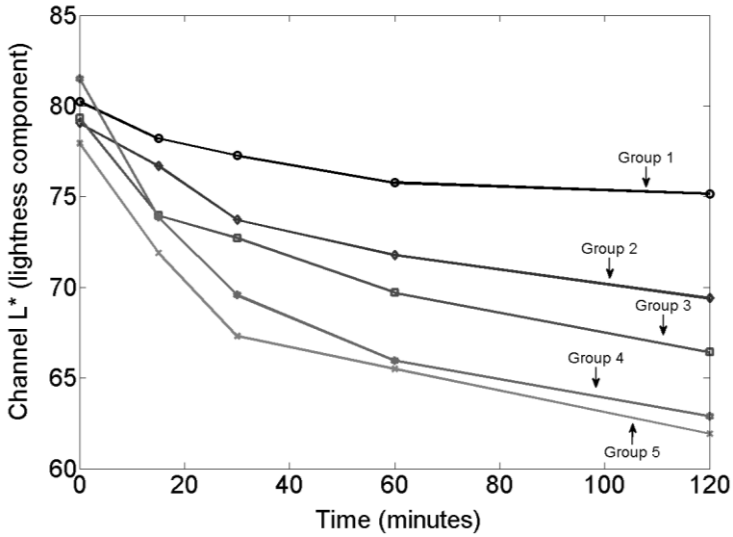

Figure 4. Increase of luminance L* with time of banana puree indicating the browning rate to different stages of ripening bananas.

As shown in Fig. 5, an increase of protein in the crude extract was observed during monitoring of banana ripening. Determining that the groups immature of fruit banana, contains a lesser amount of protein in comparison with the group 5, which is observed a high concentration.

PPO from banana fruit was semipurified by using sequential steps of fractionation with solid ammonium sulfate (40-60% saturation) and gel permeation chromatography. Specific activity of PPO corresponding evry group (1-5) was evaluated and compared among the same groups. Specific activity of PPO semipurified enzyme of each the all five groups proposed during the monitoring of banana ripening, was determined. It´s found that the specific activity of PPO extracted from banana corresponding to group 5 was 1.52-fold higher that the specific activity of PPO from group 1.

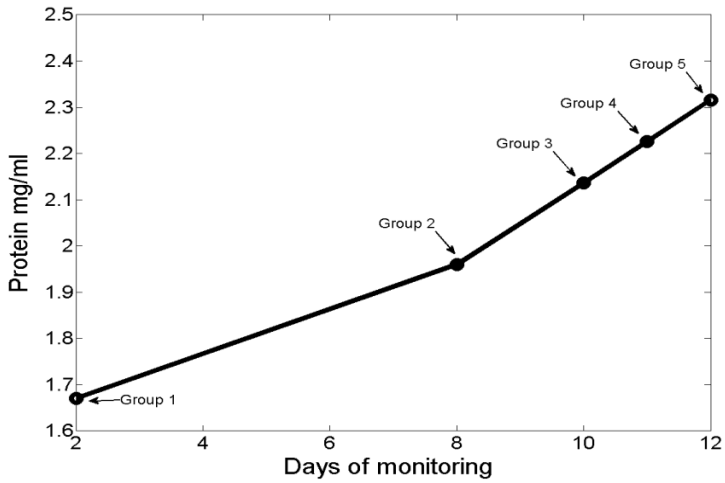

Figure 5. Increase of protein (mg/ml) of crude extract during ripening, according designated groups.

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Effect Ofmicrowave Pre-Treatment On The Frying Kinetics Of Potatoes

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Introduction

In recent years thesocial and cultural changeshad increase the comsuption of fast-foods especially fried products.However,the mainproblem associated withthe fried productsis theconsiderable amount ofoil absorptionduringfryingprocess(Song *et al*., 2007b; De Grandi*etal*., 2009). The excessive comusption of fat is associated to differentdiseases such asdiabetes, cardiovascular problems and of obesity(Mellema, 2003). Due to these problems of health, in the last years thefood processing industryhas focused in the developmentof alternative methodsfor the production of fried products with lessoil absorption(Pedreschi andMoyano,2005ab, Song *etal*., 2007a, Morales-Pérez and Vélez-Ruiz, 2011). Among the studieson alternativesto reducethe fat contentin fried foodsareohmic heating(Salengke andSastry, 2007); combination ofosmoticpretreatmentand microwave (Sahin *etal*., 2007); blanching(NaCl solution, citric acid), application ofhydrocolloidsderived fromcarboxymethylcellulose (Rimac-Brncic *etal*., 2004), dehydration invacuummicrowaveand subsequent vacuum frying(Song *et al*., 2007a).

The aimof this studywas to evaluate theeffect of microwave pretreatment (200, 400 and 600W)on the oil absorption,moisture loss andphysical changes potato stripsduring frying.

Materials and methods

Potatoes *Alpha* varietyandsunfloweroil were usedduring the experiments. Fresh potatoeswere washed, peeledand cut intorectangular stripsof0.5x0.5x4.0cm.Thereafterpotatostripswere subjected toa microwave pretreatmentusinga domestic oven of 2.5GH of frequency(Daewoo, Korea).Threepotatostripswere placedhorizontally inthe oven microwave, which operated at 200, 400 and 600W,exposure timewas considereduntil thecenter temperatureof potatostripreached 70±5°C.Thepotatostripswithout pretreatmentwere consideredas control samples.Subsequentlyto microwave pretreatments, thepotato stripswere friedinahome fryer (T-Fal, model FR4015)at 160and 180°C, atdifferent time intervals(0,60, 120, 180, 240 and 300 s). After frying processthe samples were drained and the oil excess at the samples-surface was removed by absorbent paperand cooled toroom temperature.Finally the moisture loss, oil uptake and colour of the fried samples were analyzed.Statisticalanalysis was performedand amultiple comparison testofTukeywithsignificance levelα=0.05.All analyzeswere carriedout in triplicate.

Results and discussion

Pretreatment and moisture content

The experimental dataof moisture loss were fitted to afirst-orderexponential model$MR = e^{(-k \cdot t)}$, where*k* is therate ofwater loss and *t*attimeoffrying. For the temperature of 160 °C the values of *k* were 0.007, 0.010, 0.016 and 0.027 min^{-1} for control and pretreated sample with 200, 400 and 600 W, respectively. For the temperature of 180 °C the values of *k* were 0.013, 0.018, 0.014 and 0.010 min^{-1} for control and pretreated sample with 200, 400 and 600 W, respectively. It was observed that microwave pretreatment affected the water loss rate at the frying temperature at 160 °C, being the water loss rate higher in treated samples in comparison with control samples. However, at 180 °C the microwave pretreatment did not affect the water loss rate in comparison with control samples (Fig. 1 and 2).

Thefinal moisture contentoffriedpotato stripsat 160 °C was0.379, 0.319, 0.451and0.324kg/kgdbforthecontrol andpretreatedstripsat 200,400, 600 W, respectively. At

fryingtemperature of 180°Cthefinal moisture content of potato strips was0.357, 0.262, 0.356and0.260kg/kgdbforcontrol andpretreatedsamples at 200,400 and 600 W, respectively.During thefrying processas thetemperature and time increasing themoisture content decreased, this is relatedtodrying and cooking duuring frying. This affirmation coincideswith the work ofSong*etal*.(2007b) who showed that thefryingtemperatureaffectssignificantly (P <0.05) the moisture content, the higher thetemperature, the lower the moisture content.

Effect pretreatment on oil content

The oil abosrption kinetics of microwave pretreated potato strips were fittedtoafirst-orderexponential model: $O = O_{eq}(1 - e^{-K \cdot t})$, where O_{eq} is the theoretical amount of oil potato strips; *k* is the absorption rate oil and *t* at time of frying. At a frying temperatureof160°Cthe fitted values ofO_{eq} were0.131,0.122, 0.127and0.124kg/kgdb for control and pretreated samplesat 200, 400 and 600 W, respectively, whichcorresponds to a reductionin the oil absorption25 % with respectto the samplepretreatedat 600W. In reference to thefryingtemperatureof 180°Cthe values obtained for O_{eq} were0.173, 0.181, 0.172and0.099kg/kg dbforthe control andpretreated samplesat 200,400and 600W, respectively, this corresponds to areductioninoil absorptionof 40 %with of the microwave pretreated sample at 600Win comparison with control samples.It was observed that in both temperatures tested there was a decrease in the rate of oil absorption of pretreated samples with respect to control samples (Fig. 3). The ANOVA showed that the temperature and microwave pretreatment had a significant influence (P <0.05) in the final oil content of the potato strips. The comparison of means(Tukey)indicated a significant differences (P <0.05) between the different microwave powers tested, showing that with the 600 W power the level of oil absorption was lower. Tajner-Czopek *et al*. (2008) reported that potato pre-dried by the method of microwave vacuum absorbed less oil than pre-driedpotatoby theconvectivemethod.

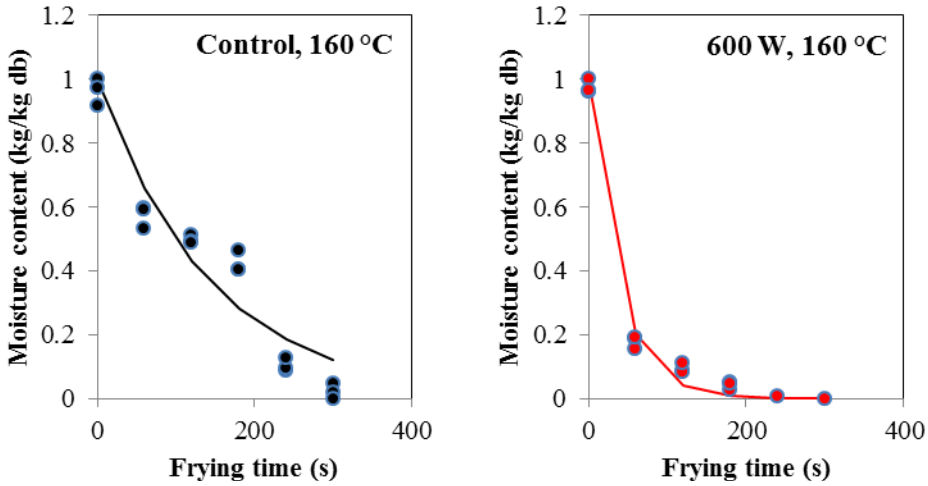

Fig. 1. Kinetics of water loss of potato strips fried at 160 °C.

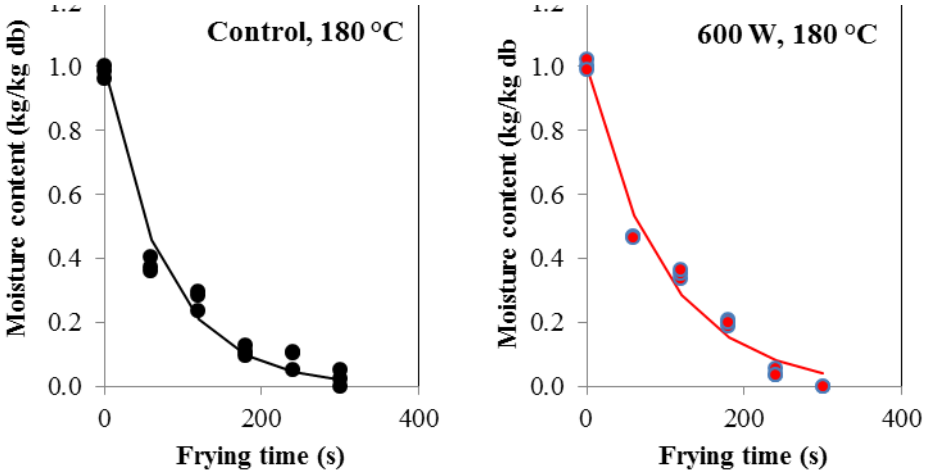

Fig. 2. Kinetics of water loss of potato strips fried at 180 °C.

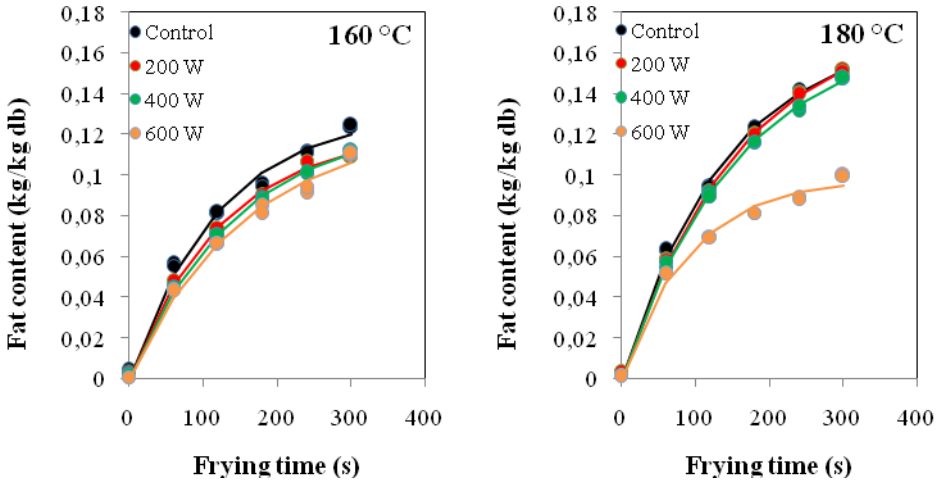

Fig. 3. Kinetics of oil absorption of potato strips fried at 160 and 180 °C.°C.°C°C.

Color measurement

The color of the fried potato strips should be light, light gold, without the presence of black points or streaks. Table 1 summarizes the values obtained for luminosity (L*), coordinates of the green-red color (a*) and blue-yellow coordinate (b*) for control and microwave pretreated samples (200, 400, 600 W) after 300 s of deep fat frying at different temperatures. It was observed that the color characteristics of potato strips were obtained as the temperature increased and fryingtime. The difference in color of the potato strips were primarily due to temperature and frying time, and not by the pretreatment applied. All values of color coordinates werein the range of the light yellow color in the CIELAB space, but the best characteristic color (light gold) was observed in potato stripsfriedat 180°Cfor 4 and 5 min.Similar conclusions obtained Sahin *y col*. (2007) who reported that the development of color (darker) was higher in conventionally French fries than microwave French fries, since themicrowavefryingtimewas shortand it was notenough to finishthe Maillard reaction.

Table. 1. Values of color parameters at 300 s of frying.

Parameters	Control		200 W		400 W		600 W	
CIE	160 °C	180 °C	160 °C	180 °C	160 °C	180 °C	160 °C	180 °C
L*	69.936	73.224	74.098	73.832	72.336	71.61	73.212	72.426
a*	-4.306	-2.856	-4.64	-3.97	-2.474	-1.32	-2.558	-1.35
b*	23.534	24.956	20.256	20.818	20.018	27.008	19.73	26.08

Conclusions

Microwave pre-treatment yielded a potato strips with lower oil content, also this pre-treatment did not affect the color of the potato strips compared with control.

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Optimization Of Process Aimed To The Solvent Extraction Of Added Value Components From Oats

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Abstract

The study covers the feasibility for obtaining added value components from oats by solvent extraction. Design of experiments was developed in order to evaluate factors such as time, temperature, solid size, and solvent in the solvent extraction process. A factorial design of 1 block and two levels with 32 runs were developed. The results were analyzed by ANOVA, having as response variables Total Polyphenols Content (TPC) and Oxygen radical absorbance capacity (ORAC). Total polyphenols were determined by Folin-Ciocalteu method and ORAC capacity by a fluorescence method. As a result from the ANOVA the factors that had significant influence in the TPC response variable were particle size and solvent polarity, while the interactions that also were significant were particle size-solvent, solvent-temperature, size-temperature-time, and solvent-temperature-time. Regarding ORAC capacity response the significant factors were time, particle size, and solvent, while the most significant interactions were time-solvent, temperature-time, and solvent-temperature-time. ANOVA analysis was developed with a significance level of95%.The results show that the higher values of TPC was 1.6 mg GAE/g and ORAC values of de 32 mmol TE/g, this while using a solvent of higher polarity such as water, smaller particle size (.5 mm), temperature of 30°C, and extraction time of 60 minutes. As a result, variables such as solvent and solid size seem to have more influence in the extraction process than temperature and time.
Key words: Optimization, solvent extraction, process development.

Introduction

In 2010 in the state of Chihuahua 65,296.20 tons of oat were harvested which has an estimated value production of $ 170,753,100 (Mexican Pesos), making the state the largest producer of oat nationwide. Therefore oatmeal is one of the main agricultural products harvested within the state (7), not only by the quantity harvested but also by the characteristics of oat grains. Since oats have a high content of unsaturated fatty acids it contains various compounds that protect lipid oxidation in grains. Oxidation gives food containing lipids undesirable characteristics such as taste, smell of rancidity, discoloration and decreased nutritional value. The substances in oats with antioxidant activity includetocopherols and several esters of long hidroxicinnamatoschain (1).Antioxidants are molecules that have the ability to avoid or prevent oxidation with other molecules. Oxidation occurs whenever an electron withdrawing species to another species that gains electrons is reduced. In these oxidation reactions free radicals can be produced, highly oxidative species may cause harm to the body.

Previously, antioxidant capacity was evaluated against LDL and the measurement of the absorption capacity of oxygen radicals (ORAC) from extracts on ground oats, oat flour, and oatmeal. Extracts were made using methanol as solvent and the results were expressed in milliTrolox equivalents (TE/g). The oat extracts pearling from the higher antioxidant capacity obtained 2.89-8.58 TE/g, while the values coming from trichome antioxidant had the lowest values of 1.74 (4). Another study evaluated the antioxidant capacity (assessed using effects on the b-carotene and linoleic acid as a reference) and quantified the content of phenolic compounds (PFT), using the Folin-chiocalteu (mg gallic acid equivalents) technique from different oat extracts (groats, shelled and whole). Extractions were made with 80% ethanol (2).

During this project we use and extend new methodologies for the search and evaluation of solvents that help meet the needs of the process, but also help make the process sustainable. This research is to obtain

through analysis and experimentation methodologies to extend key information for the early identification and evaluation of solvents for extraction of value added compounds in oats (5, 6).

Methodology

Solvent extraction: Oats were obtained in a food distribution center for cattle located in the city of Chihuahua, which has no knowledge of the culture conditions.Oat milling was performed using a commercial blender. To carry out the extractions were 4 different variables, with two levels, high and low were considered: particle size (0.5 and 2 mm), solvents (water and isopropyl alcohol), time (20 and 60 min), and extraction temperature (30 to 50 ° C). The extraction was performed at a solid liquid ratio of 1:5 (5 g. of oat/20 ml of solvent). The extractions were performed in Erlenmeyer flasks of 250 ml and placed in shaker.

Quantification of total polyphenols: Quantification of total polyphenols (PFT) was performed by Folin-Chiocalteu technique previously described by Gutierrez etal. (3). Gallic acid standards (100, 200, 300 and 400 ppm) were prepared and a solution of 20% Na_2CO_3. All standards and samples were run in triplicate. The readings obtained with the standards were used for a calibration curve.

Oxygen Radical Absorption Capacity (ORAC): This technique consists in measuring the degradation of a fluorescent molecule (fluorescein) due to the addition of compounds azo compounds (AAPH). To carry out the test equipment was used in fluorescence reader micro plates (brand flash varioscan Thermo Fisher Scientific) (8). Performing necessary standards trolox (4, 8, 16, 32 and 64 mM), a solution of 0.62 M and a solution of 140 nM fluorescein, all solutions were made with a phosphate buffer solution at 75 mM with a pH 7.5. The plates used were transparent 96-well flat bottom.Fluorescence readings were made with excitation wavelength of 485nm and an emission wavelength of 535nm every 4 minutes for two hours.All standards and samples were run in triplicate, using the estimates obtained from the oxidation kinetics (area under the curve) of Trolox standards was performed with a calibration curve which were evaluated oxidation kinetics of the extracts obtained obtaining the results in units of mMTrolox equivalents per gram of oats (TE micromol /g).

Statistical Analysis: It was using Minitab software 16 to perform an ANOVA with a significance value of 95% to evaluate which factors and interactions had a significant effect on the extractions.

Results and discussion

To carry out the calculation of the total polyphenol content (TPC) a calibration curve was made to obtain a linear equation, so it is possible to calculate the content of the extracts analyzed. During these preliminary studies it was found that the highest values of TPC were 1.6 mg GAE/g, this extraction is performed with the higher polarity solvent (water), with the smaller particle size (0.5mm) at a temperature 30 °C for a period of 60 minutes.The highest antioxidant capacity was 32 mmol TE/g. This extract was the same as the highest content of TPC.Extracts made with isopropyl alcohol as solvent showed practically zero values of ORAC and TPC. This could be explained due the low affinity of the compounds to IPA.The values of TPC and ORAC for the water extraction are higher than the values reported by Handlemanet. al (4). which may be because Handleman et. al. used methanol as a solvent. Another possible cause is the particular differences of oats used by Handlemanet. al and the oats used in our experiments.ANOVA results indicate that single factors such as solvent, particle size, and the interaction of both have a significant effect on the extraction. Small particle size (0.5mm) and high polarity solvents (water) favored extraction (Figure 2). We used a confidence level of 95%. For PFT ANOVA obtained a R2 of 97.46% while for the ORAC ANOVA obtained a R^2 of 90.33%.

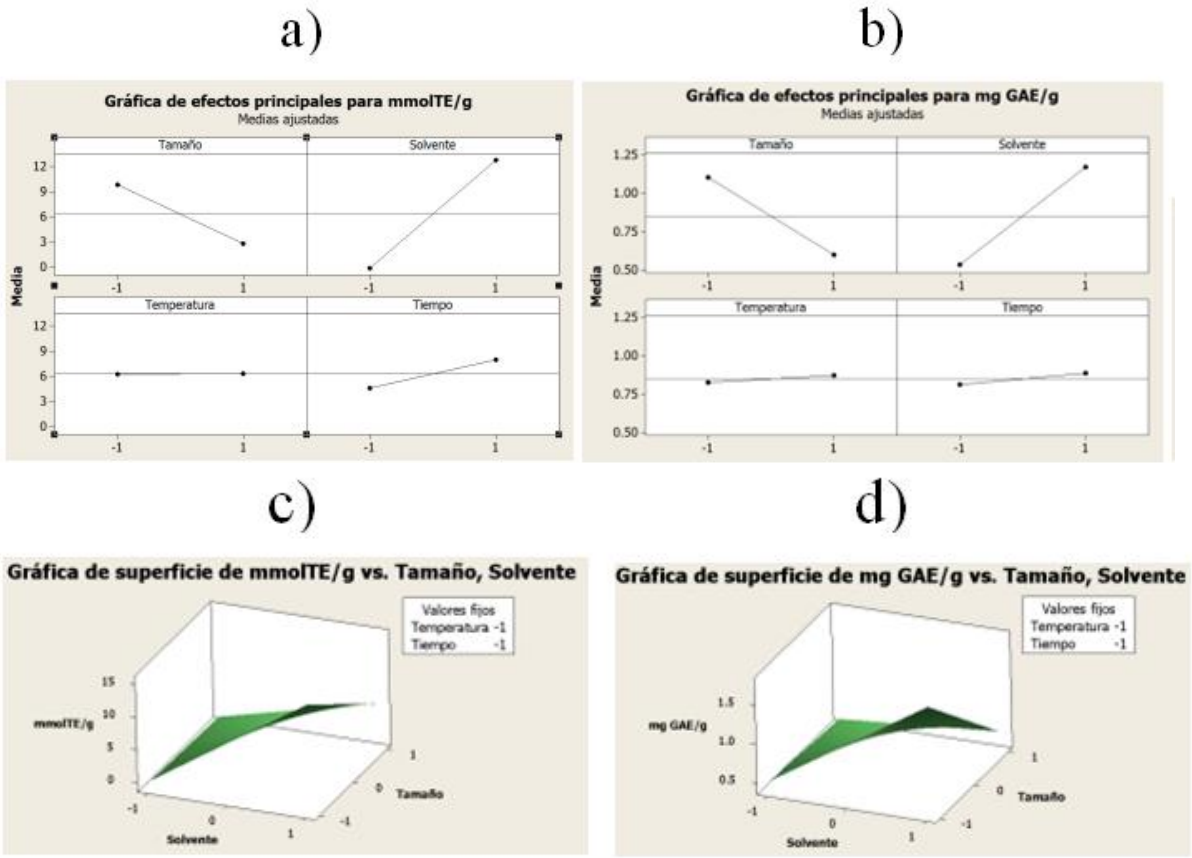

Figure 2. a) main effects plot for effects of mmolTE/g. b) main effects plot for effects of mg GAE/g. c) Guide mmolTE surface/g vs. Size, Solvent. d) Guide surface GAE/g vs. Size, Solvent.

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Elaboration Of Mango Snacks By Dehydration Operations

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Abstract

Mango *(Mangifera indica L)*cv Ataulfo is an important fruit in Nayarit due to its production volume, however losses because of the lack of industrialization are high. Fluidized bed drying (FB) reduces time and costs process and osmotic dehydration (OD) maintain sensorial properties, the texture, hardness and color were evaluated by preference test. The objective of this research was to optimize both drying operations to obtain snacks from mango Ataulfo. Mango cv Ataulfo fruits in physiological state were used. A factorial design 3^3 was applied to OD: temperature (30, 40 and 50 °C), NaCl concentration (0, 1.5 and 3 %) and sucrose concentration (40, 50 and 60 %). Mango fruit was washed and cut in cubes. OD was applied during 120 min, moisture, color changes and total soluble solids (TSS) were analyzed. A factorial design 3^3 was applied to FB: temperature (40, 50 and 60°C), flux (7, 10 and 12 m/s) and cube size (0.5, 1.0 and 1.5 cm). FB was applied during 90 min, and moisture, color changes and effective diffusivity were monitored. The optimal values obtained for OD were sucrose 60%, NaCl 0% and 50ºC and for BD flux 12 m/s, temperature 60ºC and cube size 1.5 cm, the lowest moisture content recorded was 2.65 % g H_2O/g ms, 0.534 Aw. The mango snacks could be an alternative to scholar lunches due to their natural source and sensorial properties.

Keywords: osmosis, dehydration operations, Fluidized bed drying, moisture.

Introduction

Themango *(Mangifera indica)*varietyataulfois a fruitpalatableconsumer,actualy Nayaritis the secondplace innationalproduction; however this fruitis very susceptible tohaving largepostharvest lossesof totalproduction dueto lackmarketing. The development ofa product based onthisresultwould generatea reductioninthe losseswhich would beofgreat importancefor producers.The termdryingrelates tothepartial or totallossofthefreeandboundmoisturemaking ita methodofpreserving foodwhich inhibits orreduces the proliferation ofmicroorganismsand hindersputrefaction.Thefluidizedbed dryingisrecognizedfor its efficiency, low cost of construction and operation, easy operation and maintenancealso representingcost savingsof storage, but the factorsthat influence theoptimizationprocess varyaccordingtothe raw materialdrying. The terms "fluidization" and"fluidized bed" are used to describetheconditionofcompletelysuspendedparticles, since it behaves like adensefluid. Physicochemicalfactorsas well as time, concentration anddrying temperatureare the parametersconsideredin this project aremoreimportantas they canbe handledin such away as to obtainoptimumquality productfor the consumer. Inthis paper,the studyis limitedto a fruitdrying: TheMango(*Mangifera indica L.*) varietyAtaulfousingthisfluidized beddryingandosmotic dehydrationas a pretreatment(DO).

Material and methods

Biological material and preparation of the sample

For this project were collected mangoes (Mangifera indica L.) variety Ataulfo without any pretreatment of ethylene with a degree of maturity. The handle collected was selected according to its firmness was washed and then cut cubes (1 cm^3) for determining the optimum conditions of osmotic dehydration (OD) and 0.5, 1 and 1.5 cm^3 for fluidized bed drying. This process is performed manually using household items such as slicers and knives. It weighed 133 grams of these cubes for further treatment in the osmotic solution.

Osmotic dehydration

The DO carry out using different concentrations of sucrose (40, 50 and 60%) of NaCl (0, 1.5 and 3%) and temperatures of 30, 40 and 50 ° C in addition to the addition of 1% citric acid in all solutions to prepare. The osmotic solution was stirred until completely dissolution. The obtained cubes were immersed in the solution for 120 minutes osmotic sampling every 15 minutes to measure the SST in the sample and the solution, moisture and weight loss for subsequent drying the fluidized bed which is performed when the optimal parameters are obtained from the DO. Table 4 shows the combinations of concentrations of NaCl, sucrose and temperature for obtaining optimal osmotic dehydration.

Fluidized bed drying

The fluidized bed dryer is within the Food Analysis Laboratory at the Technological Institute of Tepic. The cubes of 0.5, 1 and 1.5 cm^3 were subjected to three drying temperatures of 40, 50 and 60 ° C and three flow rates of 7, 10 and 12 m/s.The drying time of the sample will be 90 minutes whereas it is possible that in this period of time is not reached optimum product drying, taking sample every 15 minutes for the subsequent analysis of the cubes in the thermobalance and to obtain the kinetic drying. Table 5 shows the organization of the treatment for drying in a fluidized bed.

Analytical determinations

SST: total soluble solids of the sample and the solution were determined by a refractometer Atago NAR-1T mark.

Humidity: This was determined through a Sartorious MA35 Thermobalance placing a mark inside a cube set temperature of 75 ° C.

Color: was measured with a Minolta colorimeter CR-400 brand.

Size: It will record the change in size of the samples during drying using a vernier gauge.

Water activity (Aw) was determined with a hygrometer AQUALAB series 3.

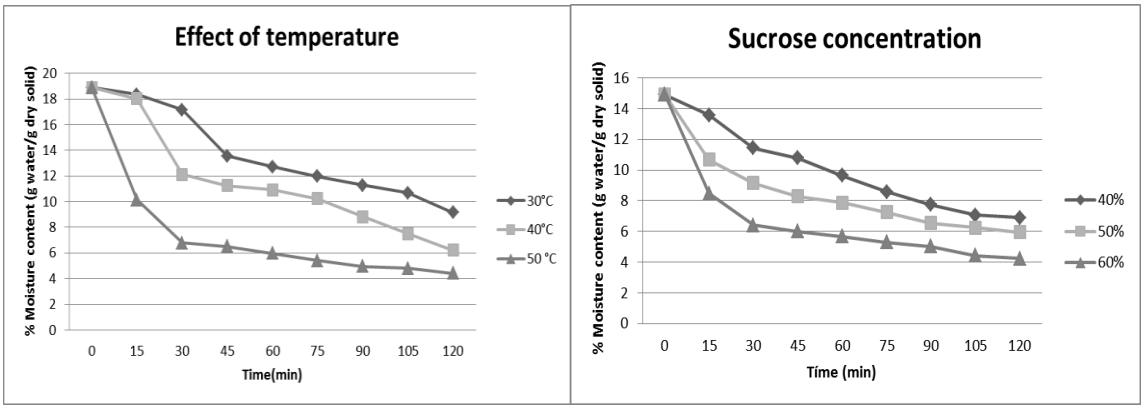
Sensory evaluation: A test sample descriptive and evaluated for color, texture and flavor.

The results were statistically analyzed with a 33 factorial design using analysis of variance (ANOVA), if there is significant difference between treatments be conducted mean comparisons by LSD test ($\alpha = 0.05$) using the SAS statistical package THE SYSTEM 9.0 and for the response surfaces STATGRAPHICS5.1 PLUS.

Results and discussion

Osmotic dehydrationstudybased onthetemperature variation, sucrose and NaCl.

The temperatureis directly related totheremovalofwater, the higher the temperaturethe greater isthe effective diffusivity ofwaterinside the fruit, regardless ofthemicrostructure(1).InFigure 1acan be seenasthelossofmoistureishigher than the temperaturebeinghigher inthiscaseat 50°Cand reaching itsequilibrium moisturein the spaceof90 to 100minutesis observedbesidesthelossdifferencebetween the temperaturesofhumidityfrom minute15.Lashigh temperaturesexceeding 50 °Ccan cause changes inthepermeabilityofthe cell wall, causing changesinitsselectivity tothe solutesin the solution.Theseresults areduetothe increasedtemperature favors thediffusionduetothe diffusion coefficientfor a concentration oftheosmoticsolutionconstanthas atemperature dependenceofArrheniusrate(2).



a b

Figure 1.(a) The effect of temperatureontheosmoticdeshidratacion(b) Effect ofdifferent concentrationsof sucroseintheosmotic dehydration.

Figure1bshows thattheosmotic dehydrationspeed, slopeinthegraphoflossofmoistureas a function oftime increaseswith increase inthe sucrose concentrationis observedatthe concentrationof60% sucroseproduces agreater effect onmoisture lossfrombeing notorious15 minutes.This is becausethe product losesmore waterwhenimmersedin solutions ofhighsucroseconcentrationbecause thedriving forcedifferencebetweentheosmotic pressurewithin theproductandthe solutionis more noticeable. There is alsothe strong influence ofthe concentrationofsolublesolids gainduetohigher concentration ofsucrose, highersolublesolids gainandwater loss(3).Mostmoisture lossseenat the highest concentrationofNaCl(3%) but no significant differencebetween the concentrationsandlow concentrations ofNaCldid notaffectosmoticdehydration (4).

Studyfluidized bed dryingfunction ofvariationtemperature, sizeand airflow

The effectof temperature onthekineticsofdrying can beseen in Figure2a wherewe noticethat the greater thehigherdehydrationtemperatureatwhichthetemperatureis60 °Cwhere we findthe lowest percentageof2.65%ofmoisture, this maybe due totheincreasedtemperaturefavorsthediffusion,since itacceleratestheremovalofwaterfrominside the foodtowardsthissurfaceporositydepending onthe fruit.The variationof the air flowis an important variablefluidized beddryingand Figure2bshowsitseffectwhereit can be seenthat the greaterairflowishigherthanthedehydrationanddryingoccurs moreuniformflowbeing12 m/ s, it was shown thatdryingin the fluidized beddependslargely onthetemperatureandairvelocity(5). Ultimatelylowhumidityand highvelocityairflowis greatermoisture lossof the product.In the caseofthebucketsize variationasmoisture lossmeasurementwas obtainedwiththelargercubewhichwas 1.5cm3,thiseffectis attributed to aphenomenonofagglomerationwithin the dryerinsmallercubes, whichprevents food frombeing driedsince theseform a compact massby reducing contact betweenthe drying airandthe feed (6).

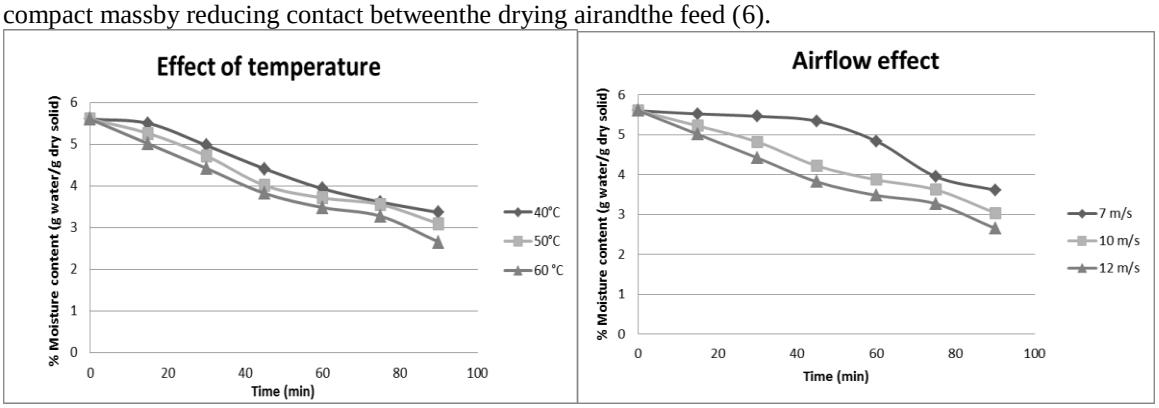


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Figure 2.(a) effectofthetemperatureinthefluidizedbed drying(b) effectof the air flowat different levels.

Water activity(Aw)

It is consideredmicrobiologicallystablefoodwhenit has aloweraw0.5, so inthe realizationof this project, we sought to know what theoptimal processvariablesforminihojuelaswithlong lifeandfit forhuman consumption. Figure 17 showstheevolutionoftheactivityofwaterin the driedmango cubesusedfor differenttemperatures. TheAwofwatertodifferent temperaturesvaried between0.8and0.5,a temperature of 60°Cwhichlower reaches isbeingAwof0.504.

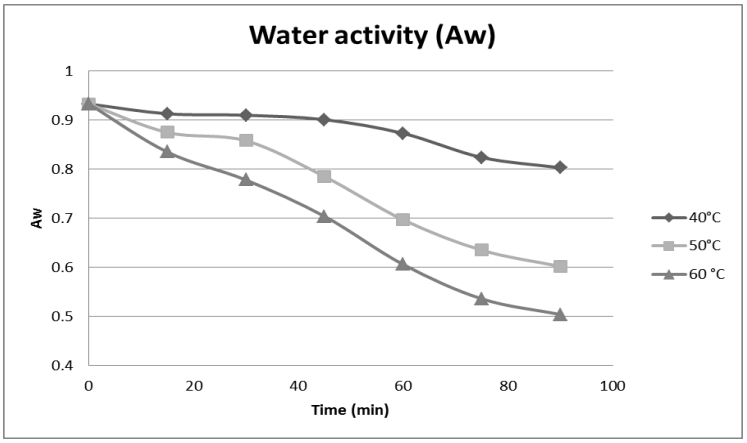
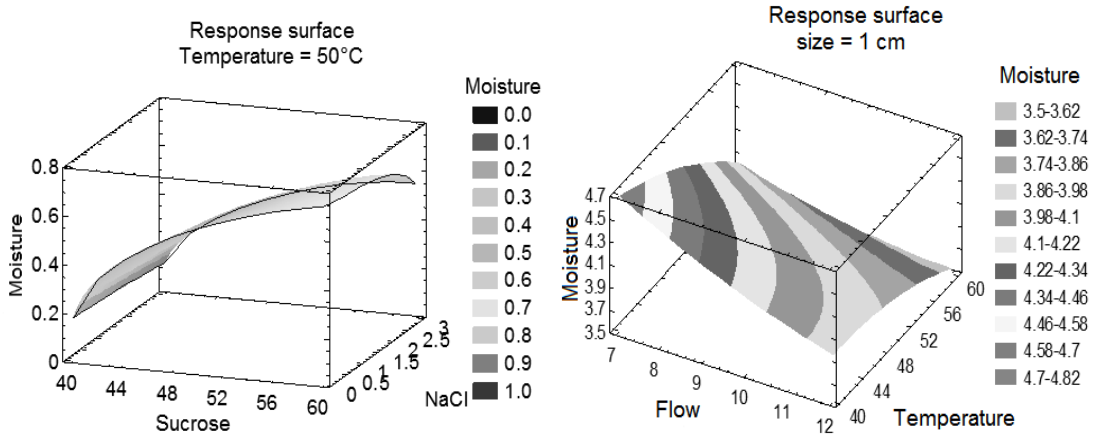


Figure 3.Water activityversus timeat different temperatures.

Statistical analysis andresponse surfaceosmotic dehydrationand drying influidized bed. Accordingtothe statistical analysisthevariables having asignificanteffectonmoisturelossmango cubesaresucrose concentration, temperature, their interactionand the interaction betweenthe sucrose concentrationandtheconcentrationofchloridesodium.The surfaceofosmoticdehydrationresponseshownin Figure 4aandthe optimum valuesobtained withthepackageStatgraphics5.1Plus:60% sucrose, 0% NaCl and50°C temperature.Inthefluidizedbed dryingvariableshaving asignificanteffectonmoisturelossmango cubesaretemperature,airflow rateandthebucketsize. Theresponse surfaceof the fluidized bedshownin Figure4b,the optimum valuesobtained for thefluidizedbed dryingare:airflow11.22m /s,temperature 60°Candsizeof 1.5cm3cube.



a b

Figure 4.(a) Response Surfaceosmoticdehydrationof(b) responsesurfacefluidized bed drying.

Conclusions

As expectedthetemperatureis directly relatedto theremovalofwater, the higher the temperaturethe greater theosmoticdehydrationdiffusivityinthe microstructureregardlessof the fruitso theoptimalvalueofthetemperatureis 50 °C, inthebedfluidizedobtain the samebehavioras the optimumthe highest temperaturecorresponding to 60°C.Accordingtoexistingdiffusivityintheprocessofweightlossofthe sample is reducedduring osmotic dehydrationassugargainis promotedagainstlossof water.Inthe case offluidizedbed dryingdehydrationOptimalconditionswerethe higher levelsoftemperatureand size of thehubtoflowthoughwas obtained asoptimalin termsof 11.22performed statisticalrevealsall the variables involvedthataffectthe drying processinthe fluidized bed.Thephysicochemicalandorganolepticcharacteristicsaremaintainedacceptablecolorbeingvariablehaving amoresignificant differenceremarkabledeformationthat occurs inthe bucketsisdue tothe water outletfromthecenter of the foodtothesurfacecausingnoareasaestheticscubeshapeterminatethe process sincethis causes areductionin size.

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A Feasibility Study For The Production Of Added Value Compounds From Apple Waste: A Simulation Case

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Abstract

Current technologies and their feasibility for the production of added value compounds from apple waste are explored in this research. Different solid processing technologies, extraction processes, water removal and downstream separation alternatives are explored in order to provide the best technology from an industrial perspective. A process simulation case study of a solid-liquid extraction was developed in the software Aspen Plus and Aspen Batch process to evaluate the performance for the different process choices in aspects such as cost, sustainability, yield, and feasibility to be scaled.

Water removal and solvent extraction experiments were developed at laboratory scale in order to obtain important parameters for the simulation of the case. Response variables such as operation time and temperature were evaluated as well as total polyphenol content (TPC) and antioxidant capacity ORAC. The data was corroborated with data available in the literature. The information was used to develop different simulation scenarios and evaluate process performance.

From preliminary results it can be highlighted that water removal and solvent selection are critical activities in the development of a feasible process. Freeze-drying operations of apple waste processing can be decisive in terms of costs; as a result vacuum drying could be a more economic option. Solvent selection and optimization can be decisive in the sustainability of the process being methanol and ethylene glycol the best choices. Finally, thiswork intend to provide key data to aid into the implementation of this kind of process in the near future and been able to obtain complex components with a cost effective technology in the supplement and additives food industry.

Introduction

Process development and plant design are usually the most complicated part of an industrial scale-up production process;it requires a great and exhausting planning which consumes a lot of time and an investment. Any changes after the process has been selected and approved turns into elevated costs and time for its implementation (1).As a result process simulation becomes an important tool for analyzing scaling issues during the development stages.Software simulation is useful for the scaling-up and planning of different scenarios in a process design.

Solid-liquid extraction is a unit operation where a solute contained in a solid matrix is to be extracted by a solvent, typically a liquid substance that has a great affinity for the solute (target compound) (2). This process has a wide application in industry, from metallurgy with copper salts recovery to pharmaceutical in leaching plants and roots for isolating nutritional compounds (3).

Regarding the extraction of added value compounds such as polyphenols from apple Kammerer et al. (4) reported the recovery of polyphenols from apple juices using an acrylic adsorption resin at a pilot-plant scale.Moreover, it has been reported the phenolic profile of apple varieties, resulting flavan-3-ols ((+) catechine) and hydroxycinnamic acids (chlorogenic acid) the principal classes of phenolic compounds in pulp apples (5).There are reports in the literature that focuses on the drying step for the process of using apple peels and pomace to recover phenolic compounds and use them as additive in the food industry. Valdenegroet.al (6) compared differences in phenolic content and antioxidantactivity on three different methods of drying,

where freeze-drying results the best option. Furthermore, Reyes et. al (7) published drying kinetics for atmospheric and vacuum freeze-drying at some temperatures and freezing rate. This is one of the most important steps in the overall process.

Solvent selection can be decisive in the feasibility of the whole process (8). There has been literature regarding the phenolic content and antioxidant activity of apple pomace extracts in alcoholic solvent (9). Shen et al. (10) reported considerable results using supercritical carbon dioxide as a solvent for oil extraction in rice bran, but is turns difficult to handle supercritical solvents in industrial scale because of their needs for pressurized systems or extra solvents (11).

So far, the majority of the analysis isfocused on the capacity of a solvent for extracting a component regardless on the sustainability of the process in an industrial scale perspective. In other words, there is a need for more research involving simulation software for a scale-up process development.

Materials and methods

A simulation study case was developed in Aspen Batch Plus software (Aspen Technology, Inc.) in order to compare feasibility. The solid-liquid extraction process was considered in three different arrangements to compare the overall energy requirements and yield of production.Laboratory extraction conditions were considered in the cases; this to explore the feasibility of scaling the process with such conditions.

Freeze-drying is a critical step. Water removal in apple pomace results vital because not only reduces volume, but also prevents rancidity of stored apple. Problems have been reported for heat and mass transfer during freeze-drying scaling-up (12-14), such as variations of shelf temperatures and atypical radiation that result in variations of heat transfer rates will not be covered in this paper.The first arrangement consists of a freeze-drying step, where water is removed from apple pomace, followed by the extraction step at atmospheric pressure and 60 °C and, finally, concentratedto powder of extract in a spray dryer at90 °C (15). Figure 1 shows the possible scheme for process conditions of the first arrangement.

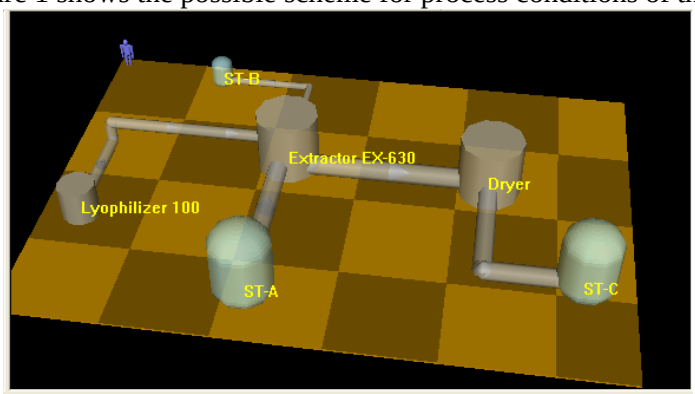

Figure 1. Schematic plant scenario for simulation case 1, in Aspen Batch Plus (Aspen Technology, Inc.)

In the second case (Figure 2), a convection drying of apple pomace is carried out, then the extraction procedure and finally, the spray drying step. Convection drying conditions were at an air velocity of 1.5 m/s and 40 °C for 7 hours (16).

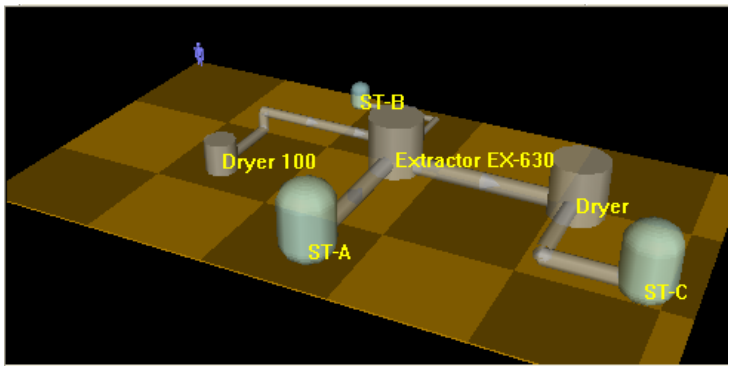

Figure 2. Plant design for process in Case 2.

Third arrangement has a freeze-drying (lyophilization) process to remove water, next by the extraction step and then, the extract is concentrated in a membrane filter (shown in Figure 3) at 4 bar of pressure, 80 °C and 50 L/m^{2}h (17). Conditions for freeze-drying process were according with Tang and Pikal (18).

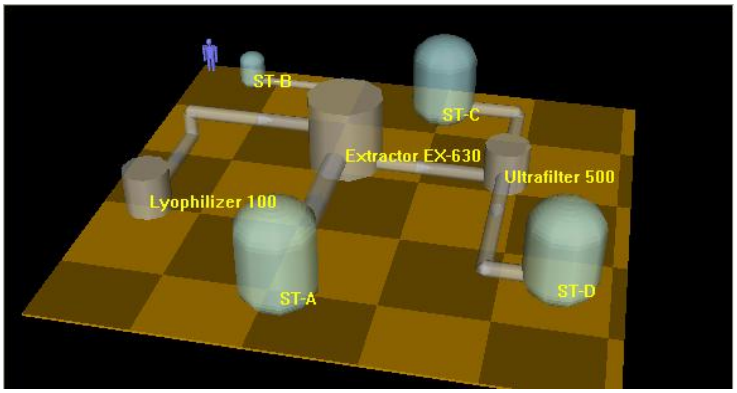

Figure 3. Possible scenario for process of Case 3.

Methanol was used as the solvent for the extraction process in all three cases. Once the simulation was carried out for all of 3 cases, yield and costs analyses were considered to compare different process designs and cost issues.

Results and discussion

Results of main parameters for an economic/yield analyses are shown in Table 1. In every three cases the yield of process (step yield), in terms of product recovery, was expected to be 80%. Considering only the economical part, third case seems to be excellent in comparison with other two cases, since it requires much less investment in equipments of plant design needed for that process scheme at given conditions. On the other hand, first and second cases turn into much more expensive processes.A more detailed analysis shows that second case takes only a fraction of time to be performed, while third case is the most time-consuming. This obviously delays process production (for being a batch process) and raises costs from energy consumption.

TABLE 1. Main results of simulation data from all three study cases.

			---------- Results ----------					
Case	**Arrangement**	**Extraction yield (%)**	**Process time (h)**	**Input materials (kg)**	**Polyphenols (kg)**	**Polyphenol rate (kg/h)**	**kWh/kg Polyphenol**	**USD/kg Polyphenol**
1	L-E-SD[a]	80	19.56	22,939	12	0.62	461.00	65.25
2	CD-E-SD[b]	80	11.56	25,011	12	1.60	449.52	63.63
3	L-E-MC[c]	80	57.09	22,925	12	0.29	239.15	33.85

[a]Lyophilization – Extraction – Spray Drying
[b] Convective Drying – Extraction – Spray Drying

[c] Lyophilization – Extraction – Membrane Concentration

Another thing to take in count is that, in first and second cases, product ends up in a powder phase, which is easier to transport and store. In third case, otherwise, the final product is a concentrated solution.Definitively, simulation is an excellent and useful tool in Chemical Engineering for scaling-up different processes at an industrial level. It helps to test different arrangements for an industrial process, changing and varying whichever equipment and/or unit operation, handling different flows and operational conditions, with the purpose of select the best choice in order to obtain the maximum yield of the entire process. All of this, considering a good balance with economical estimates.Finally, it is highly recommended to feed the simulation software with experimental data, since it can give results numerically correct but unrealistic. For this reason, untested conditions in the process may lead to problems in yields or dangerous conditions on the industrial plant.

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Water Adsorption Isotherms Of Tascalate, A Powder To Make A Traditional Beverage Of Chiapas, México

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Introduction

Hispanic food nowadays is rooted in the sixteenth century, the invasion of European foods and crops that followed the arrival of the Spanish helped to change the diets of the New World forever (1). In Mexico introduced products that had more impact on the diet were wheat, sugar and some spices like cinnamon, conversely, we can mention: corn, chili, tomato and cocoa.

By the sixth and seventh centuries cocoa was regularly cultivated on the coast of Tabasco, Campeche, Belize and the Gulf of Honduras and their beans were used as a way of trade (1). Cocoa was also one of the most precious foods by Mesoamerican peoples and was consumed in many different ways. Among the Aztecs were also used the cacao with corn, forming a powder called cacahuapinolli. The native population of New Spain produced their own beverages integrating European elements. Tejate,choroteo pozol and tascalate, from Oaxaca Tabasco and Chiapas, are some related beverages with cocoa that emerged among the peoples of the colonial era (2).

Because Tascalate is a powder that is used to prepare a traditional handmade drink, the formulation is not strictly defined, varying from place to place within the Chiapas state, however, the basis of the formulation are corn (60.6 %), cocoa (3%), sugar (36%), cinnamon (0.1%) and achiote (0.3%) (3). Thetable 1 shows the proximate composition of the main constituents of tascalate.

Table 1 Proximal composition of corn, cocoa and Tascalate (g/100g of sample)

Component	Corn	Cocoa	Tascalate*
Moisture	9.1	10.1	9.01
CrudeProtein	8.5	16.0	8.73
Fat	4.8	49.5	6.84
CrudeFiber	5.4	2.1	5.16
Ash	2.6	1.3	2.50
N.F.E.	69.6	21.1	66.27

Source: Tables from INN, 1985
* Calculatedaccording the reported composition, without considering sugar, because sample lacked of it

Adsorption isotherms

A parameter to get a measure of higher or lower water availability of the foods, hence its availability to participate in biochemical reactions and microbial development is the water activity (aw), so it is used as an indicator to predict the useful life of a food. (4)

An adsorption or desorptionisotherm, represent the relationship between the total moisture content and the water activity of any material at a given temperature (5).The utility of the adsorption isotherms, can be summarized: a) allow us to anticipate product hygroscopicity. b) it can anticipate the rehumidification to a water content corresponding to Mo, c) calculate the amount of water adsorbed in function of storage time and the food duration in an acceptable quality level (6 and 7).

The objective of the present project was to analyze behavior of water adsorption of Tascalate, using adsorption isotherm, to know the monolayer moisture values and other thermodynamic parameters for water adsorption data.

Methods and materials

The sample used was Tascalate handcrafted of Chiapas de Corzo, Chiapas, without sugar. The sample was dried in a vacuum oven at 60 ° C for 12 h. Potassium sorbate was added to prevent microbial contamination at higher aw.

The samples were placedin closed containersatconstantrelative humiditywithout air circulation, containing saturated saltswith an aw of 0.11- 0.9, preparing these bottles previously in chambers with temperature control (5, 25 and 35 ° C), reaching a steady state.Weight was recorded from the trays until a constant weight was obtained over 12 days. To determine the value of themono-layer moisturethe B.E.T. model was used.Moisture values were adjusted to mathematical models of Isocom computer program.

It was determined the heat of sorption (Qs), the Gibbs free energy, enthalpy and Sorption Entropy of samples interpolating on the calculated isotherms, the corresponding aw of the 2.5 to 20 g of water/100 g dry solid at intervals of 2.5, using the following equations:

The sorption heats from the Clausius-Clapeyronecuation:

Ec. 1 $$\frac{Qs}{R} = \frac{Lnaw_1 - Lnaw_2}{\frac{1}{T_1} - \frac{1}{T_2}}$$

Sorption Enthalpy: Ec.2 $$\Delta H = \Delta Hv + Qs$$

ΔHvis the water vaporization heat.

Gibbs free energy: Ec.3 $$\Delta G = RTLnaw$$

Sorption Entropy: Ec.4 $$\Delta S = \frac{\Delta H - \Delta G}{T}$$

Results and discussion

Adsorption isotherms.

The water adsorption isotherms of Tascalate are shown in Figure 1, This behaviour is manifested in the form of a sigmoidal shaped curve, thus reflecting a Type II isotherm , similar to that presented by corn.(7)

In the moisture range of 0 -10%, the influence of the presence of cocoa in Tascalate was also observed, because the cocoa sorption behavior shown an intersection of the curves at different temperatures(7, 8), but for the rest of the curve, the corn behavior was followed.The Tascalate´s water adsorption values were fit the Oswin model, at three studied temperatures, providing the parameters shown in table 2.

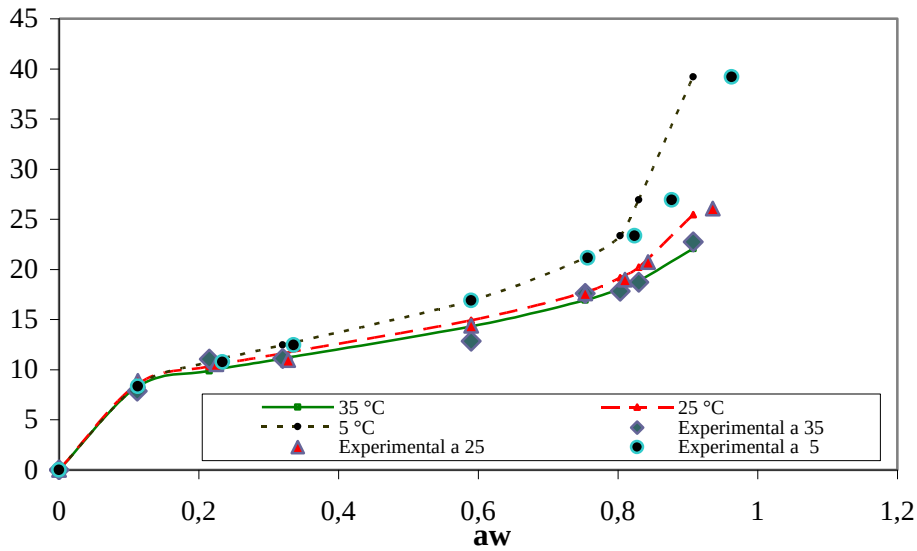

Figure 1. Tascalate Adsoprtion Isotherms at diferent temperatures, experimental data and adjusted by the Oswin model

Table 2.Oswin model parameters for adsorption isotherms of Tascalate

Parameter	**35 °C**	**25 °C**	**5 °C**
K_1	2.5789	2.6180	2.7208
N	0.2253	0.2308	0.2915
% error	4.5240	2.8280	3.5770

Monolayer moisture determination

In the aw range of 0.11 to 0.5 the BET model was used(Table 3), which showed that for Tascalate values (6.6-7.7), it was similar with those reported for corn (6.42-8.22), this could be explained because that it is the major component in the formulation of Tascalate (7).

Table 3.Parameters from B.E.T. model for adsorption in Tascalate

Parameter	35 °C	25 °C	5 °C
C	32.93	78.46	649.35
Slope	0.156	0.143	0.130
R^2	0.9899	0.9915	0.9994
Monolayer (Mo)	6.60	7.08	7.70
Corn's Monolayer[7]	6.47	7.07	8.27

The constant Cwas also calculated, Chavez and Pitombo(9) explained that when having higher value than 10 the isotherm corresponds to II type and in case of being less than 10 corresponds to a curve of II type. As can be seen in Table 3, in the three temperatures the value of constants was above to 10,it which confirms the sigmoidal behavior type corn (7).

Thermodynamic parameters

The adsorption heat values are presented in Table 4. The adsorption heat of Tascalateshowed an increase when moisture content increase, having its maximum at 10% of moisture, where the moisture monolayer value is proximately located, then reduce with increasing of moisture. This is one of the effects of temperature on the adsorption heat(4).

Table 4. Thermodynamic Parameters of water adsorption of Tascalate

Moisture (%)	aw			M[3]	- Qs[1,3]	- ΔH[1,3]	- ΔG[1,2,3]	ΔS[1,2]
	5 ºC	25 ºC	35 ºC					
5	0.04	0.05	0.06	-0.82	1.64	12.13	1.72	45.50
7.5	0.08	0.09	0.11	-0.88	1.74	12.23	1.39	45.71
10	0.14	0.17	0.21	-1.11	2.21	12.70	1.05	46.11
12.5	0.33	0.38	0.45	-0.80	1.59	12.08	0.57	42.41
15	0.48	0.59	0.63	-0.79	1.57	12.06	0.31	41.50
17.5	0.64	0.77	0.80	-0.68	1.35	11.84	0.15	40.21
20	0.73	0.84	0.87	-0.53	1.06	11.55	0.10	39.07

[1]cal/mol [2] A 25 ºC[3] x10 [3]

About Entropy, this presents a similar behavior with the parameters before mentioned, showing its maximum value in the range of humidity monolayer, because in the monolayer exists the thermodynamic balance point. Furthermore, the free energy of the system, showed a decreasing with respect to moisture, this is due to a low humidity material is more trend to adsorb water, because ΔG is higher and on the other side when the material becomes saturated, adsorption is more difficult.

As regards the classification with the Labuza's diagram, theTascalate has a moisture content of 6.46%, with this moisture value and interpolating in the adsorption isotherms, the aw value of the product, was located at these conditions and is determined it corresponds to the Zone I. So we can affirm that it is highly stable in its native state.

Conclusions

The water adsorption isotherms of Tascalate correspond to the Type II isotherm. The behavior of Isotherms with respect to temperature is generally determined by the major component in the formulation, corn; however,in the zonewith less than 10% humidity there is a marked influence of the other component next in proportion, cocoa.

The monolayer value obtained by the B.E.T. model is determined by the content of corn in the Tascalate formulation.

In monolayer value Qs, ΔH and ΔS, presents its maximum negative value, while the ΔG decreases when humidity increases, indicating Tascalate stability in the monolayer value.

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Rheological And Textural Propertiesof Native And Carboxymethylated Flamboyant(*Delonixregia*) Seed Gums

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Introduction

Among thegums that are usedinthefood, pharmaceutical, textile and paper industriesit is include thegalactomannans,thesegumsarefrom endospermof differentplant speciesandare widely usedas thickeners, binders andstabilizers(1; 2).

The galactomannan-type structure consists of alinear chainwith D-mannopyranose(β-1, 4 linkages) andramifications(α, 1-6) withD-galactose units(2). These gums present functional primary groups (OH) but no secondary,neither electrical charge(4).Chemical modifications providegreater versatilityin usingthe gums due to the modificationoftheir physicochemical properties, which will bedetermined primarily bythe degree of substitutionand distributionof the substituents(3,5).

Therheological studiescontribute to our knowledgeof the molecular structureormoleculardistributionoffood componentsand topredictstructuralchangesin the food duringthemanufacturing process.Furthermore,rheological characterizationis usedas aqualitycontrol tool, shelf life andalsoto designtheprocessing equipment(6).

The textureof a food isoneof the most importantcharacteristics, used todeterminethequalityofa productbytheconsumer(7), therefore thecharacterizationofgelsallow to knowthefeasibilityofuse of proteins andpolysaccharidesin foodstuffsduethese ingredientstoimpartpropertiesoftexture, structure andstability, etc.(8).

It isdesirable fromthe standpoint oftechnologicaland economic development,findingalternative sources ofregionalor indigenouscharacterthat could havepotentially goodfunctionaland nutritionalattributes. For thecaseofthegum obtainedfrom the flamboyantseeds, it is necessary todeterminetheir physicochemical properties, such as dispersionandrheological behavior and texturalproperties of gels.

Therefore the objective of this study was to evaluate the change of rheological behavior and textural properties of native gum of flamboyant (*Delonixregia*) (FNG) when it was carboxymethylated (CFG).

Methods and materials

Flamboyant gum extractionand carboxymethylation.

Flamboyant tree (*Delonixregia*) seeds were collected randomly from four zones in the city of Merida, Yucatan, Mexico. The extraction of the flamboyantgum was done hydrating theseedfor 12h at 70°Cunder

constant stirring(4).The FNG was modified by carboxymethylation (CFG) usingCAS10%at 70°Cfor 90min(8).The degree of substitution (DS) of the CFG wasdetermined by titration (9)

Compression strength of gel.

The samples were dispersed (4 y 6 g/100mL) in a cylindrical mold of 40 × 40mm and were heated at 80 ° C for 1 h, under constant agitation. Cooled in a water bath at room temperature for 15 minutes and then cooled to 4 ° C for 24h. The resulting gels were compressed in a Universal Testing Machine (Instron Model 4411, Canton, MA,USA) at 5 kg compression force and a rate of 5 mm/min. (5). The results are the mean of at least three reproducible runs.

Rheological behavior.

Dispersions of the two kinds of gums at 4 g/100mL of concentration and two pH values (3 and 9) were prepared and flow properties at three temperatures (5, 25 and 45 ° C) were determined using a Rheometer AR -2000.The results are the mean of at least three reproducible runs. The curves were fitted to the model of the power law: $\sigma = k\,\gamma^n$

Statistical analysis.

The rheological properties were evaluated using a 2X3 bifactorialdesignin blocks, the pH (3 and 9) and temperature (5, 25 and 45) were factors, and texture was evaluated using a 2X2 bifactorialdesign in blocks whit factors: pH (3 and 9) and solute concentration (4 and 6 g/100mL). In both cases the blocks were CFG and FNG, and ananalysis of variance was used.

Results and discussion

Flamboyant gum extractionand carboxymethylation.

Thegum obtainedshowedprotein and fibercontentof2.25 and1.87% respectively,similar to those reportedby other authors(8). The value obtained forELN(95.12%) was similarto that reported byothers (5), with a value of95.31%. Theextraction yieldofnativegumflamboyantobtained fromendospermflourwas 31%, it was similar to reported byothers (5, 8). The native gums yield respect to integralflamboyantseedwas 10%.

Withthecarboxymethylationprocessused, 0.47 ofdegree of substitution (DS)was obtained, which is within the rangeachievedby previous works(5,8)who reportedvaluesof0.33and0.63 of DS.

Gel strength.

Forthe CFG,inall cases studiedat the lowest concentration (4 g/ 100 mL), weakgelswere obtained and didn’t show aclear break in their structure, without anyphase separationobserved.

The gels formed with native gums flamboyant (FNG) were compared with the ones formed with modifiedgums (CFG), at both concentrations (4 and 6 g/ 100mL) and two values of pH 3 and 9, as seen in Figure 1. The highergel strength valueswere obtained with the native gum atpH 3 withboth of the studiedconcentrations.Theanalysisof varianceshowed thatall variables studiedwere significant ($p < 0.05$), including doubleinteractions, butexcludingpH-solids concentration interaction.

This could be explained by an increase in the solubility of the modifiedgum (CFG),in comparison to the native one (FNG), due to the carboxyl group (R-COO-)present in the CFG; then the negatively charged gums exhibit their repulsion between ionic groups, bearing molecules relatively stretched and forming more effective hydrogen bonds with water molecules (9, 10).

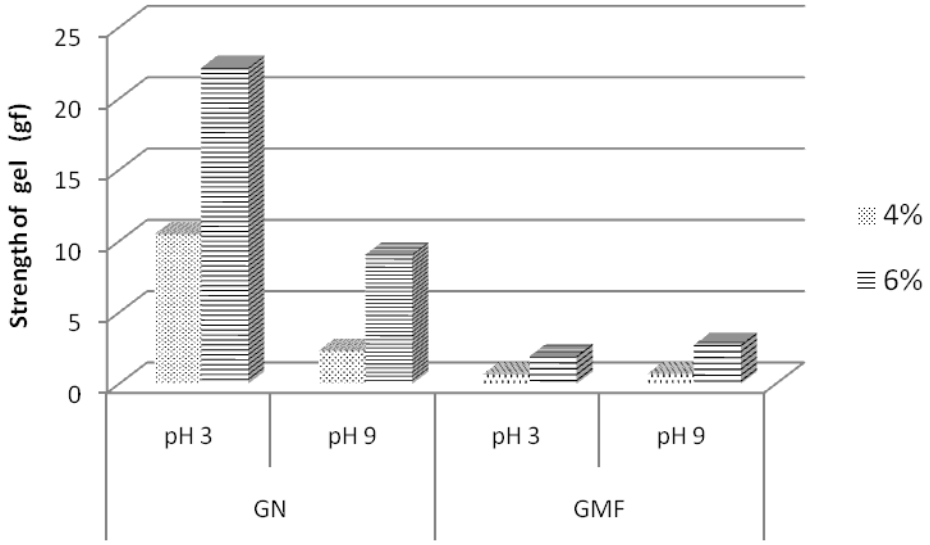


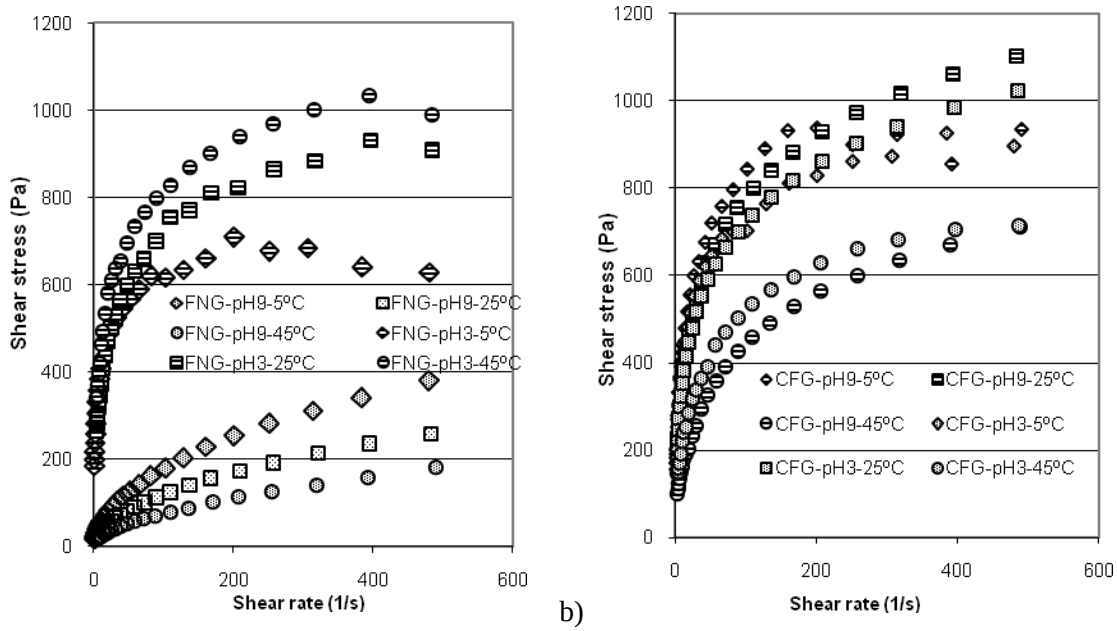
Figure 1.Gelstrengthofthe gels obtainedfrom nativeandmodifiedflamboyant gums.

Rheological behavior

The influenceof temperatureandpHonthe rheological behaviorof carboxymethylated andnativegum's dispersionswere evaluated, finding that bothgumsat different temperaturesandpHvaluesfit the model of Power Law, withvaluesR^2in a range from0.95 to 0.99. The flow curvesobtained are showninFigure 2.

Theflowbehavior index values(n) ranged between0.2and0.5forall conditions studiedinthe two typesflamboyantgums asshown inFigure 3,indicating afluidizingorpseudoplasticbehavior. For consistency indexvalues(k),a greaterdispersion of datawasfound because theyrangedfrom 16 to240,where the lowest valueswerepresented bynativegumat pH9 inthethreetemperatures studied. In the other hand, it was found thatthevalues werealsohigherforthenativegumatpH 3.

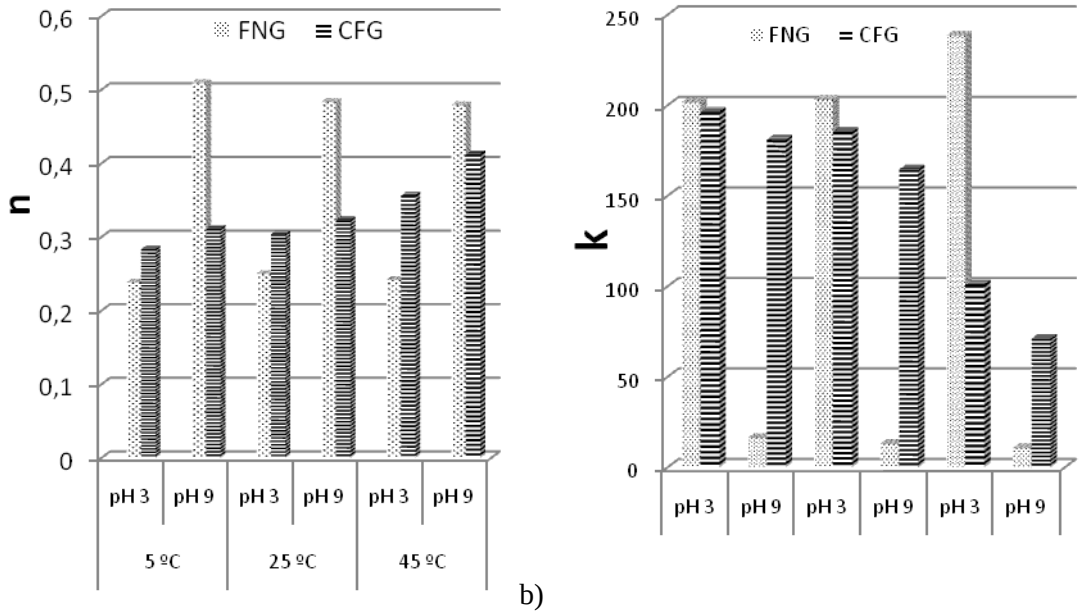
These consistency indexvalues(k =16-240Pa.s^n) are higher than those reportedby other studies(6) to4 g/100mLdispersionsof mucilageextracted fromAlyssumhomolocarpumseed(k =7-11Pa.s^n),anddifferentbehaviorwithpH values,decreasingthese **k**valueswith increasingpH values.This could be explainedbytheincreased solubilityinneutral and alkaline systems ofcarboxymethylatedgums (9,10).



a) b)

Figure 2. Flow Curves for dispersions of gum at 4g/100mL a) native b) carboxymethylated at different pH and temperature values.

The analysisof variance forkvaluesshowed thatthesignificantvariables($p < 0.05$)werethepHand temperature, andpH-type gumandtemperaturetype-gum interactions.Whilefor valuestheflowbehavior index(n)the only variablethat resulting significant ($p<0.05$) was the pH.



a) b)

Figure 3. Values of a) the flow behavior index (n) and b) the consistency index (k) for dispersions at 4% of native and carboxymethylated flamboyant gums at different pH and temperature values.

Conclusions

Under the measurements conditions, the rheological behavior ofboth gums types showed that theflowbehaviorindexvaluewas increasedandtheindex consistence was decreasedas thetemperature and pH values were increased.In the other hand, the FNG had a gelling property higher than the CFG. These changes in functionalproperties of the CFG could increase its versatility in food industry, because it could be used in fluid products with suspended solids.

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Shelf Life Estimation Of Extruded Snacks Expanded By Frying And By Using Hot Air Stored In Polypropylene And Metallized Propylene Packaging

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Introducción

In Mexico, the snacks are widely consumed and they are considered convenience products for its accessibility, high calorie content and because mitigate hunger momentarily [1]. The term snacks covers a wide range of products: expanded (popcorn), fried (chip potatoes, pork rinds and flour based snacks), extruded (pellets of flour and corn flakes), seeds or roasted grains, baked goods (cookies), coated (peanuts), candies, dried fruits, etc.[2].

On the other hand, shelf life of a food product is defined as the period of time within which its sensory, chemical, physical, microbiological, nutritional, functional and safety properties remain acceptable under expected or specified conditions of distribution, storage and display. This definition involves several intrinsic and extrinsic variables from the product [3]. Determination of shelf life is a need since it helps to reduce risks, diminishes product loss and provides assurance that the product remains consistent over time with respect to quality [4].

Because of their low moisture content and high fat content there are two main deterioration mechanisms of packaged snacks: crunchiness loss and oxidative rancidity [5]. The aim of this work was to determine the influence of two different polypropylene packaging on the shelf life of extruded snacks expanded by two methods: frying and expansion using hot air. For this, physicochemical and microbiological quality variables were monitored.

Methods and materials

Extruded snacks and packaging materials were provided by a snack manufacturer company from Hidalgo, Mexico.

1.1 Moisture content and ethereal extract determination. Moisture content and ethereal extract determination of snacks was carried out according to the methods established by AOAC [6].

2.2 Microbiological quality: Microbiological analysis was performed according to techniques established in Mexican regulations at the beginning and end of stability tests [7, 8, 9 y 10].

2.3 Shelf life study: Two different snacks were studied: one produced by frying and the other expanded by using hot air, considering the influence of two different packaging: Polypropylene (A) and metalized polypropylene (B). The study was carried out under 3 different temperature conditions (25°C, 35°C and 45°C), for 60 days. The following quality variables (Q) were assayed:

MOISTURE ABSORPTION: the gain of weight of the samples stored in two types of packaging was monitored weekly under 2 different study conditions: 25°C, 100% relative humidity (RH) and 35°C, 75% RH. For further calculations, it was required to determine the initial product moisture, the critical moisture (when the snack loses crispness) and moisture balance under the study conditions.

ACIDITY INDEX (AI): We proceeded according to NMX-Y-302-SCFI-2004 [11]. Results are reported as percentage of free fatty acids determined as oleic. The AI was measured every 7 days in snacks stored at 35 to 45°C and every 15 days for snacks stored at 25°C.

PEROXIDE INDEX (PI): For this determination some adaptations of the methods reported by Cuppett et al. [12] and Homer et al. [13] were made. Peroxide was extracted from each sample with a mixture of HAc-CH3Cl (3:2), the peroxide present in the sample oxidizes Fe (II) to Fe (III), Fe (II) remaining forms an orange complex when adding a solution of 2% o-phenanthroline, which was determined by spectrophotometry at a wavelength of 512nm. The Fe (III) determined is proportional to the amount of peroxides in the sample. The results were reported in meq peroxide /kg sample. The periodicity of the analysis was the same as in the AI.

Results and discusion

3.1 Initial moisture and ethereal extract of snacks

Table 1 shows the results of the determination of initial moisture and ethereal extract of the snacks studied. The mean and standard deviation of three determinations are reported.

Table 1. Initial moisture and ethereal extract of the snacks studied

Snack	*% Moisture*	*% Ethereal extract*
Fried	3.18 ± 0.063	27.11 ± 0.234
Expanded with hot air	4.60 ± 0.049	4.76 ± 0.097

The extruded snacks presented low moisture contents. According Colonna et al. [14] the value of moisture for fried products is 3%. Moreover, Arias et al. [15] reported values between 3.8-5.1% in snacks expanded by frying. These values are similar to those obtained in this experiment, being lower in the fried snack sample because a greater loss of water is produced by volatilization during the frying process compared to the hot air expansion [16]. Moreover, the smallest value in ethereal extract content was presented in the hot air expanded snack. This difference is due to that the fat content of the food is not affected by this method, contrary to the case of fried snacks where the fat content comes to be as high as 30% [14].

3.2 Microbiological quality of snacks

None of the samples studied presented microbial growth due to the nature of the product. It is mainly attributed to the low moisture content.

3.3 Estimation of the shelf life of the extruded snacks

Calculations to estimate the shelf life of the snacks stored under the study conditions were performed using the results of gain of weight due to moisture increasing, taking into account that for a product whose shelf life is related with the moisture increasing (i.e. crispness loss), the end of this shelf life occurs when the initial moisture content (im) reaches a critical moisture condition (mc). This period can be calculated using the equation EC.1 as a function of EC.2 equation, where the food moisture balance (m_e) also should be considered [17, 18].

$$\Theta_c = \frac{Ln(G_c)}{\frac{k}{x}\frac{A}{W_s}\frac{P_o}{b}} \qquad \text{EC.1}$$

Where:

$$Ln(G_c) = \ln\left[\frac{m_e - m_i}{m_e - m_c}\right] \qquad \text{EC.2}$$

Figure 1 was made with data obtained from the EC.2 equation, for each weight value (expressed in grams of water/100g of solids) obtained during the study period, and once the critical value (Gc) was calculated. EC.1 equation allows the estimation of the shelf life period and its denominator is a theoretical value that

is obtained experimentally through the slope of Figure 1. It should be noted that a single plot was obtained for each condition of the study but all them followed a behavior that follows the format of Figure 1.

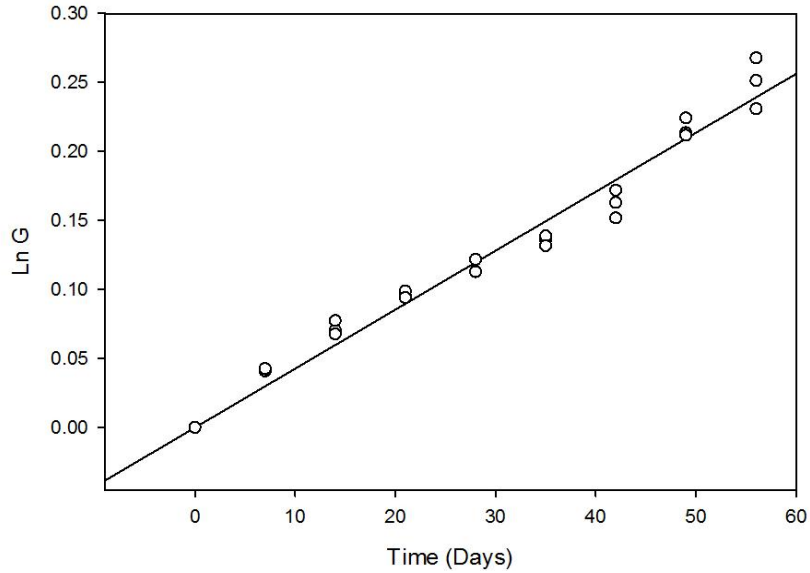

Figure 1. Moisture gain (G) for snacks stored in polyethylene at 35°C and 75 % RH; Gc is the location of critical moisture content for loss of crispness.

Table 2 presents the estimated shelf life periods of the snacks analyzed under the different study conditions. It is noted that the packaging B has lower permeability to water vapor and therefore a greater conservation.

Table 2. Estimation of snacks shelf life due to moisture gain**.**

Conditions	***Snack***	***Packaging***	***Shelf life (months)***
25 °C-100 %HR	Fried	A	1.056742586
		B	6.277610593
	Expanded by hot air	A	1.404153708
		B	6.167032205
35 °C-75 %HR	Fried	A	1.279181637
		B	3.844965671
	Expanded by hot air	A	1.069486793
		B	6.935194892

After the storage period of the samples at 25, 35 and 45°C, AI remained constant and PI showed an increase with respect to time, for this reason this variable was set as the variable quality (Q) for estimating the shelf life of the products. In all cases a best linear fit for a zero order reaction was obtained, which is the typical behavior of lipid oxidation reactions [19]. All the conditions of the study were adjusted to EC.3 equation. Figure 2 shows the behavior of snacks for all study conditions for PI assessment.

Packaging A $$[Q] = [Q]_0 \pm kt$$ EC.3

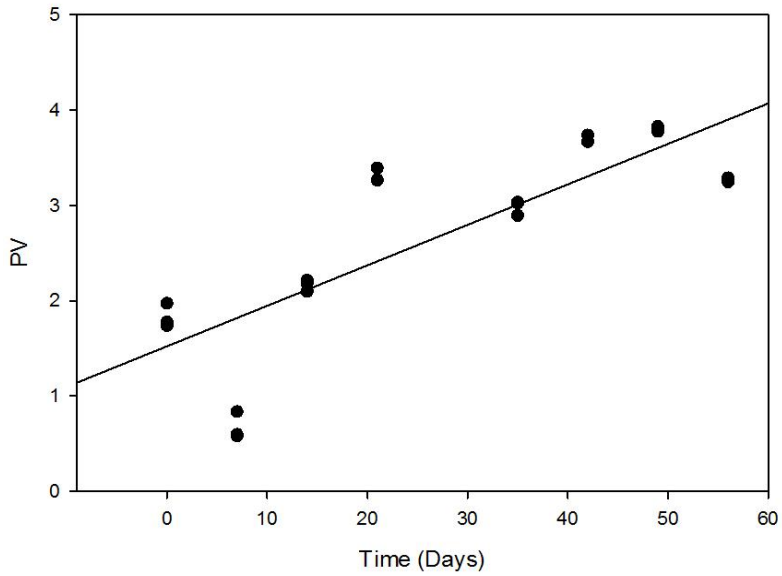

Figure 2. Peroxide index as a function of time under study conditions

All kinetic data obtained in this study are reported in Tables 3 and 4. It can be observed that as the temperature increases the reaction constant acquires a higher value in all cases studied. The reaction rate constant is function of the temperature and is described by the Arrhenius equation [20].
The activation energies calculated for each study situation are below the range reported for fat oxidation reactions, which fluctuated from 41,842 to 104,605 J/mol [19].

Table 3. Kinetic parameters and shelf life estimation of fried snacks.

Packaging	$k_{25\,°C}$ *(meq peroxide/kg. day)*	$k_{35\,°C}$ *(meq peroxide/kg.day)*	$k_{45\,°C}$ *(meq peroxide/kg.día)*	*Ea (KJ/mol)*	Q_{10}	$\Theta_{25\,°C}$ *(months)*	$\Theta_{35\,°C}$ *(months)*	$\Theta_{45\,°C}$ *(months)*
A	0.0294	0.0565	0.0618	29.5690	1.43	9.04	6.30	4.38
B	0.0301	0.0445	0.0541	23.2041	1.34	9.32	6.94	5.17

Table 4. Kinetic parameters and shelf life estimation of snacks expanded by using hot air.

Packaging	$k_{25\,°C}$ *(meq peroxide/kg. day)*	$k_{35\,°C}$ *(meq peroxide/kg. day)*	$k_{45\,°C}$ *(meq peroxide/kg. day)*	*Ea (KJ/mol)*	Q_{10}	$\Theta_{25\,°C}$ *(months)*	$\Theta_{35\,°C}$ *(months)*	$\Theta_{45\,°C}$ *(months)*
A	0.0182	0.0447	0.0447	35.7534	1.50	12.33	8.21	5.48
B	0.0201	0.0437	0.0444	31.6011	1.42	11.63	8.16	5.73

The acceleration factor, Q10 represents a practical and reliable way to predict the effect of temperature variations during food storage [21]. Snacks are dry products (with low moisture content) that exhibit low Q10 values, near 1.5 which is the minimum for dehydrated products [22]. By comparing the values of both packaging, it can be observed that packaging B presented the lower Q10 values which indicate that it has better barrier properties to prevent food deterioration by lipids oxidation.

With experimental data obtained, empirical models for estimating the shelf life of the snacks were constructed according to the temperature measured in °C.

For fried snack:

Packaging A $$\phi(\text{Shelf life}) = 10^{2.828 - 0.016(T°C)}$$ EC.4

Packaging B $$\phi(\text{Shelf life}) = 10^{2.766 - 0.013(T°C)}$$ EC.5

For snack expanded by hot air:

Packaging A $$\phi(\text{Shelf life}) = 10^{3.008 - 0.018(T°C)}$$ EC.6

Packaging B $$\phi(\text{Shelf life}) = 10^{2.927 - 0.015(T°C)}$$ EC.7

Conclusion

Un estudio de estabilidad por prueba acelerada es una herramienta eficiente para realizar una predicción de la vida de anaquel de un producto mediante la utilización de modelos matemáticos.
The nature and chemical composition of food, packaging type as well as storage conditions determine the conservation of physicochemical and sensory properties of the product and hence its shelf life. An accelerated stability study is an efficient tool for shelf life prediction of a product by the use of mathematical models.

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Effect Of Incorporating Potato Flour To Wheat Flour On The Quality Characteristics Of Cookies

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Abstract
Cooking was prepared using different levels of substitution of wheat flour and potato flour (0-20%). The potato flour was obtained previously in the laboratory from white potato variety alpha out of standard. The effect of the flour substitution was determined, on the chemical rheological and sensorial properties. The different concentrations of potato flour added in replacement of wheat flour increased the absorption of water dough, the mixing time, and stability. Also when substitution level rise the tenacity increasing and decreased the extensibility of the dough. These changes have been attributed to the decrease of the gluten in wheat potato flours mixture. The potato flour incorporation present advantages in the determine hardness. Spread factor, puncture force and fracture strength of the cookies were also determined. Potato flour lowered the spread factor from 6.6 to 6.1 with increasing its level from 0-20%. The 15% of potato flour substitution impart best characteristics of texture and sensory properties of cookies. Sensory evaluation revealed that increasing levels of potato flour (>20%) lowered the overall acceptability because of taste and distinct flavor developed during baking.
Keywords: potato flour, cookies dough, rheological characteristics, texture.

Introduction
Potatoes are one of the most popular foods potato is one of the world's important food crops. It is a low input crop and is used as vegetable, a desert, a source of starch and animal feed. Potato with items consumed because of their negligible fat content, high dietary fiber, high vitamins, a high yield, relatively low cost of production and good amount of minerals and 6-12 percent protein content adaptability to a wide variety of soil and climate types. In recent years, bakery products have been produced using composite flours. These flours are advantageous to developing countries because wheat imports can be reduced and alleviate the use of locally grown grains. Indeed, research studies have been conducted with the intention of promoting the use of composite flours, in which flour from locally grown crops and high protein seeds replace a portion of wheat flour for use in bread, thereby decreasing the demand for imported wheat and producing protein enriched bread. Potatoes flour that can be used as functional ingredients in several processed foods. Dehydrated form processed from potatoes is the main ingredient that provides the functionality required in these processed products. For the food processing industry, the unavailability of dehydrated form for diverse functionalities is a limiting factor in the utilization of potatoes in processed foods. Several excellent reviews dealing with processing and quality aspects of potato purees, flakes and powders have been published over the past 20 years. Potato flour and wheat flour blends are frequently used in the food industry. Farinograph is an empirical instrument, commonly used to evaluate the mechanical of wheat flour dough. Potato flour prepared by the cabin dried method produce good quality flour and can be used as partial replacements for wheat flour in preparation of various bakery products. Consequently, the objective of this work was to produce cookies from composite flour of wheat-potato and to evaluate the sensory qualities to determine the acceptability of the product.

Materials and methods
Sweet potatoes of variety alpha were procured locally. Roots were washed, trimmed and cured to make them free from soil and other foreign materials, rotting, insect damage. Wheat flour, sugar and shortening

were procured from the local market. The wheat flour used had protein and ash content of 8.2 and 0.50%, respectively.

Flour preparation**.-**Uniform sized potatoes having no signs of infection or infestation were thoroughly washed in running tap water to remove any adhering soil, dirt and dust. This was followed with peeling. After peeling the tuber were slices to facilitate milling and drying operation. The slices tubers were immersed into 1% sodium metabisulphite solution for 30 min in order to inactive enzymes that may cause browning reaction. These were then milling and drained. After draining the mash were spread out uniformly on a stainless steel tray and dried in cabinet dryer at 70°C for 7 hours. Following drying the flour were passed through 80 mesh sieve to obtain flour of uniform size, sieved into fine flour and packaged for further use

Cookies preparation.-Cookie dough was prepared according to the following formula: 100 g flour (contain different proportion of potato flour and wheat flour, 10-90, 15-85 and 80-20, sugar 50 g, shortening 50g, baking powder 0.5g, and various proportion of water to make required consistency of dough. Cookie dough was made in a laboratory mixer (Hobart mixer, Model N50, Canada). Fat and sugar was creamed in a mixer with a flat beater for 2 min at slow speed. Dough water containing the baking powder was added to the resulting cream, and mixed for 5 min at high speed to obtain a homogeneous mixture. Finally, flour containing various proportion of potato flour, was mixed for 3 min at medium speed. The dough was rested for 30 min before cookies was made. The dough was then sheeted to a thickness of 4.5 mm with the help of a rolling pin and an aluminum frame of standard height. The cookies were cut with a cookies die of diameter 55 mm and transferred to a lightly greased baking tray. The cookies were baked at 205°C for 12 min in a baking oven. The baked cookies were cooled and stored in air tight container for12 h before further analysis

Analysis of potato flour**.-** Proximate analysis was analyzed according to AACC (1983) methods. The rheological characteristics of dough prepared from control wheat flour and mixtures of wheat flour and potato flour were determined by Brabender farinograph model 820603 (Brabender OHG, Duisberg, Germany) according to AACC methods (1883). Spread factor: The AACC (1983) was used to determine cookies width, thickness, and spread factor. Cookie width (W) was measured by placing 6 cookies edge-to-edge to get the average width in millimeters. Density was determined by standard seed displacement method and result was expressed as g/cc (AACC 1983). The cookies prepared by incorporating potato flour were evaluated for their sensory characteristics: appearance, color, flavor, texture, taste and overall acceptability, by a panel consisting of fifteen judges. The 9 point Hedonic scale score-card method was used to determine the sensory characteristics of the cookies made. The scoring scale was: 1 (Dislike extremely), 2 (Dislike very much), 3 (Dislike moderately), 4 (Dislike slightly), 5 (Neither dislike nor acceptable), 6 (Slightly acceptable, 7 (Moderately acceptable), 8 (Highly acceptable) and 9 (Extremely acceptable). All samples (prepared from 0-20% potato flour incorporation) were given different code letters. All measurements were performed in triplicate for each sample. Data were analyzed using statistical software (SPSS for Windows Version 12.0). Significant differences between the means were estimated using Duncan's multiple range tests considered significant at $p < 0.05$.

Results and Discussion

The moisture content of potato flour was 8.7% potato flour had very low protein content (2.3%) and high fiber content (9.4%). This high fiber increases the utility of potato flour in various food products. The starch content in potato flour was 74.5%. Ulm (1988) mentioned that the total carbohydrates of the potato in roots are comprised of approximately 80% starch and 20% simple sugars. The proximal analysis of cookies (crude protein, fat carbohydrate and ash) with the highest acceptable level of potato flour (15 percent) was similar to the wheat flour (control). Wheat flour cookie and cookie with15% potato flour incorporated; %carbohydrate 64- 63.9; % crude protein 6.6 -6.1; %fat 25.9-25.8 and ash 1.4-1.7. The table 1 shows the weight, diameter; bulk density and spread factor of cookies were decreased significantly with the increasing level of potato flour replacement up to 20%.The thickness increased slightly as substitution level of potato flour increased. On the other hand diameter of cookies and spread ratio are decreased as substitution level of potato flour increased in the baked samples and this may be due to the higher water

holding capacity of potato flour. The spread was greater 6.6 for cookies made from control, and decreased significantly with increasing the proportion of potato flour. The density of the cookies, decrease gradually from 0.587 to 0.540g/cc and the decrease was significant. However, the fracture strength of cookies increased with addition of potato flour up to 30% and further decreased with increase in potato flour. This may be due to the higher fiber content in the flour. This indicated that the cookies became soft with increased potato flour content.

Table I Physical characteristic of cookies

Level of potato flour (%)	*Weight (g)*	*density (g/cm3)*	*Volume (ml)*	*Thickness (mm)*	*Diameter (mm)*	*Spread factor*
0	10.26b	0.643c	15.95a	6.5a	41.2b	6.6c
10	10.21b	0.635b	16.07b	6.6.a	41.0b	6.21b
15	10.14a	0.629b	16.12b	6.6a	40.7a	6.16b
20	10.09a	0.621a	16.24c	6.8b	40.5a	5.95a

All values are average of triplicates. The values denoted by different letters in the same column are significantly different ($p<0.05$)

The dough characteristics of mixtures of wheat flour and potato flour were investigated using a Brabender farinograph. The water absorption of control wheat dough was higher than that for all mixture dough and decreased significantly with increases in potato starch. The peak time of wheat flour-potato, increase. Dough stability significantly increased with increase of potato flour. The arrival time measures the water absorption of flour; thus, arrival times tended to decrease slightly with decrease of protein content in the mixtures. Dough stability significantly increased with increase of potato flour in the mixture. The increase of potato flour in the mixtures decreased the tolerance of the dough, indicating the weakness of the flour as the protein of wheat flour was diluted. Breakdown time tended to be increased at 20% potato flour in the mixtures. The increase of potato flour in the mixtures decreased the tolerances of mixing, indicating that they were weaker than the control flour. Breakdown time of mixtures tended to be the same as for control wheat flour up to 10 % potato flour, above which it increased significantly. The change in a characteristic can be seen in farinograph results for flour mixture at different percentage of non-wheat constituent. Increase in water absorption lead to the weakened dough and decrease dough development and dough stability. A similar trend also observed by Hamed et al. (1973). Addition of sweet potato flour to wheat flour caused a decrease in extensibility of the dough.

Table II Dough characteristics of potato flour incorporates cookies

Level of potato flour incorporation %	*Water absorption*	*Arrival time(min)*	*Stability (min)*	*Tolerance (UB)*	*Breakdown time (min)*
0	71a	18	8.8	50	30
10	67.8b	17	11	48	31
15	64.4c	14	11.5	40	33
20	60.8d	9	18	32	35

The values denoted by different letters in the same row are significantly different ($p<0.05$)

The sensory evaluation of the potato flour incorporated cookies showed that the control cookies (o percent potato flour) had the highest scores for all the characteristics; however score at 15 percent potato flour levels was found to be on a part with the control for all the sensory characteristics. But there is a significant drop in acceptable level was observed at 20% incorporation level for all the sensory attributes. Regarding the overall acceptability of the respondent highly accepted the 15% potato flour incorporated cookies. Score of the other sensory attributes like flavor of the respondent was higher for the 15%. Results of the sensory evaluation revealed that until the 20% level, there was no significant difference observed in acceptability of the product and all three levels had similar sensory scores, being judged as good for appearance, taste and color and fair for flavor, texture and overall acceptability. The cookies formed with

addition of 15% potato flour got overall acceptability (Table III). Sensory evaluation shows that the cookies made from 20% potato flour were less acceptable. However the texture of cookies made from 20% potato flour was tender and acceptable. The sensory score shows that sweet potato flour 15% is acceptable with reference to taste mouth feel, color and texture. It was observed that the controlled sample has lower odor intensity and the intensity of odor increased significantly from slight to strong with the increased proportion of potato flour. This may be due to the volatile flavor constituents in potato flour which gave characteristics flavor and enzyme reaction also involved in the change in odor as reported by Purcell et al. (1980).

Table III sensory attributes of potato flour incorporates cookies

characters	*100-0*	*90-10*	*85-15*	*80-20*
Color and appearence	8.86b	8.86b	8.66b	8.33a
flavour	8.66c	8.40b	8.46bc	7.93a
texture	8.33c	8.06b	8.33c	7.66a
taste	8.53c	8.26b	8.40bc	7.66a
Overall acceptability	8.60c	8.20b	8.46c	7.80a

The values denoted by different letters in the same row are significantly different ($p<0.05$)

Conclusions

For the large-scale cookies manufacture potato flour can be incorporated up to 15 percent level, without affecting the sensory characteristics of cookies as was accepted by the panelists. The rheological characteristics of the dough are very important, as they influence the machinability of the dough as well as the quality of the finished product. These characteristics depend on several factors such as ingredient quality and quantity, mixing conditions, resting time and temperature of the wheat flour dough

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Infectious Agents Of *Litopenaeus vannamei* (Boone, 1931) And Their Relationship With Physicochemical Parameters In Three Different Culture Systems In Gulf Of Mexico, Mexico

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Introduction

Shrimp aquaculture has been one of the most rapidly expanding economic activities along the coasts of Mexico since 1985. The production increased from 33,480t per year in 2000– 2001 to 128,000 t in 2008–2009, representing 69% of the overall production in Mexico(1). Most of the shrimp farms in Mexico are located on the Pacific coast in the states of Sonora and Sinaloa (61,905 ha), whereas Tamaulipas and Tabasco, on the Gulf of Mexico, only have a production area of 960 ha(2). *Litopenaeusvannamei* is the predominant species of farmraisedshrimp cultured on the eastern and western coasts of Mexico,yet it is a non-native species in the Gulf of Mexico(3). As it may occur in any monoculture, the conditions ofraising shrimp in close proximity in ponds, increases the spread ofdiseases(4). Indeed, diseases have been reportedas the single greatest source of economic loss in the aquacultureindustry worldwide(5). Important factors that increasethe likelihood of disease outbreaks in shrimp culture include adverseenvironmental conditions, high stocking densities, nutritional deficiencies,inadequate aeration or water exchange, heavy algal blooms,physical injury and the presence of a large number of infectiousagents(6), such as viral, rickettsia, bacterial,fungal, protozoan andmetazoan pathogens(7).The most important viral diseases have been caused by virus like the Taura syndrome virus (TSV)(8), and the white spot syndrome virus (WSSV)(9), all originating from Mexican Pacific shrimp farms. Moreover, few baseline data are available on the protozoa (parasites and epibionts), filamentous bacteria and viruses in penaeid shrimp ponds from farms under different culture systems in either Mexico or worldwide. The present study was conducted over two annual cycles of shrimp production to investigate the possible relationships linking the physicochemical water parameters with shrimp diseases in three types of culture systems (extensive, semi-intensive and intensive) associated with three different salinity regimes.

Materials and methods

Samples of *L. vannamei* were collected from 2008 through 2009during two production cycles from three shrimp farms in the stateof Tamaulipas. The Reynosa shrimp farm is located inland inthe northern area of the state (26°04′02.60″ N, 98°14′56.08″ E). It isa semi-intensive culture farm, and its ponds are supplied with lowsalinitygroundwater. The La Pesca shrimp farm (23°50′00.99″ N,97°48′06.47″ W) is located in the central area of the state. It uses anextensive culture system, and its water is supplied from the ocean'sLaguna Madre estuary. The Morónshrimp farm, located in SouthernTamaulipas (22°37′37.03″ N, 97°54′28.06″ W), uses an intensive productionsystem; its shrimp ponds are supplied with freshwater fromthe Tigre River.

Sample collection and sample size. The shrimp were collected using a 6 m^2 cast-net with a 1.25-cm mesh(3). At the farms, shrimp remained in the ponds for 3–5 months, each month 60 shrimp were randomly collected from each shrimp farm; the sample size was chosen according to international recommendations for the monitoring of exotic pathogens, which suggest the use of a sample size that is sufficient to detect the presence of pathogens at a prevalence of 2–5% with a confidence level of 95%. A total of 1260 specimens were studied during the two production cycles, July through November 2008 and April through July 2009. Specimens from each collection site were examined macroscopically, injected

and immersed in Davidson's fixative(9)and transported to the histopathology laboratory (Facultad de MedicinaVeterinaria y Zootecnia, UAT).

Physicochemical parameters of the water. Measurements of the water quality parameters for the ponds were provided monthly by the staff of each shrimp farm. The dissolved oxygen (DO, mg/L) and temperature (°C) were collected twice each day (morning and evening). The salinity, the pH and the water turbidity were measured daily.

Results

During the 2008 culture cycle, a total of 660 organisms were sampled and analyzed: 300 organisms from the Reynosa farm, 180 from La Pesca, and 180 from Morón. During the 2009 cycle, a total of 600 organisms were analyzed: 180 from Reynosa, 180 from La Pesca, and 240 from Morón.At La Pesca, the correlation of the *Nematopsis* sp. with the morning andevening temperatures was highly significant (r=0.997 and 0.999) andproduced high determination coefficients with a significance of Pb0.01. The *Epistylis* sp. was associated with turbidity at La Pesca andshowed high determination coefficients (Pb0.05). At Morón, the*Zoothamnium s*p. showed a strong inverse relationship with the morningand evening DO. In addition, the *Epistylis* sp.showed strong inverse relationships with the evening DO and aninverse relationship with the pH at Morón, with high determinationcoefficients. At Reynosa, the *Acineta*sp. showed a strong significantcorrelation with morning temperature (Pb0.01) and high determinationcoefficients (Table 1).

Table1. Coefficients of correlation or association with regression analysis between the prevalences of pathogens (%), number of samples, with physicochemical parameters recorded in culture.

	Collect.	DO6	DO 18	°C 6	°C 18	pH 12	S‰ 12	Tdty 12	association
Gregarinassp	6	0,466	0,472	0,644	O,648	0,003	0,005	0,115	Coefficient R^2
		0,135	0,131	0,054*	0,053*	0,916	0,892	0,511	p =variable
Zoothamsp	7	0,012	0,097	0,216	0,088	0,595	0,401	0,092	Coefficient R^2
		0,818	0,496	0,293	0,518	0,042*	0,127	0,508	p =variable
Acinetasp	5	0,262	0,010	0,021	0,097	0,591	0,091	0,158	Coefficient R^2
		0,378	0,876	0,817	0,609	0,129	0,622	0,507	p =variable
Epistylissp	10	0,154	0,357	0,243	0,270	0,119	0,000	0,365	Coefficient R^2
		0,262	0,068	0,148	0,126	0,328	0,961	0,065	p =variable

DO: dissolved oxygen, °C: temperature, %S. salinity, Tdty: Turbidity.

Additional studies addressing variations in water quality parameters in shrimp ponds and their relationship with infectious agents and epibiont microorganisms are needed to prevent the adverse impacts on the health of the cultured shrimp. Therefore, the results of this study should help to preventing the spread of disease through the appropriate management of water quality in extensive, semi-intensive, and intensive shrimp cultures (Table 2).

Tabla 2.Associationofprevalenceof pathogens, with population densities in northernfarm, Reynosa; South Farm, Morón and central farm, La Pesca.

La Pesca	Watersource	Year	Extensive	*Gregarinassp*	*Epistylis sp*
	Laguna M	2008	13 /pl´s	21,10%	12,70%
		2009	12 /pl´s	18,30%	10,00%
				39,40%	22,70%

Morón	Watersource	Year	intensive	*Zoothsp*	*Epistysp*
	Rio	2008	100 /pl´s *	6,60%	---------
		2009	80 /pl´s *	3,80%	22,20%
				10,40%	22,20%

Reynosa	Watersource	Year	Semi-intensive	*Acinetasp*
	Sub suelo	2008	44 /pl´s	4,00%
		2009	40 /pl´s	---------
				4,00%

*pl's=Post-larvae.

Discussion

This study also represented the first geographical record of the presence of *Zoothamnium* sp., *Epistylis* sp., *Acineta* sp. and *Nematopsis* sp. in *L. vannamei* raised on farms in Tamaulipas. In the Northern Gulf of Mexico region of the USA, Vitella*et al*., (1970) found *Zoothamnium* in *F. duorarum* in Florida(10), and Overstreet in 1973 reported this as the most common ciliate in cultured brown, white and pinkish shrimp in Louisiana and Texas(11). Due to the extensive distribution of this infectious agent in wild and cultured shrimp, it could easily be introduced at the La Pesca farm, which uses marine water from the Laguna Madre estuary, and at the Morón farm, which uses fresh or brackish water from the Tigre River. *Zoothamnium* disperses readily and spreads quickly, as demonstrated by previous reports that noted common peritrich ciliates (*Epistylis* and *Zoothamnium*) and suctorian ciliates (*Acineta*) infecting hosts reared in both freshwater and sea water(11,12,13).

Of the three farms investigated, the Reynosa farm exhibited the least impact of infectious diseases. This finding may serve to encourage the use of low-salinity underground water in shrimp farming. The optimum salinity recommended for shrimp culture is 15–25 ppt(14), whereas the water sampled at the Reynosa farm had an average maximum salinity of 3.2 ppt and a minimum of 2.3 ppt. At the La Pesca and Morón farms, salinity was the most variable parameter during both culture cycles, due to climatic events, such as hurricanes and rainfall, which are associated with large decreases in salinity in the Gulf of Mexico(15). The prevalence of the *Acineta* sp. was significantly correlated with the temperature. Similar results were reported by López-Tellez *et al*. in 2009, who found a determination coefficient of 0.26 for the relationship between these variables(16). In this study, the correlation between the *Acineta* sp. and temperature was nearly perfect (r^2=0.960). However, the turbidity was not significantly correlated with the prevalence of *Acineta*; a result that is consistent with the findings of Hudson and Lester in 1992(12). The suctorian ciliates (*Acineta*) are saprophytes, they feed on ciliated protozoans, and their occurrence shows no relationship with turbidity. The presence of *Epistylis* sp. and *Zoothamnium* sp. in pond-cultured shrimp has been associated with excess organic matter (12), and in the present study, the prevalence of the *Epistylis*sp. showed a significant relationship with the turbidity (La Pesca farm). However, the

significance of this result was greater than that reported by López- Tellez *et al.* in 2009(16); yet, turbidity has been reported to be an excellent indicator of potential peritrichous infestation in commercial crayfish ponds (13). A significant correlation between the *Epistylis* sp. and temperature was found at the Morón farm, a finding that is consistent with the results of Hüsey and Selcuk in 2005(17). In our study, the DO showed a significant inverse correlation with the *Zoothamnium* sp. and *Epistylis* sp., indicating that the prevalence of both ciliates decreases as the DO increases. The *Epistylis* sp. was also negatively correlated with the pH. Although Jayasree*et al*. in 2001 reported that the prevalence and mean intensity of infection of ciliates were negatively correlated with the salinity (r=−0.74 and −0.56), these authors did not find any correlation between ciliate abundance and any of the parameters tested in the present study(18). In contrast to the findings of Saavedra-Buchel*et al.*in 2008,we observed a highly significant dependence of decreases in the temperature that were associated with decreases in the general prevalence of *Nematopsis*sp(19). At La Pesca, the values of the prevalence of this organism were similar in 2008 and 2009, but the temperature ranges were different. We found significant correlation and determination coefficients greater than r^2=0.994 (Table 1), and our results are consistent with the findings of Chakraborti and Bandyapadhyayin 2011(20). The current study appears to be the first report that the prevalence of gregarines, namely, *Nematopsis* sp., is directly associated with the temperature. Because these organisms are endoparasites, it is possible that the other factors analyzed do not affect their biological cycle, as shown by the statistical analysis.

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Determination Of The Floral Origin And Content Of Pyrrolizidine Alkaloids In The Honey Bee Of Yucatan

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Abstract

The honey of the Yucatan Peninsula is valued internationally for its nutritional and organoleptic qualities. Recently producers of honey, has issued warnings about the risk of contamination with pyrrolizidine alkaloids (PAs) due to the floral origin of honey. In this study 35 plant melliferous species and 60 samples of honey bee of the Yucatan peninsula were analyzed for the presence de (PAs) in relation at your floral origin. The sample preparation was based on the solid phase extraction method with cartridge (. Two honey samples were spiked with the internal standard Crotaline for the selection of the and the condition used for previous conditioned of two cartridges (MC1 and MC2). In the best conditions are obtained 96% efficiency and detection limit (LD) of 13.92 ppb using HPLC-UV. In the honey samples collected in separate apiaries, 80% were classified as multifloral. However, the predominate in honey pollen was to. *Viguera dentata: Tajonal* (FA-28), and *Febaceae, Piscidia piscipula Ja'abin* (C-2-2) plus the honey sample AG-6 where the predominant family the *Asteraceae*.

Introduction

Pyrrolizidine alkaloids (PAs) are secondary metabolites that are found naturally in plants, their effects are hepatotoxic, teratogenic and carcinogenic in humans and livestock. The contain of PAs it is originated from the families *Astereaceae, Boraginaceae*, and *Fabaceae* (1), analysis indicate that more than 660 have been isolated, of which approximately 50 % are considered potentially toxic. (2) As a consequence, a large variety of different extraction and concentration procedures, as well as analytical methods were developed over the 16 years. Inconsistent quantities of alkaloids are present in the honey products sold in international market . A simple process for extracting and analyzing the alkaloids is highly desirable to evaluate product quality.

In Yucatan the species of most importance considered for honey production are: *Asteraceae, Viguera dentata: Tajonal, Polygonaceae, Gymnopodium floribundum: Ts´íit´sílche´ and Febaceae, piscidia piscipula Ja´abin.* (3). Actually la mellisopalinology is the study of pollen contained and source in honey. By studying the pollen in a sample of honey, it is possible to gain evidence of the geographical location and genus of the plants that the honey bees visited. The morphological characteristic features in the description of pollen are: form, size, the number and type of openings and structure of the exine. (4) Therefore, in this paper we analyze the content of alkaloids and relate its botanical origin, to establish the possible source of contamination.

Methods and materials

Collection of the samples

The harvest honey was on a location with high abundance of *Asteraceae, Viguera dentata: Tajonal, Polygonaceae, Gymnopodium floribundum: Ts´íit´sílche´ and Febaceae, Piscidia piscipula Ja´abin*. Colonies were selected in locations in the Yucatan Peninsula on 2011-2012. Were visited (4) apiaries bees to collect plants and honey in different seasons. Honey samples were coded according to their place of origin and stored in plastic bottles for later analysis. The collection of plant melliferous species was conducted in the vicinity of selected apiaries.

Conservation of plant samples and honey

The honey samples were filtered through a 500 µm sieve No. 35 (Fisher Scientific Company) to remove organic matter in the bulk sample and subsequently kept in plastic bottles at room temperature.
Inflorescence at the time of collection is divided into three portions, fraction A was stored in anhydrous MeOH Corning tubes (JT Baker), to retrieve pollen, fraction B was stored in plastic bags for subsequent drying and extraction. Finally the fraction C was placed in a press for drying and subsequent botanical classification. This fraction is preserved in the Center for Sustainable Development and Utilization of Wildlife CEDESU, Autonomous University of Campeche.

Solid phase extraction (SPE)

It weighed 5 g honey, and homogenized with 0.05M H2SO4 then centrifuged at 6000 rpm, then proceeded to the solid phase extraction cartridge (MC1 and CM2) previously conditioned with MeOH/ H2SO4 and the supernatant was loaded centrifugation in the cartridge, then reconstituted with MeOH/NH4 to evaporate and concentrate finally with 1 mL of water was quantified by HPLC.
The flowers samples(fraction B) were extracted with methanol for 7 days and then concentrated in cation exchange cartridge. Finally the extract was evaporated and reconstituted in 1 mL of HPLC water.

Palynological analysis and melisopalinological

The sample inflorescences fraction A and honey diluted with 0.05 M H2SO4 were centrifuged at 2500 rpm for 3 minutes, the pellets were transferred to an Eppendorf tube to which was added 1 mL of acetic acid (J.T. Baker) after 5 min, centrifuged at 2500 rpm for 5 min, decanted and acetolizo with 1 ml of a solution of acetic / sulfuric acid (9:1) (J.T. Baker) in a water bath at 85 ° C for 10 minutes. Acetolizado pollen was centrifuged at 2500 rpm, and washed with a mixture of glycerol / water, recovered again by centrifugation. The samples of Pollen sediments were deposited uniformly on slides clean and degreased, drying at 40 ° C and sealing the preparation with a glycerin gel for subsequent observation optical microscope (Wesco).

Determination of PAs by Liquid chromatography high-resolution

Detection and quantification of pyrrolizidine alkaloids so far has been conducted using an HPLC model Thermo Scientific surveyo brand with a diode array detector and a C-18 5µ Phenomenex 15 mm mark, used two phases, A: H_2O/ formic acid 0.1% (99:1), and B: MeOH/H_2O (99:1). In both phases were added with 200 µL oxalic acid (0.5 M) and 2 mL of ammonium acetate (1 M).

Results and discussion

They have collected 60 samples of honey and 35 plant species. In Figure 1, observed the distribution map of collection zones. The three collection centers visited were: Honey and Wax Campeche, Yucatan Beekeeping Industrial, Honey and Flower Dzitbalché, getting 45 samples of honey from different municipalities of the Yucatan Peninsula. The apiaries were visited Mayatecum, Carrillo Puerto and Dzitbalché municipalities, port in the state of Campeche, they obtained 15 samples of honey and 35 samples of honey species

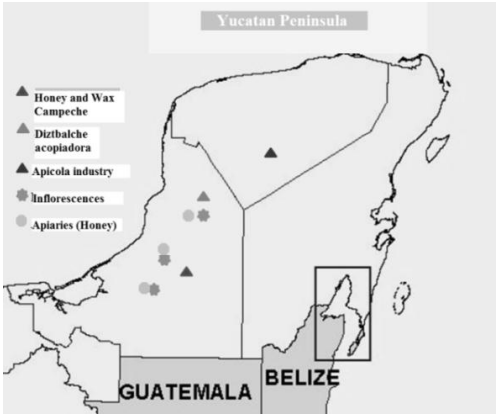

Figure 1. Sampling of the Yucatan Peninsula

In the process of extraction on solid phase, was observed that the cartridge MC2, obtained a lower yield compared with cartridge MC1 (36 and 96% respectively). (Figure 2).

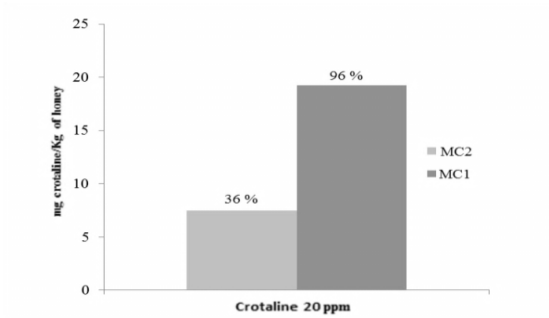

Figure 2. SPE of sample M-8 with 20 ppm added of crotaline

Based on these results was selected cartridge MC1 to verify limit crotaline recovery by standard addition in the sample (M-1). This HPLC method was applied in the validation of the process of extraction on solid phase method. Linearity, precision, and alkaloid recovering were investigated. The precision of the method is excellent, and recovering of the crotaline alkaloid ranged from 92 at 98%, excellent for a quantitative method. The linearity was very good (Figure 4A, 4B) and sensitivity was good. From the standard data, the calculated LOD (limit of detection) for crotaline was 13.93 µg/mL and the LOQ (limit of quantization) was 8.60 µg/mL, so that accurate quantization down to low levels is possible, making it easy to test the quality of honey and well as of different plant species melliferous.

Figures 4A and 4B showed the calibration curve and standard addition of crotaline respectively. In both curves the crotaline was quantified depending on the peak area of the signal in the HPLC.

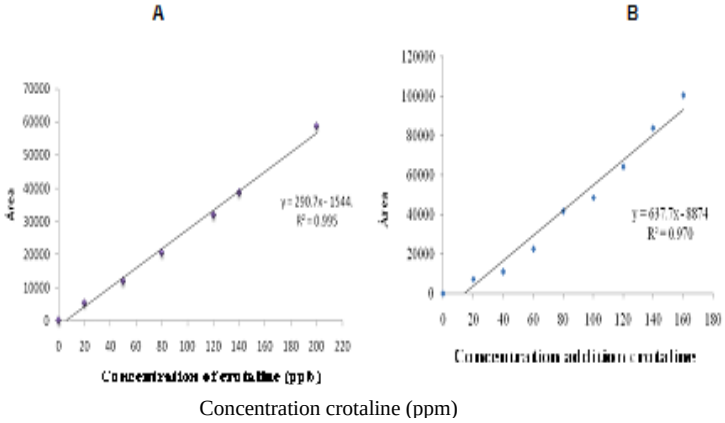

Figure 3. Calibration curve A) direct reading of different concentrations of crotaline. B) reading standard addition to the honey sample (M-1) and SPE with the cartridge MC1.

It may be noted that the affinity of the MC1 cartridge is bit variable depending on the components of the plant (Figure 4). The species that showed a significant difference in the extract recovered by SPE are FA-39, FD-1 and FD-2. This was confirmed statistically with the graphic atypical values and box and whiskers

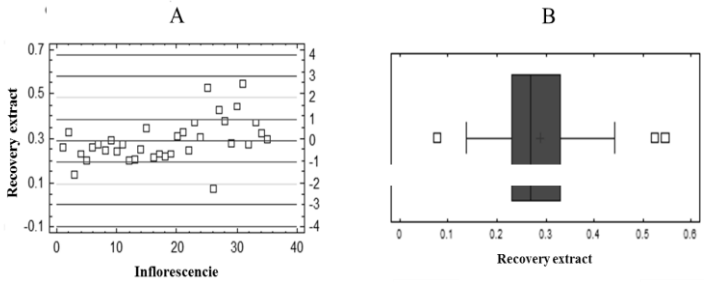

Figure. 4. Graphics A) graphic atypical values B) box and whiskers for inflorescences

In Figure 5, we present the results of the 2 species of honey melisopalinological considered as important for honey production in the Yucatan peninsula, showing the main components of the pollen and positions: polar and equatorial Position, and ornamentation of the exine colpo. One can observe that the FA-28 inflorescence classified botanically as *Viguera dentata*, of the *Asteraceae* family and commonly known as *Tajonal*, has a spherical pollen with spines. The Figure 6 shows the mellifera species C-2-2 whose pollen is spheroidal, with the ornamentation of the exine and tricolporate psilate characteristic of *Piscidia piscipula* belonging to the *Fabaceae* family and commonly known as *Ha'abin.* That is why in Figure 7, we present palynological analysis of a sample of honey (AG-6), and this shows the main dominant species by the type of pollen belonging to the Asteraceae family, which corresponds to those described above.

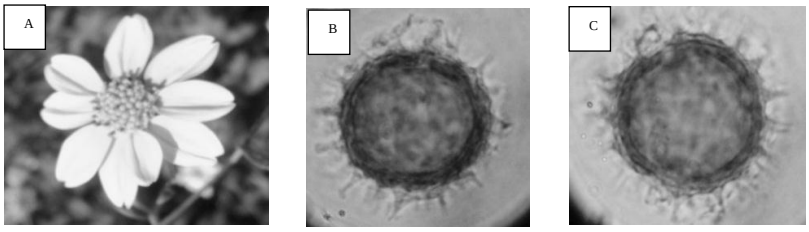

Figure 5. FA-28 *Tajonal* (a) inflorescence b) Equatorial c) Polar

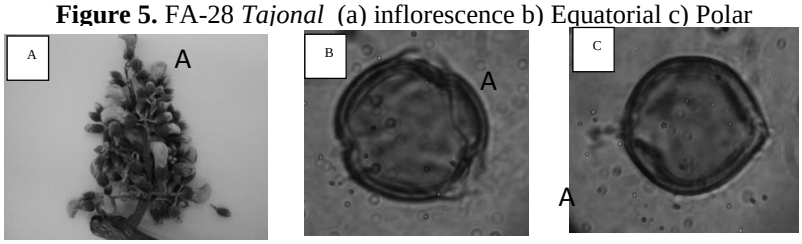

Figure 3. C-2-2 *Ja´abin* (a) Inflorescence (b) Polar (c) Equatorial

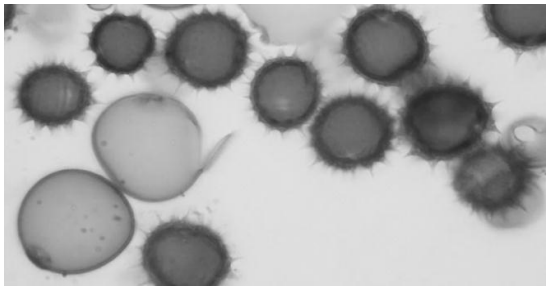

Figure 4. Honey sample AG-6 A= *Asteraceae*

Conclusions

The extraction solid phase (SPE) of alkaloids was carried out more efficiently using the cartridge MC1. Recovered (up to 96%) in honey samples added with crotaline standard. The quantification was achieved using HPLC-UV, with detection limit of 13.92 ppb and 5.31 ppb for addition and the direct reading curve standard respectively. With a 98% efficiency in recovering honey samples added with crotaline. The mellisopalynological analysis, achieved identify the main species melliferous present in honey, but more studies are yet to establish a correralation between alkaloid content and the dominant pollen type in the honey.

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Evaluation Of Antimicrobial Activity In Aqueous Extract Of *Muntingia calabura* L.

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Abstract
According to popular belief, puam (*Muntingia calabura* L.) fruit is recognized for its anti-inflammatory and anti-pyretic properties, possibly related to the presence of phenolic compounds. In agreement with to preliminary data, phenolic compounds have recognized bactericidal activity; therefore, the main objective of this study was to determine the antimicrobial activity of the aqueous extract of the dried shell of *M. calabura* against pathogens as *Escherichia coli*, *Staphylococcus aureus* and *Aspergillus niger*. Total phenolic compounds in the extract were determined by Folin-Ciocalteau method and antimicrobial testing was carried out using the method of Minimum Inhibitory Concentration (MIC) in agar dilution. The data obtained from the study showed a phenolic compounds content of 252.47 ± 0.8 mg GAE/L, and significant inhibitory effects against *S. aureus* with the MIC values ranging from 30 to 50 mg/mL. However, the extract was not effective against microorganisms as *E. coli* or *A. niger*.
Keywords: *Muntingia calabura*, antimicrobial activities, phenolic compounds.

Introduction
At present, a strong interest in food intake with nutritional value, besides properties of health benefits and control the growth of pathogens, it has generated. Recent focus of research for new source of safer and more effective antibacterial agents has been shifted towards natural products of plant sources.
Muntingia calabura L. commonly known as puam, is the name given to a tree species, native to America and belongs to the family *Muntingiaceae*. The *M. calabura* L. fruits besides having antioxidant properties are used as an antitussive, antipyretic, and to relieve conditions such as cough and bronchitis. The beneficial effects of puam consumption are attributed to the presence of antioxidant compounds (1). Scientifically, several types of flavonoids and flavones have been isolated and identified from the leaves of this plant (2), this fact justifies the folklore claims of its medicinal values.
Based on the facts that no extensive studies have been carried out to explore the pharmacological benefits of the fruits of *M. calabura* L., the present study was realized to investigate the antimicrobial activity of the aqueous extract of puam dehydrated shell on deteriorative microorganisms. The previous thing in order to contribute to the future use of puam extract as a natural antioxidant and preservative for food and pharmaceutical industries.

Materials and methods
Preparation of *M. calabura* L. extracts
The fruits of *Muntingia calabura* L. were collected in the months of May and June of 2011 from its natural habitat in Ciudad Valles, S.L.P. The shells of the fruit were manually withdrawn and were blanched at 90°C for 20 seconds in order to inactivate the polyphenoloxidase enzyme; there were subsequently subjected to drying in a convection oven (Lindberg / Blue UT150) at 55°C until obtain a moisture content of 13% ± 3. Then, there were dried, crushed and stored in amber bottles.

Determination of total phenolics

To determine the total phenolic compounds, an extract (aqueous infusion) was obtained a solid-liquid ratio of the sample and water 1:5 (w/v), which was filtered through Whatman No. 2 filter paper, this is, infusion obtained at a time of 12 minutes.

Total phenolic contents in the extracts were determined by Folin-Ciocalteu method (3), this method is based on the oxidation of phenolic groups by the phosphotungstic and phosphomolybdic acids, forming a bluish complex that is measured at 740 nm. An aliquot of the extracts was mixed with 5 ml of Folin-Ciocalteau reagent (previously diluted with water 1:10 v/v) and 4 ml of Na_2CO_3 (7.5g/l). The tubes were allowed to stand for 2 h at room temperature, total phenols were determined using UV/VIS Spectrophotometer (*AquaMate Plus*) at 740 nm. The results were expressed in terms of gallic acid equivalent (mg GAE)/L, according to the calibration curve obtained. All experiments were performed by triplicate.

Evaluation of antimicrobial activity

To evaluate the antimicrobial activity of extracts, puam dehydrated rind were employed. Extracts were obtained from the extraction process by maceration of the dry sample with 70% ethanol in a ratio 1:10 (shell:ethanol), under constant stirring in a homogenizer at 150 rpm for 24 hrs at 30°C. To remove solid plants material, the first supernatant was filtered using cotton and followed by a filter paper (Whatman No. 2). The filtrate was concentrated by evaporation in a vacuum rotavapor (RII BUTCHI) at 60°C.
The resulting extract was dried in an oven at 45°C to remove the excess of solvent. The weight and percentage of yield (%) for the crude dried extracts of *M. calabura* L. were approximately 10%, 30% and 50%, respectively. The solution was sterilized using a microfiltration membrane with a pore diameter of 0.2 microns. From this solution, necessary volume was taken to add to the culture medium.

Bacteria and yeast were assessed for susceptibility to extracts using the agar dilution assay, which was performed according to method M7-A7 from the Clinical Laboratory Standards Institute (4), to determine the minimum inhibitory concentration (MIC), using a filamentous fungus of the genus: *Aspergillus* grown on potato dextrose agar (PDA), a Gram negative (*Escherichia coli*) and a Gram positive (*Staphylococcus aureus*) bacteria grown in trypticase soy agar (TSA) were also used. Bacteria were revitalized in nutrient broth for 24 hours at 37°C. A portion of each culture was taken with an inoculating loop and it was sown in 20 mL of TSA. Colonies were taken from this isolation, and were suspended in 0.85% saline solution; this suspension was used in the control Mac Farland, which relates the turbidity (measured by nephelometry) of barium sulphate standar (mixture of 1% barium chloride and 1% sulfuric acid) with the number of bacteria present in a sample by reference to the test tube number three, whose concentration of $9x10^8$ cells/mL (5). To determine the MIC, TSA plates with different concentrations of the extract (10 mg/mL, 30 mg/mL and 50 mg/mL) was prepared. Each plate was inoculated in 0.1 mL of bacterial suspension whose concentration of CFU/mL test tube corresponds to the MacFarland scale ($9x10^8$ cells/mL) and incubated at 37°C for 24 h. TSA and sodium benzoate at 0.1% by weight where used as a negative control and positive control.

The fungus, in turn, was spread on potato dextrose agar plates and maintained at room temperature for 10 days to prepare a spore suspension that were inoculated in the center of the culture medium. Inoculated systems were incubated at 30°C and PDA and potassium sorbate 0.3 % by weight was used as negative control and positive control.

Statistical analysis

The experimental data were mean ± standard deviation of three measurements (n = 3). The data were processed using the program *KaleidaGraph*.

Results and discussion

Determination of total phenolics. In the dehydrated rind extracts was found that total phenol content was EAG 252.47 mg/L. Figure 1 shows that the content of phenols showed an increasing tendency with increasing the time of infusion, which revealed a correlation coefficient of r=0.92. This trend is consistent with that reported by Reyes *et al*. (2010) (6), who obtained an increase in the concentration of liberated phenols with increasing the infusion time (linear increase).

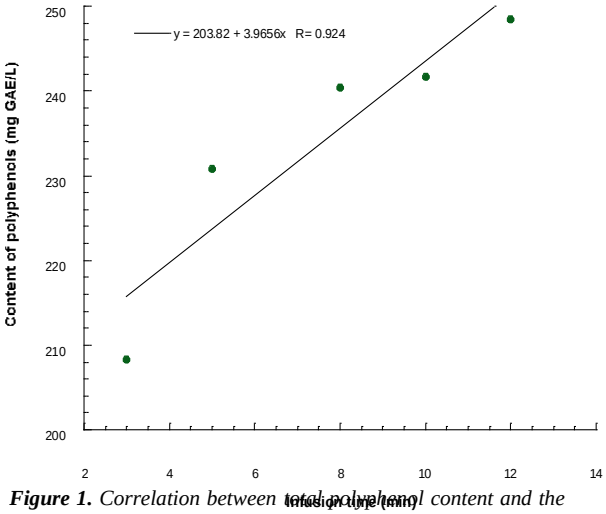

Figure 1. *Correlation between total polyphenol content and the infusion time of dehydrated skin extract.*

Comparing the content of polyphenols obtained in the dehydrated rind puam with other species, it is observed that it has a polyphenol content higher than has been reported for samples peel of potato (*Solanum tuberosum* var. Rosamunda, 4.3 mg EAG/dry weight) and tomato (*Lycopersicum esculentum*, 2.0 mg GAE/L dry weight) and similar amounts of guava (*Psidium guajava* L., 232.9 mg GAE /L) and mango (*Mangifera indica* 224.5 mg GAE/L) (7).

Antimicrobial activity. The results show a significant inhibitory effect against *Staphylococcus aureus*, a Gram positive bacterium, with a MIC of 30 to 50 mg/mL. This inhibition can be attributed to the metabolites present in the skin of the fruit and that is directly related to the polyphenols present in the phytocomplex. The sensitivity to antimicrobial effect may be attributed to their cytoplasmatic membrane structure, which allows to easily antibacterial substances destroy the cell membrane causing a departure from the cytoplasm. One of the main mechanisms of action of polyphenols consists in the disruption of the bacterial cell membrane by three possible ways: by increasing membrane permeability to small ions, affecting the structural stability of the membrane and disrupting the packing of the lipid bilayer, any of these three factors causes bacterial cell death (8).

However, the extract was no effective against microorganisms such as *E. coli* or *A. niger*, as shown in Figure 2. This result is consistent with that reported by Zakaria *et al*. (2006) (9) who observed no inhibition of *E. coli* extracts to different puam sheets extracts. This resistance may be related to the hydrophilic surface of its outer membrane, said membrane rich in lipopolysaccharide molecules displays a barrier against the penetration of numerous antibiotic molecules and is associated with enzymes in the periplasmic space, which are able to break down molecules introduced from outside (10).

The no antifungal activity against *Aspergillus niger*, is because this is a complex organism and additionally has various mechanisms of resistance among which are comprised of cell wall lipopolysaccharides, proteins, lipids, inorganic ions and mannans.

Figure 2. Determination of the inhibitory effect of extract on the growth of *Escherichia coli* (1), *Staphylococcus aureus* (2) and *Aspergillus niger* (3) at a concentration of 50mg/mL. At a concentration of 30mg/mL only *S. aureus* was inhibited again (data not shown).

Conclusions

The results obtained on dehydrated skin extract expose polyphenol content with value of 252.47 mg GAE/L, and significant antibacterial activity against human pathogenic bacteria *Staphylococcus aureus*. These results make it clear that this fruit is undoubtedly a source of phenolic compounds with potential use in the food and pharmaceutical industries.

The fact that the extract does not have broad antimicrobial activity against Gram-negative bacteria and fungi is that phenolic compounds have recognized bactericidal activity, probably associated nonspecific mechanisms that are not necessarily related to its antioxidant activity, which is consistent with other authors, who attributed to this plant more anti-inflammatory and antipyretic that antimicrobial.

Acknowledgement

The authors would like to thank PROMEP system for granting financial support through the project PROMEP/103.5/11/3671.

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Assessment Of Probiotic Strains Ability To Reduce The Bioaccessibility Of Aflatoxin M_1 In Artificially Contaminated Milk Using An *In Vitro* Digestive Model

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Introduction

Aflatoxins (AFs) are toxins that affect human and animal health through cirrhosis and acute damage to liver, tumor induction, as well as immunosuppressive, mutagenic, teratogenic and carcinogenic effects (3). Aflatoxin B_1 (AFB_1), the most toxic AF, is of particular interest because it is a frequent contaminant of many food products and one of the most potent naturally occurring mutagens and carcinogens known to man, and when ruminants consume feed contaminated with aflatoxin B_1 and B_2, they are metabolized and excreted in milk as aflatoxins M_1 and M_2, respectively (1).Aflatoxin M_1 (AFM_1) is stable during milk processing, cheese ripening and storage, and may be present in milk and dairy products. Therefore, consumption of milk and milk products increases the risk of exposure to this toxin in the population.According to the Codex Alimentarius (2), maximum levels of AFM_1 in milk and dairy products should not exceed 0.05 µg/kg. However, the total amount of a contaminant ingested does not always reflect the amount that is available to the body for absorption. Only a certain proportion of the contaminant is bioaccessible(11).Different studies have demonstrated the potential of probiotics to remove aflatoxins in liquid media using *in vitro* and *in vivo* model systems (4,5, 7, 8, 9). The aims of this study were to assess the ability of five probiotic strains to bind AFM_1 in PBS, and to reduce its bioaccessibility from artificially contaminated milk using an *in vitro* digestive model. These strains are widely used in the dairy industry and have previously demonstrated their ability to remove AFB_1 in liquid medium (10, 4, 7, 8, 9).

Materials and methods

Probiotic strains. Five strains of probiotic bacteria were used: *Lactobacillus acidophillus*NRRL B-4495 (Ac), *Lactobacillus reuteri*NRRL B-14171 (Lr), *Lactobacillus rhamnosus*NRRL B-442 (Rha), *Lactobacillus johnsonii*NRRL B-2178 (Jh) and *Bifidobacteriumbifidum*NRRL B-41410 (Bb), these strains were obtained from the Agricultural Research Service, USDA (Peoria, IL, USA) in freeze-dried form and were activated in De Man, Rogosa and Sharpe broth (Difco™) in static incubation at 37 °C for 12 or 8 h. For each bacterial culture, three sub-cultures were made, in order to obtain appropriate cell concentrations prior to each experiment. An aliquot of 1% was taken from the third sub-culture and added to 500 mL of fresh MRS medium (pH 6.2) contained in a glass bottle (PYREX®), incubated for 20 h at 37 °C without shaking. Bacterial cells were recovered by centrifugation (2000 g, 10 min, 10 °C), and washed twice with phosphate buffer saline (PBS 0.5 M, pH 7.2). Finally, the bacterial pellets were re-suspended in 20 mL of sterile PBS. One milliliter (1-2X10^9 CFU/mL) of this suspension was used to perform the tests (7).

Preparation of AFM_1 standard solution. Powderedaflatoxin M_1 (Sigma Chemical Co., St Louis, MO) was dissolved in a mixture of HPLC grade benzene/acetonitrile (97:3 v/v) to a concentration of 10 µg/mL. Subsequently, a standard solution was prepared by diluting the mixture in PBS (0.5 M, pH 7.2). The final concentration of the standard solution (5 µg/mL) was calculated using the Lambert-Beer equation and an aliquot (1 mL) of this standard solution was diluted in PBS (pH 7.2) and in reconstituted (12 %) nonfat dry milk, to reach a concentration of 10 ng/mL and to perform the AFM_1 binding assay.

AFM_1 binding assay in PBS. The binding assay was carried out following the procedure reported by Hernandez *et al*. (2009a) (7). One mL of each active culture (suspended in 20 mL of sterile PBS) was centrifuged (2000 g, 10 min, 10 °C), the bacterial pellet was washed twice with 1 mL of sterile PBS (0.5 M, pH 7.2), and then suspended in 1.5 mL of the working solution of AFM_1 (10 ng/mL), and incubated for 0, 4 and 12 h at 37 °C. Subsequently, cells were removed by centrifugation (2000 g, 10 min, 10 °C) and the supernatant fluid containing the residual AFM_1 was collected and analyzed by HPLC. For each strain, a bacterial control (bacteria suspended in PBS) and an AFM_1 control (working solution of AFM_1 without bacteria) were also incubated.

Stability of the bacteria-AFM_1 complex. To determine the stability of the bacteria–AFM_1 complex, bacterial pellets with bound aflatoxin were consecutively washed (three times) and the amount of AFM_1 released among washes was determined by HPLC. The repeated washes were performed as follows: the bacteria–AFM_1 complex was suspended in PBS (1.5 mL), vortexed for 15 s and incubated at 37 °C for 5 min; thereafter, bacterial cells were centrifuged (2000xg, 10 min, 10 °C) and the supernatant collected for quantification of released AFM_1 (7).

Assessment of bioaccessibility reduction of AFM_1 in an *in vitro* digestive model.The assay was performed according to the *in vitro* digestion method proposed by Green *et al.* (2007) (6). For that, three nonfat dry milk-based groups were established as follows: 1) AFM_1-free milk without probiotic bacteria, 2) Milk containing AFM_1 and, 3) Milk containing AFM_1 and 1 mL of probiotic bacteria (1-2 x 10^9 CFU/mL). All tested strains grew after the simulated digestive process, at this time an aliquot of 10 µL was taken from the final digestive material and added to glass tubes containing 5 mL of fresh MRS medium (pH 6.2) and incubated for 20 h at 37 °C without shaking, all tubes presented turbidity in MRS broth suggesting that all strains had grown survived through the digestive process

Quantification of AFM_1 by HPLC. The amount of AFM_1 present in collected supernatants collected was measured by HPLC (Hewlett-Packard HP1050) with fluorescence detection setting at 365 nm and 435 nm excitation and emission wavelengths, respectively (Hewlett-Packard model HP1046A). Separations were isocratic using a C18 reverse phase column (Waters X-Terra RP 18 3.5 um 3.9x100mm), with a mobile phase of methanol:acetonitrile:acetic acid 60:40:2. Flow rate was set at 0.8 mL/min and an injection volume of 20 µL was used. All data were collected using an ESACoulArray V1.04 acquisition module. The percentage of AFM_1 bound by the bacterial suspension was calculated using the following formula:

$$\% \, AFM_1 = \left[1 - \left(\frac{AFM_1 \text{ peak area of sample}}{AFM_1 \text{ peak area of toxin control}}\right)\right] \times 100$$

Statistical Analysis. To determine significant differences in the results, a one way ANOVA and Tukey's means comparison test ($p \leq 0.05$) were performed, using the statistical package Minitab v. 15. All experiments were performed in duplicate.

Results and discussion

Ability of probiotic strains to bind AFM_1 in PBS. Results of the ability of the probiotic bacteria tested in this work to bind AFM_1 are shown in Figure 1. The amount of bound AFM_1 was specific for each bacteria strain and ranged from 19.95 to 25.43 %. No significant differences ($p < 0.05$) were found in the amount of the AFM_1 bound after 4 h; at this time, Rha and Ac showed the highest values, with 23.88 and 24.09 %, respectively. On the other hand, Lr showed the highest binding ability after 12 h, with 25.43 % of the AFM_1. The binding process proceeded rather quickly given that binding of AFM_1 in all strains ranged from 19.95 to 25.28 % at 0 h. On the other hand, AFM_1 binding was significantly different for the strains Ac, Rha and Lr at different incubation times evaluated, and the strains Bb and Jh did not show significant differences ($p < 0.05$) at 0, 4 and 12 hours of incubation in AFM_1 solution. Variations among strains suggest that either the architecture and/or the cell wall composition of bacteria could have influenced the binding ability of AFM_1 by bacterial strains, In this regards, previous studies have propose that peptidoglycans and/or teichoic acids (8) from bacterial cell wall are probably the primarily

components responsible for AFB_1 binding. Additional studies have confirmed in a clear visual way the AFB_1-bacteria interaction by microscopy and fluorescence techniques (10).

Stability of the bacteria-AFM_1 complex. The effect of washing on the release of the AFM_1 is presented in Table 1. The highest percentage of AFM_1 released after the first wash with PBS was significantly ($p < 0.05$) higher for Lr and Rha, which released 4.37 and 3.18 % of AFM_1 initially bound. After two washes no additional AFM_1 was detected in the wash solution. Although all strains tested were capable to bind AFM_1, these strains did not bind AFM_1 of irreversible way.

Ability of probiotic strains to reduce AFM_1 bioaccessibility in an *in vitro* digestive model. The results for the reduction of AFM_1 bioavailability are depicted in Table 2. The probiotic strains tested showed a reduction in the relative bioaccessibility of AFM_1 from 22.72 to 45.17 % within 3 hours. The strain of Bb showed the highest reduction (45.17 %). According to the statistical analysis, no significant differences ($p < 0.05$) were found among the four evaluated *Lactobacillus*strains, which had percentages of reduction that ranged from 22.72 to 26.50 %.Jh was the *Lactobacillus* strain that produced the higher reduction in bioaccessibility of AFM_1, with up to 32.20 %. In this work, the relative bioaccessibility of AFM_1 in milk was 100 % at the end of digestive assay since we did not detect any decrease in the initial concentration of AFM_1 in the positive control (milk containing AFM_1). On the other hand, the highest reduction in relative bioaccessibility of AFM_1 was obtained for the strains Bb, which showed a percentage of reduction of 45.17 % at the end of the assay. Within *Lactobacillus* strains, strain Ac showed the best ability to reduce the bioaccessibility of AFM_1 (32.20 %). The Bb strain showed better ability to reduce the bioaccessibility of AFM_1 than to bind it in PBS solution. The improved ability was observed after Bb strain became in contact with bile solution in the digestive assay. These observations are in agreement with an earlier work (8), in which author reported that exposure to bile increases the binding ability of *Lactobacillus* strains.

Table 1. Percentage of aflatoxin M_1 released during the washes with PBS AFM_1-free. The detection limit of AFM_1 was 0.27 ng/mL.

	Washes		
Strains	**1**	**2**	**3**
*Lactobacillus acidophillus*NRRL B-4495	1.84 ± 0.04^{ac}	ND	ND
*Lactobacillus reuteri*NRRL B-14171	4.37 ± 0.09^{e}	ND	ND
*Lactobacillus rhamnosus*NRRL B-442	3.18 ± 0.04^{d}	ND	ND
*Lactobacillus johnsonii*NRRL B-2178	1.94 ± 0.03^{a}	ND	ND
*Bifidobacteriumbifidum*NRRL B-41410	1.46 ± 0.04^{b}	ND	ND

Different superscripts mean significant differences ($p \leq 0.05$).

Table 2. Percentage of AFM_1 reduced after digestive process by probiotic bacteria *in vitro* in Milk (10 ng/mL.s, 37 °C). Treatments with different letters in each column are statistically different by each bacteria strain ($p < 0.05$).

STRAINS	**% AFM_1 REDUCED AFTER DIGESTIVE PROCESS**
*Lactobacillus acidophillus*NRRL B-4495	22.72 ± 2.60^{a}
*Lactobacillus reuteri*NRRL B-14171	26.50 ± 1.80^{a}
*Lactobacillus rhamnosus*NRRL B-442	24.54 ± 4.10^{a}
*Lactobacillus johnsonii*NRRL B-2178	32.20 ± 2.81^{b}
*Bifidobacteriumbifidum*NRRL B-41410	45.17 ± 2.64^{c}

Different letters mean significant differences ($p \leq 0.05$).

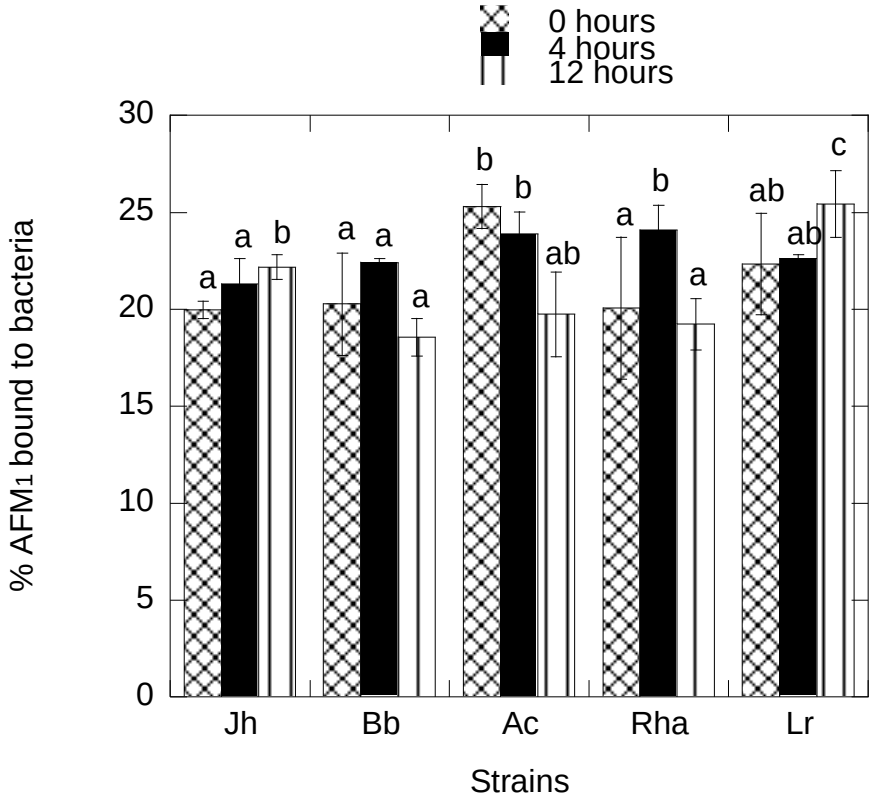

Figure 1. Percentage of AFM_1 bound to probiotic bacteria in PBS (10 ng/mL, 0, 4 and 12 hours, 37 °C). Treatments with different letters in each column are statistically different by each bacteria strain ($p < 0.05$).

Conclusions

Results have shown that all probiotic strains assessed in this work have the ability to bind AFM_1 in PBS and to reduce its bioaccessibility after simulated digestive process using skimmed milk as a food model, but to different extend. *Lactobacillus reuteri* was the strain with highest AFM_1 binding ability in PBS after 12 h of incubation, while *Bifidobacteriumbifidum* showed the highest reduction of AFM_1bioaccessibility in the *in vitro* digestive model. Although all tested strains showed ability to remove AFM_1 at different incubation periods, and that the binding process proceeded rather quickly given that binding of AFM_1 was observed at 0 h binding of AFM_1, it was a partially reversible process since all strains tested released a small portion of bound AFM_1 after a single wash with PBS. Variations in binding ability among strains support the previous suggestion that different mycotoxin binding sites could be present in different strains providing distinctive AFM_1 binding properties to each bacterium. The current findings support the notion that more detailed studies on the impact of probiotics and the food matrix on AFM_1 intestinal absorption and in vivo assessment of bioavailability in relevant animal models are needed.

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Risk Assessment Of Heavy Metals Content In An Agricultural Soil Irrigated With Wastewater In Toluca, Mexico State, México

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Introduction

The metals are inherent components of the earth's crust; however, currently soil pollution with heavy metals is a global environmental problem which is having a significant increase with rising industrialization (Salvatore et al 2009). Industrial activities such as mining, electroplating and manufacturing produce a large volume of wastewater effluents containing toxic substances as metals, including heavy metals, that damage the quality of the aquatic system where they are downloaded (Tiwari et al 2008). Metals such as Pb, Zn, Cu, Cr, Cd, Hg, Mn and As, among others, can be ingested directly or indirectly by animals and humans, creating a serious health problem worldwide (Raskin et al 1997). Undoubtedly, some metals play significant physiological functions but at higher levels than those required theycan cause different effects on human health. Exposure to zinc (Zn) even in low concentrations can cause poor muscle stiffness, loss of appetite and nausea. Chromium (Cr) can cause cancer in the digestive tract and lungs, while manganese (Mn) leads to neurotoxicity problems, decreased levels of hemoglobin and accumulationin the gastrointestinal tract. Copper (Cu) can cause severe toxicological problems due to accumulation in the brain, skin, liver and pancreas, damage to the kidneys leading to anemia. Lead (Pb) is one of the most toxic heavy metals as it has health impacts in the long term, can cause anemia, encephalopathy, hepatitis and nephritic syndrome. Another important toxic metal is cadmium (Cd), because it can cause cancer, liver damage, destruction of the mucous membrane, vomiting, diarrhea, bone degeneration, besides affecting the production of progesterone and testosterone. Exposure to nickel (Ni) can cause serious problems kidney and lung, pulmonary fibrosis and skin dermatitis. One of the more toxic metals to humans is mercury (Hg) which causes degeneration of the nervous system (Lesmana et al 2009). The great toxicity of metals is related to many factors that include extremely persistence in the environment because they are not biodegradable so quickly build up to toxic levels. Also,they can accumulate in the soil due to toxic application of wastewater having high metal levels for extended periods. In addition the industrial downloads continually enter to the water supply consumed by humans and animals, endangering their health and growth (Freedman and Hutchinson 1981). Moreover, in many areas these discharges are used by local farmers to irrigate their crops, so they introduce these contaminants to crops (Warning et al 1986). Often, plants growingin arable soils either contaminated or not, as shown visually undamaged but its chemical composition is different. In this scenario, the reuse of domestic and industrial wastewater in irrigation of agricultural crops appears to be a lucrative business because appreciable amounts of plant nutrients are present in these waters (Rattan et al 2005). Several studies have documented the use of diluted effluent and industrial sludge as promoting productivity and growth of agricultural crops (Quartacci et al 2006; Abbas et al 2007; Chandra et al 2008). Unfortunately these waters contain not only nutrients; several studies have reported the heavy metal pollution of wastewater and soils irrigated with them (Tiwari et al 2008; Cao & Hu 2000, Nan et al 2002). In many developing countries, due to the that fact farmers have no alternative source to irrigate their crops, they do

it with industrial effluents (Tiwari et al 2008; Abbas et al 2007)which containhigh levels of various toxic metals (Cu, Cd, Cr, Zn, Fe, Ni, Mn and Pb) (Tiwari et al 2008; Abbas et al 2007; Kumar et al 2004) mainly. However, from the biological point of view the overloading ofheavy metals often leads to degradation and contamination of the food chain mainly through the plants grown in these soils (Rattan et al 2005). In the Valley of Toluca, State of Mexico is located the Upper Lerma River, which receives significant discharges of municipal and industrial wastewater, mainly from the pharmaceutical, food, textile, chemical, cosmetic, electromechanical, metalworking and automotive industries, among other. Also, the river containing material sedimentable highly contaminated fromthe agricultural residues obtained from tributaries such as fertilizers and pesticides, this are dragged to the José Antonio Alzate Dam. The river is regularly desludged whosesludge are used as a source of organic matter bysome local farmers found throughout the basin, while the waters of the dam are used to irrigate the agricultural fields due to shortage of drinking water for irrigation. Crops that are growing along the dam, and probably contaminated with heavy metals, are mainly maize (*Zea mays*), oats (*Avena sativa*), bean (*Viciafaba*) and beans (*Phaseolus vulgaris*). In this paper we study the physicochemical characteristics of an agricultural soil irrigated withwastewater discharges to determine their potential risk on safety of agricultural products.

Methods and materials

Collection of soil samples

Soil samples were collected from the surface layer (0-30 cm) of an agricultural area located in the vicinity of José Antonio Alzate Dam. The agricultural zone is characterized by cultivating a greater proportion, corn, but has a lesser proportion of growing beans and oats. Soil samples were collected in the month of July 2011, dried at room temperature and stored in polyethylene bags until analysis.

Physicochemical analysis

The physical and chemical properties of soil were determined by routine analytical methods established by the Mexican Official Standard NOM-021-SEMARNAT-2000 (Norma Oficial Mexicana NOM-021-SEMARNAT-2000).

Determination of heavy metals

The samples were ground, sieved, digested and the metal content was determined according to the NOM-147-SEMARNAT/SSA1-2004 (Norma Oficial Mexicana NOM-147-SEMARNAT/SSA1-2004).

Statistical Analysis

All experiments were performed in batches of 5 test tubeswith 3 replications. All data and determined were subjected to analysis of variance and comparison of means was performed using the Tukey test at 5% probability level.

Results and discussion

The physicochemical characteristics of the agricultural soil studied,showedthat itwasan acidic soil with a cation exchange capacity regarded as average, very low content of organic matter and carbon, with electrical conductivitynormal, medium content of nitrogen and phosphorus, low potassium content, with arelationship of carbon / nitrogen deficient, while the exchange cation content of calcium and magnesium were very low, with an average bulk density, and sandy clay loam and no sodiumsoil (Table 1). The characteristics of the soil adjacent to José Antonio Alzate Dam show that is not suitable for agricultural crops, due to deficiency of nutrients and organic matter, so the crop productivity is diminished.

Table 1. Physicochemical characteristics of agricultural land located in the vicinity of José Antonio Alzate Dam, State of Mexico Toluca.

Parameter	Concentration
pH	4.95 ± 0.36
Cation Exchange Capacity (Cmol/100 gss)	27.5 ± 4.86
C (%)	1.95 ± 0.72
OrganicMatter (%)	3.36 ± 1.23
ElectricalConductivity (mS/cm)	0.52 ± 0.67
N (%)	0.12 ± 0.05
P (ppm)	20.81 ± 1.92
K (ppm)	134.2 ± 16.18
C/N (%)	16.25 ± 1.55
Ca (ppm)	3.14 ± 0.85
Mg (ppm)	76.11 ± 11.11
Na (ppm)	23.1 ± 4.62
BulkDensity (g/cm^3)	1.15 ± 0.04
Texture	Sandy clayloam
Sand (%)	56.8 ± 1.65
Clay (%)	21.6 ± 2.39
Loam (%)	21.6 ± 1.76

The values represent to the media ± SD.

While the concentrations of heavy metals in the soil in overall shape are not toxic, because they do not exceed the concentration ranges considered as permissible in soil (Raskin et al 1997). However, the presence of Cr, Cu and Fe in the soil studied may represent long-term risk of bioaccumulation in agricultural products, which can happen to continue using the waters of José Antonio Alzate Dam as a major source of irrigation of agricultural crops (Table 2). Several reports in the literature have documented the movement of heavy metals contaminating water and soil (Rattan et al 2005; Abbas et al 2007; Cao & Hu 2000; Nan et al 2002), to agricultural crops (Feng et al 2012; Salazar et al 2012), and dairy products from cattle (Rodríguez 2003), who graze and drink water in the banks of water bodies contaminated with industrial waste and sewage, which have high concentrations of heavy metals such as Cr, Cd, Hg and Zn, among others.

Conclusions

In all soils tested were detected the heavy metals as the cadmium, copper, chromium, iron, nickel, lead and zinc. Although the concentrations do not exceed the official Mexican standards, it is important to take precautionary measures to avoid the risk of displacement of metals, mainly cooper, chromium, and iron as they can in a medium and long term represent a public health problem by consuming agricultural products contaminated with these elements.

Table 2. Content of heavy metals in agricultural land located in the vicinity of José Antonio Alzate Dam, State of Mexico Toluca

Metal	Concentration (mg kg^{-1})
Cd	0.36 ± 0.04
Cu	45.50 ± 1.48
Cr total	23.61 ± 5.79
Fe	12338 ± 627.68
Ni	16.12 ± 1.32
Pb	8.72 ± 1.79
Zn	23.57 ± 1.96

The values represent to the media ± SD.

Acknowledgements
The authors thank the National Council for Science and Technology for supporting this work through the project "Biorremediaciónambientalpara la conservación de la biodiversidad" in Complementary Support Programme for the Institutional Strengthening Research Groups.

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Antimicrobial Evaluation of Eight Spices Essential Oils Against *Escherichia coli, Pseudomonas fluorescens* and *Listeria monocytogenes*

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Abstract

Spices in food technology had been used as additives.However, other potential use is in storage meat preservation.Meat usually is attacked by pathogens as *Escherichia coli*, *Pseudomonas fluorescens* and *Listeria monocytogenes*. The objective of this study was to determine the antimicrobial activity of eight essential oils extracted from spices (oregano, garlic, thyme, clove, cardamom, cinnamon, mint and rosemary) which had demonstrated to have inhibitory effects against some pathogens expressed as MIC and MBC. The methodology included a pre-test in TSA with concentrations 0 – 1500 ppm of essential oil and the microorganism (conc ~0.6 abs, 620nm). The MIC and MBC were determinated in a secondary test which was realised in TSB as a kinetic with essential oil concentrations based in the first test. As results, it was founded that essential oils cardamom and mint presented the less activity while,oregano and garlic presented the best inhibitory activity, the same behaviour was observed in the three pathogen strains analysed (Duncan, $p<0.05$). As conclusion, the addition of essential oils such as oregano, garlic and rosemary in processed food could be used either additive or preservative. As a perspective based in these previous results, the addition of essential oils in minimal processed meat could permit longer storage periods and even the elaboration of a hydrocolloid coat,which could be used as package not only for meat products also for cheese and other foods with high humidity level.

Keywords:Antimicrobial activity, essential oil, spices, food pathogens.

Introduction

In the last three decades, the increased awareness ofresistance to antibiotics especially in multidrug resistantpathogenic microorganisms, as well as the emergence ofundesirable side effects caused by inappropriate antibioticuse, has excited great interest in looking for new sources ofuseful agrochemicals and pharmaceuticals. Plantshave been the main source of new structural types ofcompounds with potential antimicrobial properties todevelop novel chemical prototypes.Searching for bioactive natural products requiresappropriate bioassays, focused on the desired activity (e.g.antifungal, antibacterial), as well as various techniques forisolation and identification (1).The antimicrobial properties of plant volatile oils andtheir constituents from a wide variety of plants have beenassessed and reviewed. It is clear from these studies that these secondaryplant metabolites have potential uses in medical proceduresand applications in the cosmetic, pharmaceutical and food industries. Biological activityof essential oils depends on their chemical compositionwhich is determined by the genotype and influenced by environmental and agronomic conditions (2). Natural antimicrobial agents derived from sources such as plantoils have been recognized and used for centuries in food preservation.EOs and spices were used by the early Egyptians and have beenused for centuries in Asian countries such as Chinaand India.Someofthe spices, such as clove, cinnamon, mustard, garlic, ginger and mintare still applied as alternative health remedies in India. Most spices have eastern origins; however, some of them havebeen introduced after discovery of the New World – spices such aschili peppers, sweet peppers, allspice, annatto, chocolate, epazote,sassafras, and vanilla, which have been used for food flavoring andmedicinal purposes (3). The antifungal and antibacterial activity exhibited bySpices essential oil has been demonstrated by severalresearchers, but unfortunately,there are few quantitative data (minimal inhibitoryconcentration or minimal bactericide concentration)related to the antimicrobial activity of essential oilobtained from different aromatic plants cultured against foodborne pathogens (4).The objective of this study was to determine the antimicrobial activity of eight essential oils extracted from spices (oregano, garlic, thyme, clove, cardamom, cinnamon,

mint and rosemary) to demonstrate their inhibitory effect against *Escherichia coli*, *Pseudomonas fluorescens* and *Listeria monocytogenes*used as reference pathogens expressed as MIC and MBC.

Methods and materials

Biological materials

Essential oils of oregano (*Origanumvulgare*), garlic (*Allium sativum*), thyme(*Thymus albicans*), clove (*Syzygiumaromaticum*), cardamom (*Elletariacardamomum*), cinnamon (*Cinnamomumzeylanicum*), mint (*Menthapiperita*) and rosemary (*Rosmarinusofficinalis*) were purchased from Marlin Chemical Products (Mexico). Organisms used in this work were *Escherichia coli* (ATCC O157:H7), *Pseudomonas fluorescens*(ATCC CD BB B-96) and *Listeria monocytogenes* (ATCC Scott A) as pathogen strains. Strains were growth 48 h in tryptic soy broth (TSB, Sigma-Aldrich, USA) at 38 °C before use.

Antimicrobial activity

An initial test with essential oils was carried out in order to find a suitable values rage. Samples were prepared in concentrations from0 to 1500 ppm of essential oil in 20 mL of sterile tryptic soy agar (TSA) and the pathogen microorganismswere sown on the surface, samples were incubated 24 h at 38 °C. After that timethe bacterial worth was observed. Positive control was streptomycin 5 mg/mL.Subsequently, EOs were added in concentrations determined as the range in the first test in 5 mL of sterile TSB and the microorganism 10^6CFU (conc ~0.6 abs, 620nm), samples were incubated 24 h at 38 °C as Jimenez-Arellanes*et al*. (2011) described. Finally absorbance was measured at 620 nmin a spectrophotometer Lambda XLS (Perkin Elmer, USA). Results are presented as minimal inhibitory concentration (MIC)defined as the concentration of antimicrobial that maintained the initial population of pathogen without change at the end of the incubation period ($p<0.05$) and Minimal Bactericidal Concentration (MBC) defined as the lowest concentration at which 99.9% of the initial inoculum was killed in the medium and no growth was observed when 100 μL of the samples were plated onto TSA and incubated at 38 °C for 24 h (6).

Statistical analysis

Means comparison data were analysed by Duncan's test considered significantly different when $p<0.05$. The analysis and graphics were performed using the GraphPad Prism software (USA).

Results and discussion

Although the preliminary test was carried out for the tree strains, Table 1 shows the TSA plate preliminary test only for *Escherichia coli* because the results were similar between *P. fluorescens*and *L. monocytogenes*strains.Oregano and garlic have not bacterial growth with the addition of 200 ppm, rosemary and cinnamon 400 ppm, thyme and clove 600 ppm, mint 800 ppm and cardamom was supposed higher to 1000 ppm. So,concentrations for the kinetic test in TSB were establish as: Oregano and garlic 100, 200, 300 and 400 ppm; rosemary and cinnamon 100, 200, 300, 400, 500 and 600 ppm, thyme and clove 200, 300, 400, 500, 600 and 700 ppm, mint 400, 500, 600, 700, 800 and 1000 ppm; and cardamom 800, 1000, 1500, 2000 and 2500 ppm.

Table 1.Preliminar test in TSA for EOs range determination using *E. coli*.

Qualitative test to determine Essential Oil concentrations (ppm)						
Spice	100	200	400	600	800	1000
Rosemary	X	X				
Oregano	X					
Garlic	X					
Thyme	XX	X	X			
Mint	XXX	XXX	XX	X		
Cinnamon	XX	X				
Cardamom	XXX	XXX	XXX	XXX	XX	X
Clove	XX	XX	X			

Results of a triplicate test are presented.

The pathogen elimination studies are considered important because foodborne diseases continue to be a serious threat to public health all over the world. The incidence of illnesses appears to be increasing on a global basis including developed and industrialized countries. Foodborne illness outbreaks associated with *Escherichia coli* O157:H7 on meat and fresh produce products, some refrigerated ready-to-eat, fresh-cut fruits and vegetables, often eaten with minimal processing, are a potential source of foodborne infection. *E. coli* O157:H7 are associated with feces from livestock and wildlife, it is clear that they are transported in surface runoff, and accumulate in sediments and soils contaminated by these animals. These bacteria may also migrate into groundwater. Eliminating pathogens from livestock would aid in reducing soil and water contamination with various fecal pathogens. Researchers are currently investigating several approaches to eliminating *E. coli* O157:H7 from livestock, including antibiotics, antimicrobials, probiotics, vaccines and bacteriophage (7). Other important reference pathogen is *Listeria monocytogenes,*which concern to the food industry due to its high mortality rate and wide distribution in the environment. It can be found in a wide variety of rawand processed foods. This Gram-positive pathogen has the ability to survive under a wide range of conditions such as refrigeration temperatures (2-4 °C), acidic pH and high salt concentrations (up to 10 g/100 g) in foods. Control of *L. monocytogenes* in various foods usually includes the application of 'hurdle' technology by combining factors such as low temperature, acidification, low Aw and chemical or natural preservatives (8). And *Pseudomonas fluorescens* that is a psychotropic spoilage bacterium which can reduce the shelf life of refrigerated fruits and vegetables. Despite their commensal nature, *P. fluorescens* are nonpathogenic and lack virulence factors of other plant pathogens. Some *P. fluroescens* enzymes can degrade plant cell walls and their components and it is capable of breaking down some plant-derived carbohydrates, fatty acids, and oils and can hydrolyze proteins causing the spoilage of milk, meat, and fish (4).

In Table 2 MIC and MBC results are presented. After be having run the kinetics for each concentration, EO and microorganism, it was observed similar behaviour between *E. coli* and *P. fluorescens* MBC and few in the MIC. Otherwise when *L. monocytogenes*was compared an increase in the MIC and MBC values was observed.

Table 2.Minimal Inhibitory Concentration and Minimal Bactericidal Concentration of EOs in TSB.

MIC and MBC determined of Essential Oil (ppm)						
Spice	*E. coli*		*P. fluorescens*		*L. monocytogenes*	
	MIC	MBC	MIC	MBC	MIC	MBC
Rosemary	100	200	100	200	200	300
Oregano	<100	100	<100	100	<100	100
Garlic	<100	100	<100	100	<100	100
Thyme	300	400	200	400	400	600
Mint	500	600	500	600	600	800
Cinnamon	200	400	300	400	300	500
Cardamom	1500	2000	1500	2000	2000	2500
Clove	300	400	300	400	300	500

Means of the results of a triplicate test are presented.

It is possible to notice that oregano and garlic are the spices with higher antimicrobial potential independently of the strain due to their thymol and carvacrol contain, then rosemary and cinnamon which contains camphor/1,8-cineole/borneol/camphor and cinnamic aldehyde/eugenol, respectively. Thyme, mint and clove showed similar behavior thymol, carvaclor and eugenol(3). Finally cardamom which is not a common use spice presented the lower antimicrobial activity. This activity could be linked with aromatic groups in the active molecule. Also, it was observed that when the essential oil is sweeter the less antimicrobial activity was obtained.

There is high difference between the spices like oregano and garlic with spices like cardamom. Consequently, further research on the antimicrobial activity of spices essential oils and its main components is needed to understanding the mode of action in order to evaluate its usefulness in the shelf life extension of packaged foods such as bakery, meat, poultry, seafood, and cheeses. To know the specific compounds which own potential activity could permit in future, the replacement of synthetic and chemical food additivesusing natural compounds instead, which are considered less dangerous and less toxics in human health.

Conclusion

The addition of spices essential oils with bioactive compounds could be added in processed food either additive or preservative in order to improve human health. In this work the spices tested showed the next antimicrobial behaviour: oregano = garlic (less than 100 ppm EO)> rosemary > clove > cinnamon > thyme > mint > cardamom (between 1500 to 2500 ppm EO).

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Isolation And Molecular Identification Of *Curvularia lunata/ Cochliobolus lunatus* Causal Agent Of Leaf Spot Disease Of Cocoa

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Introduction

Cocoa (*Theobroma cacao* L.) is a crop with great commercial value in many tropical regions worldwide, whose fruit is processed in the industry in many products, cosmetics and medicine industry. However, plantations generate a large volume of waste that generally remain scattered into the plantation and cause the phytopathogen propagation, affecting the cocoa plant and plays an important role in the destruction of their fruits. The most important are the frosty pod rot caused by *Moniliophthora roreri* (1), witches' broom caused by *Moniliophthora perniciosa* (2), black pod caused by *Phytophthora* spp. (3), and leaf spot caused by *Curvularia lunata*/*Cochliobolus lunatus* (4). *Curvularia* is a hyphomycete fungus which is a facultative pathogen of many plant species. Most *Curvularia* are found in tropical regions, though a few are found in temperate zones. The teleomorph state of the type species *Curvularia lunata* is *Cochliobolus lunatus*. In Mexico, few works has been focused on the molecular identification of fungi cocoa diseases (i.e. *M. roreri*). In particular in the Chontalpa region, located in the center-west of the Tabasco state, Mexico, the fungi have been the most aggressive. The identification of phytopathogenic strains is important for developing strategies for its prevention and control. Molecular techniques such amplification of DNA conserved sequences by Polymerase Chain Reaction (PCR) have proven to be useful in studies of genetic diversity, life cycle, ecology, phylogeny and identification of microorganisms. The technique known as PCR-ITS (Internal Transcribed Spacer-PCR) allows replicate *in vitro* thousands of times, specific sequences of the inner region of ribosomal genes of fungal species. Therefore the sequences of the nucleotides are compared to others from GeneBank, allowing its taxonomic identification. The genes are amplified and compared the ribosomal DNA (18S, 5.8S and 28S), which are highly preserved (5). The objective of this study, was the molecular characterization of five strains of fungi, by means of the sequenciation of the ITS I/5.8s/ITS II region of the DNAr of all fungus previously identified as *Curvularia lunata*, *C. hawaiiensis* and *Bipolaris tetramera* by morphological tecniques, obtained through diseased fruits of cacao plants from the Chontalpa area of the state of Tabasco, Mexico.

Materials and Methods

Fungal strains

The strains HT-ITV03, HT-ITV40, HT-ITV45, HT-ITV46 and HT-ITV48 used in this study were isolated from diseased fruits of cocoa trees collected during October 2007 and November 2009, respectively. The samples of diseased fruits were taken from three commercial plantations located in the municipality of Huimanguillo, Tabasco. For isolating fungi, fragments of diseased fruits were placed in a moist chamber and incubated at 25ºC for 3 days in darkness. After incubation, individual fungi were recovered as they grew out of the diseased tissue and then subcultured on potato dextrose agar (PDA) at room temperature for 5 days to obtain pure cultures. All fungal strains were maintained on PDA at 4°C and as a suspension of spores at -74ºC. In addition, the strains were subcultured every month on PDA medium.

DNA extraction, PCR and sequencing

Extraction of DNA from *Cochliobolus lunatus*, *C. hawaiiensis* and *B. tetramera* strains were performed as described previously Kurzatkowski *et al*. in 1996 (6). A region of nuclear DNA, containing the ITS I,

5.8S, and ITS II sequence, for each strain were amplified by PCR using the primers ITS1 (5'-tccgtaggtgaacctgcgg-3') and ITS4 (5'-tcctccgcttattgatatgc-3') (7). PCR amplifications were performed in a total volume of 50µl reaction, which contained: 10 µL of 5x Colorless buffer, 200 µM dNTPSs, 0.2 µM of each primer, 2.5 units of Taq DNA polymerase, and 10–50 ng of template DNA. The PCR reactions were placed in a Bio-Rad thermal cycler (Bio-Rad Laboratories, Hercules, California, USA) under the following parameters: 5 min initial denaturation at 95ºC, followed by 30 cycles of 1 min denaturation at 95ºC, 1 min primer annealing at 57ºC, 1 min extension at 72ºC and a final extension period of 12 min at 72ºC. The resulting products were purified with the Geneclean® II kit (Bio101 Inc., California, USA) according to the manufacturer's protocol. DNA samples were sequenced by the Instituto de Biotecnología, Cuernavaca, Mor., Mexico.

DNA sequence analysis

DNA sequences from each strain were assembled by use of Chromas 1.45 (School of Health science, Griffi th University, Gold Coast Campus, Southport, Queensland, Australia), and then aligned using the ClustalX inference package (8). The sequences were subjected to the basic local alignment search tool (BLAST) available at http://blast.ncbi.nlm.nih.gov/Blast.cgi against sequences available in GenBank. Phylogenetic analysis of the aligned sequences was done by Neighbour-Joining analysis with 1000 bootstrap replicates and was supported by bootstrap method. Unrooted phylogenetic tree was constructed with help from the PhyloDraw program 0.8 (9).

Results and Discussion

Isolation of strains

Five random samples of cocoa fruits with symptoms of disease of each of the three plantations were collected. One hundred and twenty eight strains were isolated of which three were identified by their morphological characteristics as *C. lunatus* and one as *C. hawaiiensis* and *B. tetramera*, respectively. The isolated species of the *Cochliobolus* genus (*C. hawaiiensis* and *C. lunatus*), were already reported by other authors as pathogen of plants like corn (10), barley (11), oat, sorghum, banana, pineapple (12) and many fescue (13). These fungi cause the leaf spot and the putrefaction of the vascular tissue and fruits reasons because these pathogen produce cocoa losses. This genus of fungi are the teleomorph state of many species of fungi of the genus *Bipolaris* (14) and *Curvularia* (15) genus that were reported as pathogen or the cocoa crop by others authors (4). By other hand, the anamorph state of *C. hawaiiensis* was reported and isolates in Tabasco state by others authors (16) represented the first report for the isolation of the teleomorphic state in Mexico.

Molecular identification and phylogenetic analysis

For the three strains of *C. lunatus* and the individual isolates of *C. hawaiiensis* and *B. tetramera*, were obtained the entire sequences of the ITS I/5.8s/ITS II region, together with the ends of the 18S and 28S subunits. The BLAST search confirmed the morphological identification of the sequences of *C. lunatus*, *C. hawaiiensis* and *B. tetramera*. The sequences examined in this study for the ITS I/5.8s/ITS II region, for the strains of *C. lunatus*, *C. hawaiiensis* and *B. tetramera* and other related sequences from GenBank had a length of between 506 to 629 bp for the species examined in this study of *Cochliobolus* spp. and *Bipolaris* sp. and the length of the consensus region for alignment of all sequences was 697 bp (Table 1). For the analysis of 5.8S subunit was observed to be highly conserved between all species of *Cochliobolus* and *Bipolaris*, with change limited to the variations of a few bases in the sequences of *C. lunatus*. On the other hand, in the 18S and 28S subunits, was observed much variability, which was limited to point mutations, insertions or deletions, predominantly among isolates of *C. hawaiiensis*, *B. tetramera* and *B. spicifera*. Much variation was observed in terms of deletions, insertions and base substitutions within the ITS regions, especially in the ITS II, although many conserved regions were present in the ITS region I and ITS II. In general the length of the sequences of the ITS I and ITS II regions was highly conserved in all species of *Cochliobolus* and *Bipolaris*, with limited variations to the change of a few bases. Phylogenetic analysis of sequences of strains of *C. lunatus*, *C. hawaiiensis*, *B. tetramera* and others 24

sequences of *Cochliobolus* and *Bipolaris* related of GenBank, generated a tree by the Neighbor Joining method that grouped isolated from the strains HT-ITV03, HT-ITV40 and HT-ITV45, in one same clade and were aligned along a brother clade formed by sequences of the strains IP 1417.82, IP 2328.95, IP 613.60 and AHB-1 of *C. lunatus* (DQ836799, DQ836800, DQ836798 and GQ280375) the first three isolated in France by Desnos-Ollivier *et al*., in the year 2006 (15) and the fourth isolated in Mexico by Tapia-Tussell *et al*., in the year 2009 (17), supported by a bootstrap value of 929. On the other hand, for sequences of *C. hawaiiensis* and *B. tetramera* isolated in this study, we observed that lined up in different clades. The HT-ITV46 strain, was aligned with the sequence of strain Alcorn 7612(b)-6 of *C. hawaiiensis* (AF071324) isolated in Canada by Berbee *et al*. in the year 1998 (18) and HT-ITV48 strain was aligned with the strain BMP 51-31-01 of *B. tetramera* (AF229477) isolated in the United States by Pryor and Gilbertson in the year 2000 (19).

Table 1. *Bipolaris* and *Cochliobolus* genus, ITS I/5.8s/ITS II region and 18S and 28S genes for the aligned sequences

GenBank Access number	Gene 18S length (bp)	ITS I length (bp)	Gene 5.8S length (bp)	ITS II length (bp)	Gene 28S length (bp)	Total length
HT-ITV45	44	162	158	162	103	629
HT-ITV40	46	163	159	164	74	606
HT-ITV03	55	162	156	162	78	613
DQ836799	55	160	158	163	59	595
DQ836800	55	162	158	164	59	598
GQ280375	21	161	158	165	50	555
DQ836798	55	162	158	165	59	599
AY253918	39	161	158	161	42	561
EF503562	45	161	158	161	38	563
AJ303084	32	161	158	161	40	552
AF229477	11	161	158	161	41	532
HT-ITV48	8	161	158	161	47	535
HT-ITV46	30	161	158	161	33	543
AF071324	11	161	158	159	17	506
FR717533	30	161	158	161	65	575
AF158106	27	161	158	159	21	526
GU480767	32	165	158	161	60	576
EF540752	29	161	158	161	60	569
AF071322	11	161	158	159	17	506
AJ853762	8	161	158	161	40	528
HM195268	31	161	158	162	60	572
FJ040180	30	161	158	162	58	569
AY923860	30	161	158	162	59	570
HM195265	31	161	158	162	60	572
HM195264	30	161	158	162	60	571
HM195263	22	161	158	162	61	564
HQ248191	0	150	158	164	60	532
GU183125	16	161	158	161	114	610
HT-ITV45	0	114	158	164	114	550
Length range :	0 to 55	114 to 165	156 to 159	159 to 165	17 to 114	506 to 629
Consensus sequence:	1-55 bp	56-227 bp	228-385 bp	386-555 bp	556-697 bp	1-697 bp

This result, confirms that the strains identified as *C. lunatus*, *C. hawaiiensis* and *B. tetramera* may belong to different specimens of these species due to bootstrap values that support these alignments (Figure 1). Also, with the phylogenetic analysis performed, was corroborated the findings by Worapattamasri *et al*. (20) since it was found that *C. hawaiiensis* is a heterothallic fungus and that *B. hawaiiensis* is its anamorphic state. On the other hand, Weikert-Oliveira *et al*. in 2002 (21) reported that *Bipolaris* species are genetically distinguishable and showed that the teleomorph of the analyzed species have high genetic similarity in their electrophoretic profiles when were compared with their respective anamorphs. According to Burdon and Silk (22), a major source of genetic variation in phytopathogenic fungi, are mutation and recombination. Furthermore, within a species, genes flow between populations and

supplementing this process as propagules which diffuse from one epidemiological area to another and from one deme to the next.

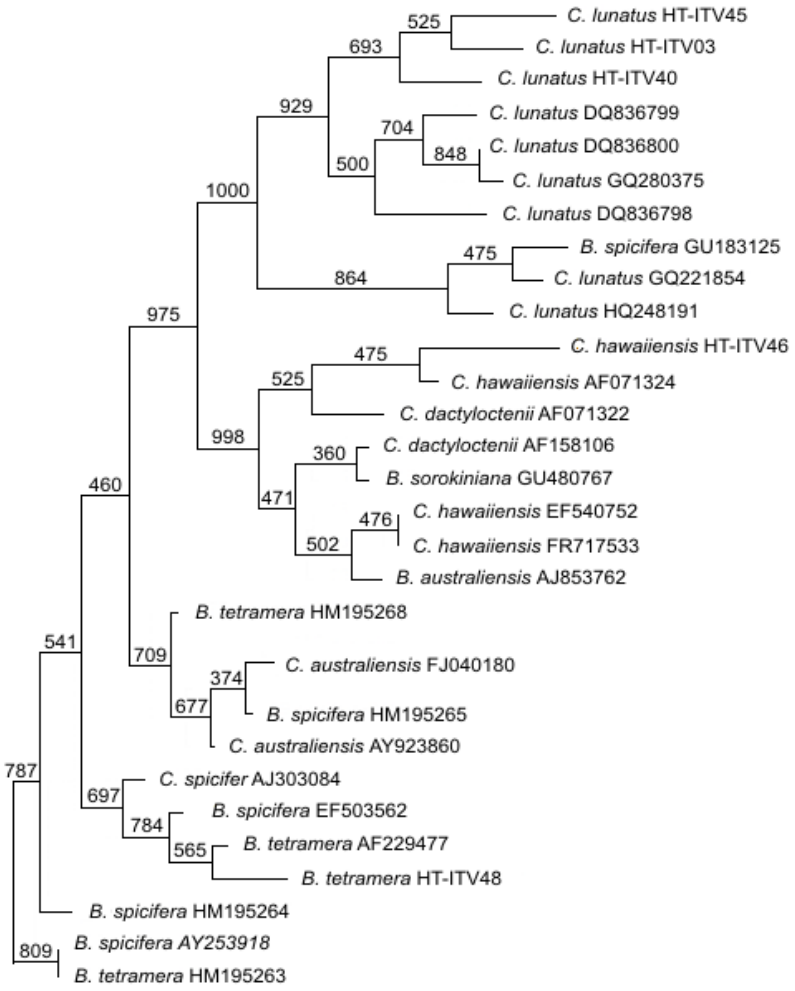

Figure 1. Neighbour-Joining tree derived from fungal ITS sequence data. Numbers on nodes are bootstrap values, i.e. The frequency (%) with which a cluster appeared in a bootstrap test of 1000 runs with Kimura 2-parameters. HT-ITV03, HT-ITV40, HT-ITV45, HT-ITV46 and HT-ITV48 isolated from *T. cacao*.

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Study of Quality and Shelf Life of Ground-Sheep Patties Using a Combination of Enterocin 22 with Sodium Lactate

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Introduction

Raw meat can be easily contaminated by several microorganisms, including pathogens associated to serious food-borne illnesses. In order to extend the shelf life time of refrigerated meat products, synthetic additives are being added into their formulation. However, in recent years the use of synthetic additives has been questioned and consumers increasingly demand the use of natural products as preservatives in foods (1). The application of natural antimicrobials as preservatives have increased the attention of scientists, because consumers demand higher quality and minimally processed foods (2). However many natural antimicrobials have a limited spectrum of antimicrobial activity and are effective only at very high concentrations. A possible solution may be the combined use of natural antimicrobials with chemical preservatives in the food industry. The combination may help to reduce the concentration of chemical preservatives, offering an alternative to satisfy the increasing consumer demand for foods safer, fresh and minimally-processed foods (3,4). Research into synergistic effects of the combined action of natural antimicrobials could increase microbial lethality and achieve an improved level of product safety according to the hurdle technology concept for food preservation (4). Lactic Acid Bacteria (LAB) have been divided into many genera, and those important in food include *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Pediococcus*, *Leuconostoc* and *Streptococcus*. The genus Enterococcus is of particular interest, due it produces natural antimicrobials called bacteriocins. The Bacteriocins are considered as antimicrobial peptides that can be degraded by their transit into the intestinal tract. The enterocin 22 is produced by *Enterococcus faecalis* MXVK22 and it has a great potential as natural biopreservative as they can inhibit spoilage and pathogenic bacteria. The Sodium lactate (SL) is a natural antimicrobial commonly used in meat products. The objective was to evaluate the antimicrobial effect of enterocin-extract (EE) in combination with SL on the quality and shelf life of Ground-Sheep Patties inoculated by *Escherichia coli* JMP 101.

Methods and Materials

2.1 Microorganism and media.

The activation of *E. coli* JMP 101 stocks (donated by Dr. M. Collins, Queen's University of Belfast, North Irland) was performed in trypticasein soy broth (TSB), and for microbiological analysis EMB media (eosin methylene blue) was used as a selective medium for *Escherichia coli* JMP 101, standard count agar (SMA) was used for mesophiles, violet red bile agar (VRBA) was used for coliforms, Casein glucose broth (CGB) was used for *Enterococcus faecalis* MXVK22. All strains were incubated at 37 ºC for 24 hours.

2.2 Bacteriocin extraction.

The EE was obtained from overnight culture of *E.faecalis* MXVK22 in CGB medium under anaerobic conditions, after the culture was subjected to a thermal treatment at 70 ºC during 30 minutes, for proteases inactivation(7). It was centrifuged at 3,100 x g during 20 minutes at 4 ºC and the supernatant was collected. The filtrate was pH adjusted to 6.5 and freeze-dried. The freeze-dried crude extract was label EE and stored at -80°C until its use.

2.3 Bacteriocin inhibitory activity.

The EE was dissolved in sterile water (500 mg/mL) and sterilized by filtration using 0.45 µm membranes (Durapore® Millipore membrane filters, GV, Ireland). Then two seriated dilutions were made and stored in refrigeration until its use. The inhibiting activity was carried out by the diffusion of agar method (8), with modifications, using *Listeria innocua* ATC3309 as the sensitive strain. The technique consisted of depositing 30 µL of the EE dilutions, in wells done on the upper semisolid soy trypticasein agar (TSB) layer (0.8% of agar), which was previously inoculated with the sensitive strain (70 µL of *L. innocua* ATC3309 culture in the logarithmic phase 10^6-10^7cfu/mL, D.O 600nm = 0.3). Bacteriocin activity was evaluated by measuring the inhibition halo formed after incubation at 37°C during 24 h, for the growth of the sensible strain. The inhibiting activity was reported in arbitrary units by milliliter (AU/mL), defined as: the inverse of the dilution (in 2 bases) in which it is obtained an inhibition halo of 2 mm in diameter, between the volume of antimicrobial compound placed in each well, by the factor of conversion of µL to milliliter (9,10).

2.4 Antimicrobial susceptibility testing.

A checkerboard was used to evaluate the combinations of different concentrations from EE (50 to 400 AU/g) and SL (0.096 to 0.77 mL/g), (Table1). The combinations were tested in ground-sheep patties previously inoculated with *E.coli* JMP 101 (5 x10^5 CFU/mL). The meat model system consisted of ground ovine meat inoculated with *E. coli JMP 101* and added with each of combination of preservative solutions previously prepared and molded into the form of burgers (20 g). Afterward, samples were packaged using an oxygen-permeable film and stored at 4°C; leaving a control without antimicrobials. Samples were taken at 0, 3, 6, and 9 days of storage. The combinations were labeled with three numbers, where the first number indicates the day, the second number indicates the concentration of EE and the third number indicates the concentration of the antimicrobial SL, in accordance with a checkerboard (Table1). The variables in study were viable count of *E. coli* JMP 101, mesophiles and coliform, pH and color (CIE a*, b*, L*, H* and C*).

Table1. Checkerboard Array in which serial dilutions of EE with SL

ANTIMICROBIAL		Sodium Lactate (mL/g)				
		0 **1**	0.096 **2**	0.192 **3**	0.385 **4**	0.77 **5**
Enterocin 22 (AU/g)	400 **5**	400/0 **51**	400/0.096 **52**	400/0.192 **53**	400/0.385 **54**	400/0.77 **55**
	200 **4**	200/0 **41**	200/0.096 **42**	200/0.192 **43**	200/0.385 **44**	200/0.77 **45**
	100 **3**	100/0 **31**	100/0.096 **32**	100/0.192 **33**	100/0.385 **34**	100/0.77 **35**
	50 **2**	50/0 **21**	50/0.096 **22**	50/0.192 **23**	50/0.385 **24**	50/0.77 **25**
	0 **1**	0/0 **11**	0/0.096 **12**	0/0.192 **13**	0/0.385 **14**	0/0.77 **15**

The first number indicates the day, the second number indicates the concentration of EE and the third number indicates the concentration of the antimicrobial SL

2.5 Statistical analysis

All variables were analyzed by principal components analysis (PCA) using the Unscrambler v9.8 statistical software.

Results and Discussion

The Figure 1 shows the diagram corresponding to the PCA of the combination of EE with SL. The first and second principal components (F1 and F2); explain 86% of the data variability, where F1 is a component that contains the tonality, chromaticity and a*, while F2 contains the variables L and b*. The diagram shows a negative correlation (inversely proportional relation with time) between chromaticity and a* and pH and viable account (Mesophiles, coliforms and *Escherichia coli* JMP101); also shown a positive correlation (increased proportionally with the time) between the variables L and b, and the variable pH and viable account which are located in continuous sites. The increase in pH due to the

deamination of amino acids (11), as well as microorganisms hydrolyze proteins and amino acids, that make the load of the media change (12).

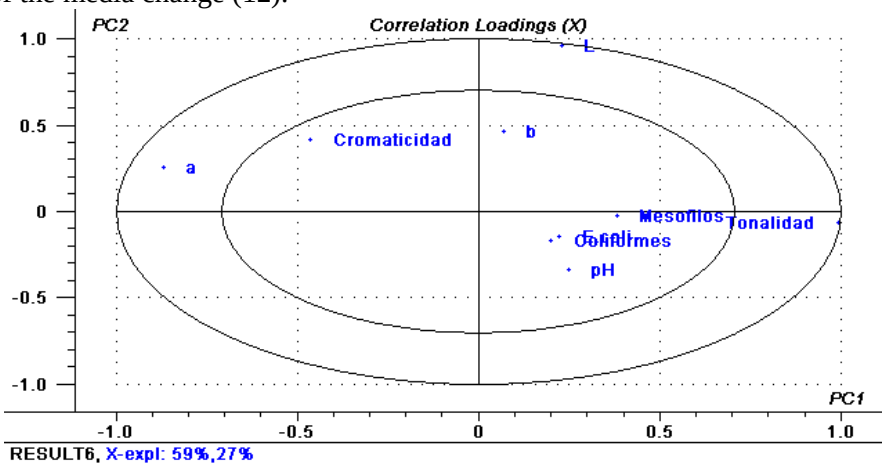

Figure 1. Principal components analysis (F1: 59%; F2: 27 %) of the combination of EE with SL in Ground-Sheep Patties during storage at 4 ° C.

The Unscrambler statistical program showed nine graphics, one for each variable (a, b, L, cromaticidad, tonalidad, coliforms, E.coli y pH). For the study, we selected the graph corresponding to the variable a*, because it have a greater influence on the variability of the first component (F1; figure 1). The PCA of the variable a* was divided into 5 groups significantly different according to the values that were presented during storage. The best combinations were 953 (EE 400 UA/g / SL 0.192 mL/g), 952 (EE 400 UA/g / SL 0.096 mL/g), 943 (EE 200 UA/g / SL 0.192 mL/g), 933 (EE 100 UA/g / SL 0.192 mL/g) y 924 (EE 50 UA/g / SL 0.385 mL/g, figure 2), all of these were taken at nine day. The combinations selected have conserved the parameters of quality according to the bibliography for storaged ovine meat at 4 °C (13). The variable a* showed a decrease during storage, so it was observed a change in the meat of red colour (oxymyoglobin) to brown (metmyoglobin) due to the oxidation (14).

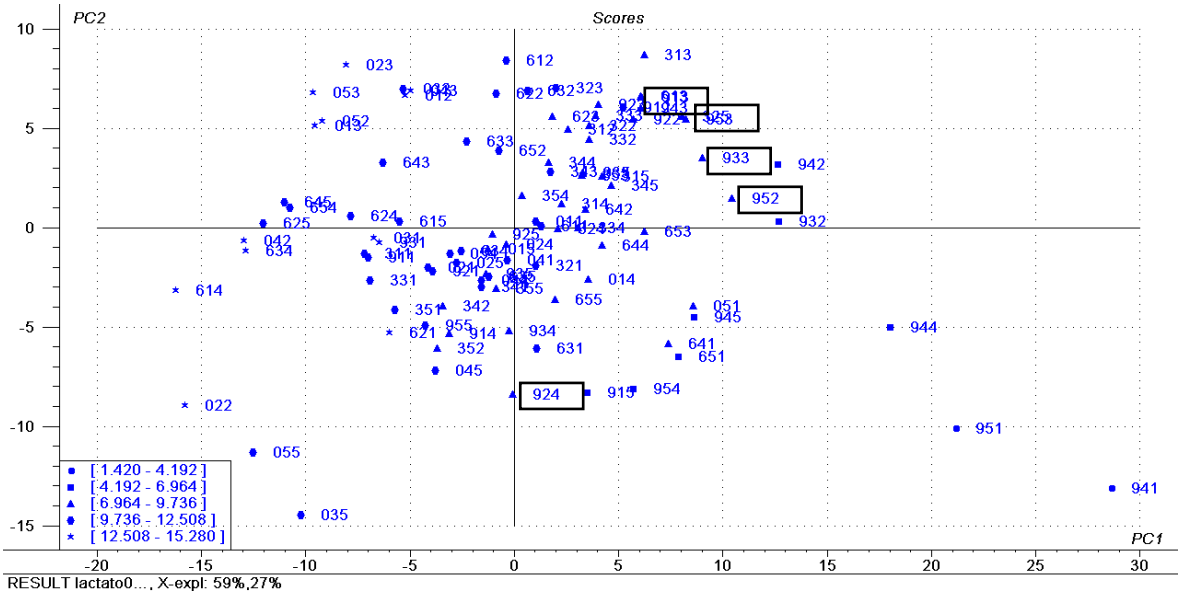

Figure 2. Principal components analysis of the combination of EE with SL of the variable a* in Ground-Sheep Patties during storage at 4 °C (F1: 59%; F2: 27 %). Each symbol represents a group significantly different according to the PCA. The first number indicates the storage day, the second number indicates the concentration level of EE and the third number indicates the concentration level of SL.

The best combinations that kept the levels low of concentration of E. coli (›5.69 log UFC/mL) were 954 (EE 400 UA/g / SL 0.385 mL/g), 952 (EE 400 UA/g / SL 0.096 mL/g), 953 (EE 400 UA/g / SL 0.192 mL/g), 944 (EE 200 UA/g / SL 0.385 mL/g), 945 (EE 400 UA/g / SL 0.77 mL/g), 943 (EE 200 UA/g / SL 0.192 mL/g), 942 (EE 200 UA/g / SL 0.096 mL/g), 933 (EE 100 UA/g / SL 0.192 mL/g), 932 (EE 100 UA/g / SL 0.096 mL/g) and 924 (EE 50 UA/g / SL 0.385 mL/g). The table 1 shows the best combinations that were within parameters of quality for each variable. The results indicated that only the combination 952 (EE 400UA/g / SL 0.096mL/g) conserved all the parameters of quality according to the NOM-034-SSA1-1993, unlike the positive control (inoculated) that only meet the conditions by 3 days of storage. Also the combinations 933 (EE **1**00 UA/g / SL 0.192 mL/g), 945 (EE 400 UA/g / SL 0.77 mL/g), 953 (EE 400 UA/g / SL 0.192 mL/g) and 954 (EE 400 UA/g / SL 0.385 mL/g) maintained the microbiological variables below the limit of 6.7 log UFC/g during storage nine day.

Tabla 1. Selected combinations of bacteriocin with sodium lactate that met the quality standards established

Combination	Mesophiles	*E.coli*	Coliforms	pH	L	a*	b*	hº	C*
924	✓	✓	✓	✓		✓	✓		
932		✓			✓		✓		
933	✓	✓	✓			✓			
942	✓		✓		✓			✓	✓
943	✓	✓		✓		✓			✓
944	✓	✓			✓		✓		
945	✓	✓	✓		✓			✓	
952	✓	✓	✓	✓	✓	✓	✓	✓	✓
953	✓	✓	✓	✓		✓		✓	✓
954	✓	✓	✓	✓	✓		✓	✓	

Limit allowed for the viable counts for ovine meat in storage at 4 °C (11,14,15): account microbiological (6.7 log UFC/g), pH (6 - 7.5), L* (40), a* (8-10), b (9-13), C* 17 (red) and hº (34-54).

Conclusion

The combinations that meet the quality conditions were selected according to PCA. Of all combinations studied only one treatment preserved the quality until the nine day of storage (EE 400UA/g / SL 0.096mL/g). The results indicate that the combination of EE and SL may have a synergistic effect, since it increased the shelf life and maintained quality and food safety.

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Disinfectant Action Of Plant Extracts In Avocado, Tomato And Lemon Inoculated With *Salmonella* spp And *Listeria monocytogenes.*

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Abstract
In spite of the abundant evidence of the antimicrobial activity of plant extracts *in vitro* systems, few studies about its *in vivo* activity have been reported. The aim of this study was to evaluate the antimicrobial activity of flour essential oil reduction in *Listeria monocytogenes* and *Salmonella* spp, both inoculated into the epicarps of hass avocado, saladette tomato and lemon. Native biota present in the fruits before and after washing was determined. Rubbing method was used for fruit inoculation. Inoculates were made with microbial mixtures of *Salmonella* inoculum consisting of three varieties of *Salmonella* and *Listeria* inoculum made with four strains of *L.monocytogenes*. After inoculation, fruits were incubated for 24 hours at 35°C. The fruit surface bacterial concentration was determined with a serial dilution using the technique plate surface extension on ASTEL and TSA agar. The statistical evaluation of extracts disinfectant action was carried using 2x3x4x4 multifactorial experiment, along with method of rising. The factors included: inoculum (*Salmonella* and *Listeria*), fruits (avocado, tomato, and lemon), essential oils (*Cymbopogom citratus*, *Ocimun basilicum*, *Lippia graveolens* and *L.graveolens* phenolic fraction) and essential oil concentrations (0.05, 0.10, 0.15, 0.2 µl/ml). Difference in log UFC/cm^2 between initial and final microbial population in fruits surfaces was obtained. Results were analyzed using ANOVA and LSD. The greatest reduction (p=0.0010) of *Salmonella* spp was obtained with 0.15 µl/ml of *L.graveolens* essential oil in all the evaluated fruits. The greatest reduction (p=0.0005) of *Listeria monocytogenes* was obtained with 10 µl/ml of *C.citratus* essential oil. The microbial inhibition was 3 log for *Salmonella* and 4 log for *Listeria*. According to the UNE-EN-1276, a disinfectant must reduce 3 log of the initial concentration. Thus, the essential oils of *L.graveolens* and *C.citratus* have properties of disinfecting agents for the surface of avocado, tomato and lemon fruits.
Keywords: (*Lippia*, *Cymbopogum citratus*, *Salmonella* spp, *Listeria monocytogenes*)

Introduction
Essential oils, because of their sensory properties and biological activity are used in both traditional and alternative medicine, as in the food, pharmaceutical and cosmetics. Constituted by a complex mixture of up to 43 components (Pino *et al*., 1996; Figuérédo *et al*., 2006; Hernández *et al*., 2009). The main biological activities have been found in essential oils or in some of its individual components are antimicrobial, antifungal, insecticide, antioxidant (Bakkali *et al*., 2008, Soto Hernández, 2007). The *in vitro* antimicrobial activity of essential oils has been amply demonstrated in gram positive bacteria, gram negative bacteria and pathogenic fungi, spoilage bacteria, fungi and phytopathogenic fungi present in the post-harvest storage, as their activity in tomato seed disinfectant (Zapata et al., 2003; Hernández et al., 2009; Cueto-Wong et al., 2010; Derwich et al., 2010; Jasso de Rodríguez et al., 2011; Adams et al., 2011). The essential oil of Lippia graveolens showed fungistatic activity against *Memmoniella* spp, *Penicillium* spp and *Hansfordia* spp, processed meat isolated (Saucier *et al*., 2004), on *Mycosphaerella fijensis* isolated from banana leaves (Obledo et al., 2004), *Erwinina carotovora* and *Fusarium oxysporum* present in diseased plants agave (Obledo *et al*., 2002). The essential oil *Cymbopogon citratus* (lemon grass) has shown *in vitro* antimicrobial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus*

subtilis, Escherichiq coli, Klebsiella pneumoniae, Candida albicans y *Aspergillus niger* (Guerra Ordoñez et al., 2004). For its part *Ocimum basilicum* L., has shown *in vitro* antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis* (Colivet *et al*., 2011). The essential oil may contain biologically active components such as sensorial active. Such is the case of *Lippia graveolens* essential oil has shown antimicrobial and antioxidant activity and is a widely used condiment in Mexican and Italian food for their sensory properties (Arcila-Gónzalez *et al*., 2004).

The sensory components of the essential oil of *Lippia graveolens* are clustered mostly in the terpene fraction (Ricciardi *et al*., 2000) and antimicrobial activity in phenolic fraction. The major components of the phenolic fraction are thymol and carvacrol (Zapata *et al*., 2003; Burt, 2004; Kintzios, 2002). It has been reported that the carvacrol has antifungal and antibacterial activity against *Salmonella* Thyphimurium, *Escherichia coli* O157:H7 y *Listeria monocytogenes* (Thompson, 1998; Lagouri et al, 1993; Burt, 2004). It has been reported that thymol has bactericidal and fungicidal activity (Ninkov, 2001) against *Absidia* spp, *Aspergillus* spp. With the process of fractionation of essential oils can get two or more products with different commercial application (Padilla *et al*., 2007; Zambrana & Hinojosa, 2003). In the case of the phenolic fraction *Lippia graveloens* may have application as disinfectant marketed natural and terpene fraction as a food seasoning flavor. Few study the *in vivo* effectiveness of antimicrobial essential oils as well as the assessment of the bioactivity of fractions of essential oils. There are no reports to date antimicrobial studies in vivo efficiency of essential oil of *Lippia graveolens* full or fractional essential oil or lemongrass and basil. That is why the aim of this study was to determine the in vivo antimicrobial activity of the essential oil of Lipia graveolens entire and its phenolic fraction and essential oil of lemon grass and basil in reducing *Salmonella* inoculated fruits avocado, tomato and lemon.

Material and methods

2.1 Strains used. - We used four strains of *Listeria monocytogenes* (isolated from broccoli i-ii-insulated commercial guacamole, iii-iv-California and Scott A) and 4 *Salmonella* serovars: Agona 039, Montevideo and Typhimurium 0236 (isolated hydroponic tomato) and Typhimurium 0118 (commercial tomato isolated). The serovars were provided by strains Collection Center and Engineering Sciences, University of Guadalajara. The strains were stored at 4 ° C (refrigerator American) Soya Agar Tripticaseína (AST, pH 7.3) (Bioxon, Becton, Dickinson de México).

2.2 Obtaining the fruit- 96 were used hass avocado fruits, tomato saladett 96 and 96 of Mexican lime untreated or apparent physical damage and commercial origin. Prior to its use waterjet washed for 20 sec. With rubbing, and allowed to dry at room temperature.

2.3 Preparation of inoculum. - Three transfers were made every serovar, two 5 ml and the third in 500 ml Tripticaseina Soya Broth (CST Bioxon, Becton, Dickinson of Mexico) for Salmonella, and *Listeria monocytogenes* in Soy Broth with Tripticaseina yeast extract (CSTEL, Bioxon, Becton, Dickinson of Mexico) incubation conditions were 35 º C for 24 h. Serovars were mixed and yielded 4 2 liters of inoculum to a final population (approx. 10^8 CFU / ml). The population was determined by serial dilution in sterile peptone diluent 0.1%, by the method of extension in surface (0.1ml) en Agar Soya Tripticaseína (TSA, Bioxon) y Agar ASTEL, supplemented with 50 µl/ml of Ac. Nalidíxico (AN) (Acros Organics), and incubated at 35ºC por 24h.

2.4 Inoculation of frutos.-The fruits, in three groups, was completely submerged in the two liters of inoculum manually rotating them for a minute. Subsequently allowed to dry for 30 minutes in a laminar flow hood (Beuchat, *et al*., 2001)

2.5 Sanitizing agents evaluated: We used four types of essential oils as disinfectants; entire essential oil of L. graveolens essential oil phenolic fraction of *L. graveolens* essential oil of *C. citratus e*ssential oil and *O. basilicum*. Each extract was used at concentrations of 0.05, 0.1, 0.15 y 0.2 ul/ml.

2.6 Sanitization treatments: - 96 were used sanitizing treatments, comprised of the four types of essential oils, the four concentrations of each essential oil, the three types of fruit and two types of inoculum. Essential oils treatments were conducted by preparing a suspension of each essential oil in pH 6.0 phosphate buffer, stirred vigorously for 5 minutes at room temperature. The fruits, in groups of three, were immersed in the suspension for a minute, rotating them manually. Then placed in a drying rack and allowed to dry for 30 minutes in a laminar flow hood (Zhuang & Beuchat, 1996).

2.6 Evaluation of the efficacy of the plant extracts. - To quantify the initial microbial population on the surface of the inoculated fruits and dried fruits used. Recovered microorganisms adhered to their epidermis using the rinse method, for which is individually placed in each fruit Ziploc ® bag with 50 ml of 0.1% peptone diluent (Bioxon, Becton, Dickinson and Mexico) and manually rubbed for a minute. With the diluent decimal dilutions were inoculated in surface extension in culture media AST (Tripticaseína Soya Agar) with 50 ul / ml nalidixic acid (NA) (Acros Organics). Incubated at 35 ° C/24h (Felisa). Colonies were counted using a Quebec counter. Then quantified the microbial population in the fruits end with essential oil treatment and determined the difference in log UFC/cm2 between initial and final populations for the reduction achieved by each extract.

2.7 Experimental design. - Each of the 96 treatments were performed in triplicate, with three replicates (n = 9). The results were analyzed using ANOVA and multiple range test least significant difference (LSD) (P <0.05), using the computer program Statgraphics Plus 5.1.

Results and Discussion

The results of the in vivo antimicrobial activity of essential oil of *C. citratus*, *O. basilicum L. graveolens* L. and phenolic fraction graveolens on *Salmonella* and *Listeria* are shown in table 1.

Table 1.- Population reduction of *Salmonella* and *Listeria* inoculated in avocado, tomato and lemon due to the addition of essential oils of *C. citratus*, *O. basilicum*, *L. graveolens* L. and phenolic fraction graveolens.

Reduction (log UFC/cm^2)							
		Avocado hass		**Tomato saladette**		**Lemon**	
Concentration (ul/ml)	**Essential oil**	***Listeria***	***Salmonella***	***Listeria***	***Salmonella***	**Listeria**	***Salmonella***
0.05	Phenolic fraction of *L. graveolens*	2.09 ^{b}	2.06 ^{c}	1.44 ^{a}	1.91 ^{c}	1.53 ^{a}	1.89 ^{c}
	L. graveolens	0.43 ^{a}	0.56 ^{a}	1.30 ^{a}	0.81 ^{a}	0.73 ^{a}	0.76 ^{a}
	C. citratus	1.54 ^{a}	0.84 ^{a}	1.28 ^{a}	1.08 ^{a}	1.25 ^{a}	1.69 ^{b}
	O. basilicum	0.38 ^{a}	1.0 ^{a}	0.68 ^{a}	1.07 ^{a}	0.90 ^{a}	0.70 ^{a}
0.1	Phenolic fraction of *L. graveolens*	2.10 ^{b}	3.63 ^{c}	3.07 ^{d}	2.83 ^{c}	2.68^{c}	2.09 ^{c}
	L. graveolens	2.03 ^{b}	1.06 ^{a}	2.34 ^{c}	1.25 ^{b}	1.38 ^{a}	1.23 ^{b}
	C. citratus	3.87 ^{e}	4.73 ^{d}	4.24 ^{e}	3.78 ^{d}	3.82 ^{e}	4.05 ^{e}
	O. basilicum	1.08 ^{a}	1.36 ^{b}	1.54 ^{a}	0.94 ^{a}	0.90 ^{a}	1.85 ^{b}
0.15	Phenolic fraction of *L. graveolens*	4.20 ^{e}	4.46^{c}	4.37 ^{e}	4.72 ^{d}	3.78 ^{e}	3.71 ^{d}
	L. graveolens	4.05 ^{e}	4.46 ^{d}	4.37 ^{d}	4.73 ^{d}	3.64 ^{e}	5.22 ^{e}
	C. citratus	4.90 ^{e}	5.13 ^{e}	3.99 ^{d}	5.33	4.49 ^{e}	5.31
	O. basilicum	2.39 ^{c}	3.00 ^{c}	2.32 ^{b}	3.31 ^{c}	2.49 ^{c}	3.44 ^{c}
0.20	Phenolic fraction of *L. graveolens*	5.00 ^{e}	5.13 ^{e}	5.14 ^{e}	5.33 ^{e}	5.14 ^{e}	5.31 ^{e}
	L. graveolens	5.00 ^{e}	5.13 ^{e}	5.12 ^{e}	5.33 ^{e}	4.45 ^{e}	5.31 ^{e}
	O. basilicum	3.98 ^{e}	4.74 ^{d}	3.13 ^{d}	3.86 ^{d}	3.41 ^{e}	4.31 ^{d}

The initial population of *Salmonella* attached to the fruits of avocado, tomato and lemon was 5.23, 5.43 and 5.41 log10UFC/cm^2 respectively. Data are mean values of population size reduction of *Salmonella* and *Listeria* (log10UFC/cm^2) (n = 9). Values in the same column showing the same letter do not differ significantly by least significant difference for P> 0.05

All essential oils evaluated decreased microbial population inoculated into the skin of the fruit, as shown in Table 1. The reduction is presented in both *Salmonella* and *Listeria* from 0.05ul/ml which was the lowest concentration studied of essential oils. In the case of 0.2ul/ml concentration, which was the highest, the reduction of the microbial population was almost complete, with the use of essential oil of *L. graveolens* and its phenolic fraction in all fruits. Using this concentration caused no apparent damage to the fruit. However, use of high concentrations of essential oils increase the cost of natural disinfectants. Reductions in microbial population greater than or equal to 3 log, which are those expected for a disinfectant agent (López *et al*., 2001), were obtained from the concentration of phenolic 0.1ul/ml using the essential oil fraction of *L. graveolens* and essential oil of *C. citratus*. In the case of essential oil of *C. citratus*, these logarithmic reduction was obtained in the three fruit inoculated with *Salmonella* and *Listeria* and in the case of the phenolic fraction of *L. graveolens* in avocado fruits inoculated with *Salmonella* and *Listeria* inoculated tomato. By using the concentration of 0.15 IU / ml were obtained greater than 3 log reduction. Using the phenolic fraction of the essential oil of *L. graveolens* and essential oil of *L graveolens* and *C. citratus* both *Salmonella* and *Listeria* on three fruits studied. These results were similar to those reported by Park y Beuchat, 1999; Ukuku y Sapers, 2001 when using sodium hypochlorite cantaloupe (3 logUFC/cm^2) and higher than that reported in lettuce and sliced tomatoes where sodium hypochlorite concentration of 200 mg / L did not reduce the population of *Salmonella* (Weissinger et al., 2000; Burnett y Beuchat, 2002).

The reduction of microorganisms varied according to the type of fruit and the type of microorganism inoculated. The population of *Salmonella* showed greater susceptibility to the use of essential oils *Listeria* as shown in Figure 1. This behavior is presented in the fruits of avocado and lemon. In the case of tomato susceptibility essential oils tested were similar in *Salmonella* and *Listeria*. The reduction in microbial population was greater in avocado, followed tomato. The reduction was smaller in lemon fruit. The results obtained in this study show the importance of studies in vivo antimicrobial activity of essential oils and fractionated full, as they show a clear influence on the type of fruit to the antimicrobial efficacy of the essential oils. Likewise, there is evidence of interaction between the microorganism and the type of fruit type in relation to the efficiency of antimicrobial essential oils. With the results of the work is concluded the feasibility of using the phenolic fraction of the essential oil of *L. graveolens* and essential oil of *C. citratus* as natural disinfectants fruits of avocado, tomato and lemon.

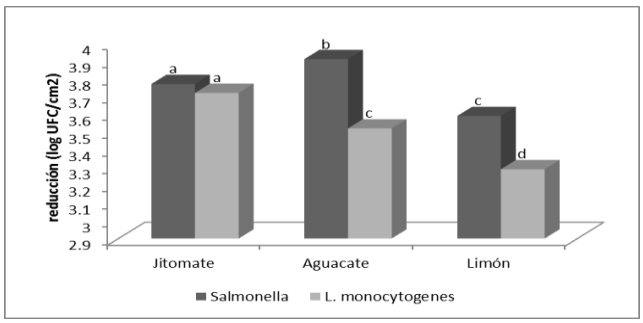

Fig.1. Mean values of reduction of microorganisms (log10UFC/cm2) in fruits avocado, tomato and lemon inoculated with *Salmonella* and *L. monocytogenes*. Columns that display the same letter do not differ significantly (P <0.05)

Acknowledgment

This work was supported by the sectoral fund SAGARPA-CONACYT through the project SAGARPA-2002-C01-0821.

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Identification Of *Staphylococcus aureus* In Milk Of Goat Herds In Three Municipalities Of Matamoros And Viesca, Coahuila; Mexico

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Introduction

Mastitis caused by *Staphylococcus aureus* (*S. aureus*) is one of the most important intramammary infections affecting both the quality and quantity of milk production in dairy cattle worldwide (7). These bacteria live in and out of the udder, in the skin of the nipple and can cause clinical and subclinical mastitis (1). The degree of swelling can vary this depending on the severity with which to react udder irritation source (2).

Mastitis in goats occurs mainly by inadequate care during milking, it is because the udder is exposed to various sources of pollution, which may be directly or indirectly as the bed, meadows, small pens, water, sponges and cloths to wash and dry the teats, the milking and milker's hands (5).

Most infections occur when environmental bacteria come into contact with material contaminated with manure and food, and the presence of *Staphylococcus* spp and mainly Coliforms (*E. coli*), and some gram-negative bacilli (4).

According to SAGARPA in 2011, the Comarca Lagunera was the first in the nation in of goat milk with production reach more than 78 millons liters and usually goat milk on the farm is sold without control in pollution (3) so it is important to know the microbial agents that could interfere with the quality and production of milk, such as *S. aureus* is the main bacteria that causes infection of subclinical and clinical mastitis in goats.

According to this background and considering that there are no studies of the causal agents of mastitis in goats in the Comarca Laguna, the objective of this research was to determine the frequency of the presence *Staphylococcus aureus* in milk obtained directly from the udder and storage tubs.

Materials and Methods

The study was conducted from of February to June 2011, in three dairy herds in the Ejidos Sacrificio, Irlanda and Gilita from municipalities of Matamoros and Viesca; Coah., respectively.

We analyzed 54 samples of milk storage tub and 294 obtained directly from the udder by milking hand, samples were analyzed in the laboratory of Inocuidad Alimentaria y Valor Agregado del INIFAP-Campo Experimental La Laguna, en Matamoros, Coah; The samples were inoculated on Baird Parker agar, recommended by the NOM-115-SSA1-1994.

Analysis of the milk samples in the laboratory

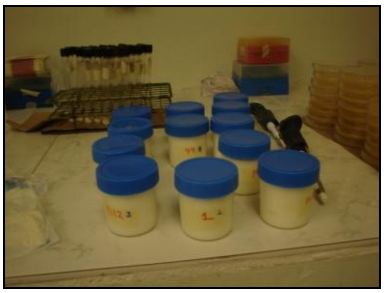

Figure 1. Samples of milk directly from the udder and storage tub

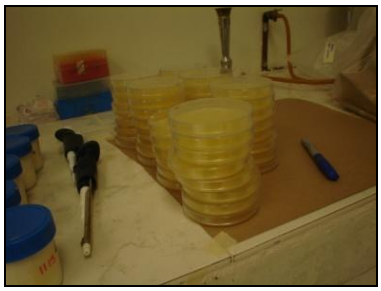

Figure 2. Boxes Baird Parker agar prepared

Figure 3. Inoculation of milk boxes Baird Parker agar

Figure 4. Extending of the dilution with asa of Digitalsky

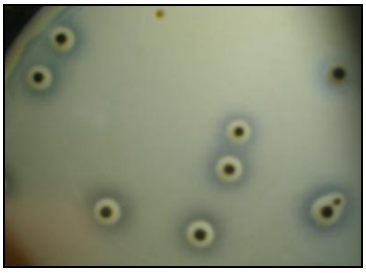

Figure 5. Colonies with characteristics of *Staphylococcus spp*

Results and Discussion

The results indicate that milk storage tub were Staphylococcus spp herd 1 of 179 Colony Forming Units (CFU)/100mL, herd 2 of 127 CFU/100mL and herd 3 of 270 CFU/100mL. For milk obtained directly from the udder were of 192 CFU/100mL of herd 1, 96 100CFU/mL of herd 2, and 53 CFU/100mL of herd 3 and shown in figure 6 and 7.

In figure 6 is observe that in the herd 3 was identified the highest number of colonies with characteristics of *Staphylococcus* spp followed the herd 1 and herd 2.

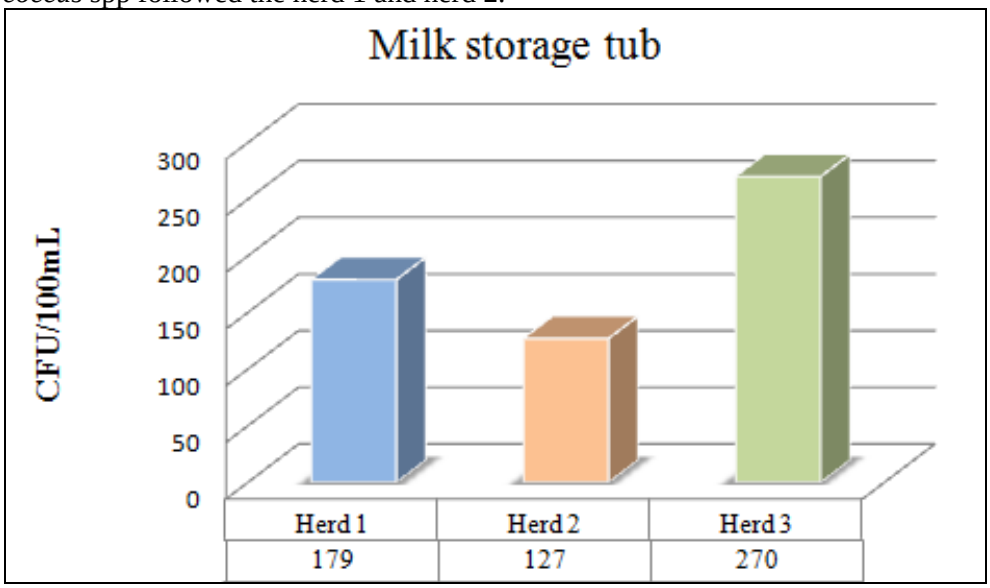

Figure 6. Total average characteristics of bacteria with *Staphylococcus* spp in milk from the storage tub in three dairy herds.

In figure 7 is observe that in the herd 1 was identified the highest number of colonies with characteristics of *Staphylococcus* spp followed the herd 2 and herd 3.

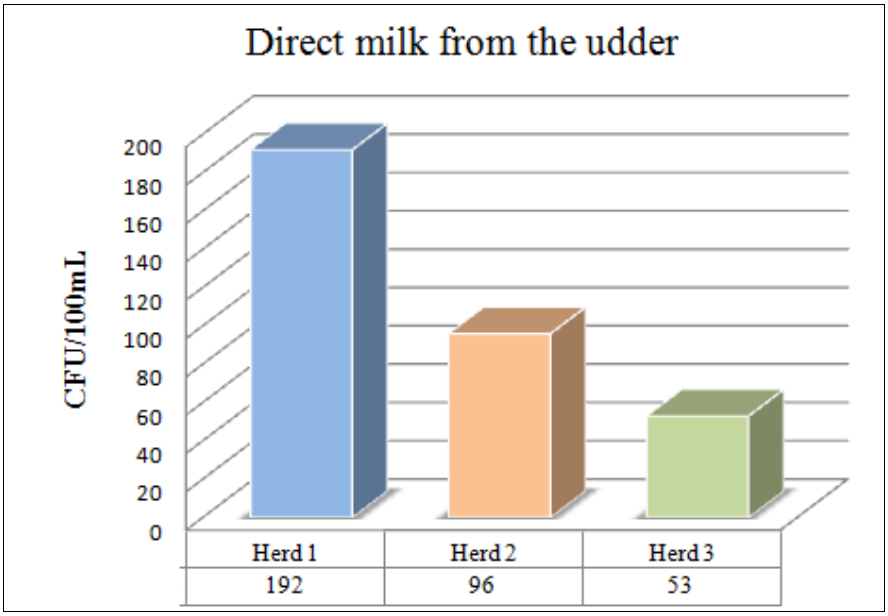

Figure 7. Total average characteristics of bacteria with Staphylococcus spp in milk obtained directly from the udder in three dairy herds.

Giving a total of 576 CFU and 341 CFU, respectively, were selected with typical 121 CFU of *Staphylococcus aureus* (FIG. 8), which are being confirmed through Gram stain positive (+) in clusters (Figure 9) Catalase positive (Figure 10) and coagulase-negative *Staphylococcus* (ECN).

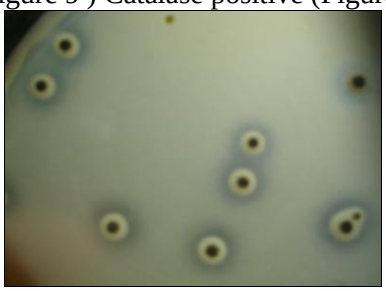

Figure 8. Colonies typical of *S. aureus*

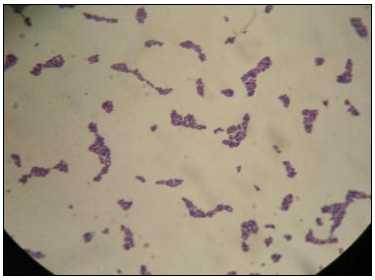

Figure 9. Gram stain +

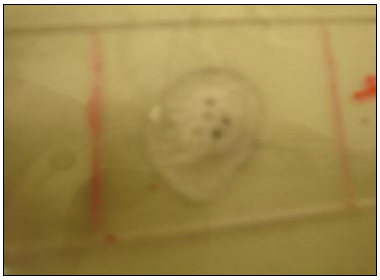

Figure 10. Catalase positive

Of the 121 CFU with characteristics typical of *Staphylococcus aureus* cultures were obtained 88 Gram positive bacteria, 65 were Catalase positive and for ECN zero (Figure 11).

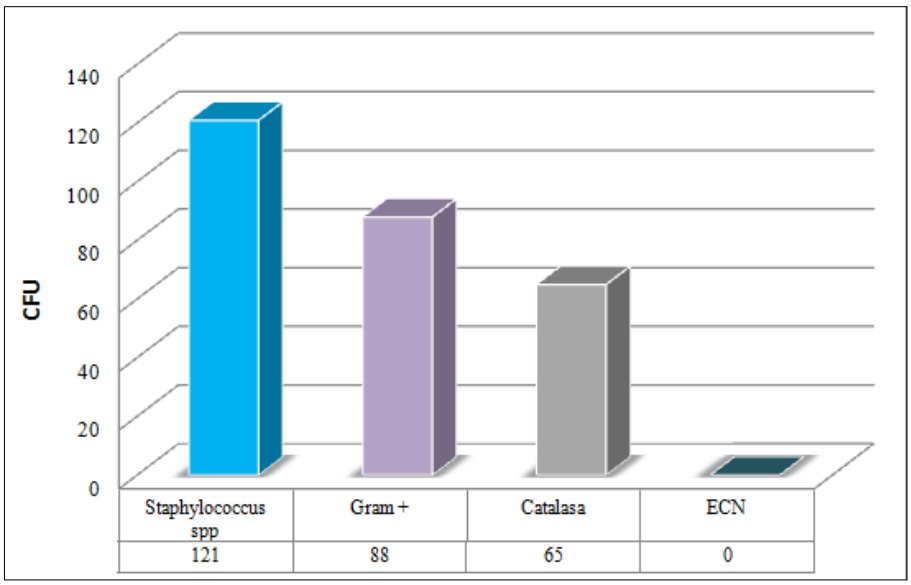

Figure 11. Average colony tests identified by Gram +, Catalase positive, *Staphylococcus spp* and ECN.

Conclusions

With the results we can infer that *S. aureus* was not found in milk storage tubs and direct from the udder, however found were *S. intermedius* and *S. hyicus*. It is recommended to study in other regions in Comarca Lagunera and implement other techniques to identify other bacterias involved in mastitis in dairy goats.

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Carotenoids Production By*Rhodotorula glutinis* YB-252 In Solid-State Fermentation

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Abstract

Carotenoids are an important group of natural pigments with specific applications as colorants, feed and food supplements, and nutraceuticals. Nonetheless, due to the increasing importance of this molecule, alternative methodologies to produce higher and more stable carorenoids-rich extracts have been developed. Polyurethane foam (PUF) and sugarcane bagasse (SB) were tested as supports and selection was based on determination of water absorption index (WAI), critical humidity point (CHP) and packing density (PD). Carotenoids production was carried out in column and tray bioreactors. Carotenoids production in SSF had the highest pigment production at 72 h of culture tme, with a yield of 2.41 mg/kg using PUF as support and a column bioreactor, while the lowest yield (0.3 mg/kg) was obtained using SB and tray bioreactor.
Keywords: *Rhodotorula glutinis*, carotenoids, polyurethane foam, bioreactor.

Introduction

Carotenoids are an important group of natural pigments with specific applications as colorants, feed and food supplements, and nutraceuticals (1). Carotenoids are derived highly unsaturated from isoprene (2). Around 600 different carotenoids of carotenogenic microorganisms, mainly produced by filamentous fungi and yeasts, and some species of bacteria, algae and lichens have been reported (3). Despite the availability of a variety of natural and synthetic carotenoids, only few of them have been exploited commercially, including β -carotene, astaxanthni, canthaxantin, lutein, capsanthin and lycopene.

Commercial production is mainly based on chemical synthesis and extraction from natural sources. Nonetheless, due to the increasing importance of this molecule and the mixture of the carotenoids produced in plants and algae, alternative methodologies to produce higher and more stable carotenoids-rich extracts have been developed (4). One alternative is the use of solid-state fermentation (SSF), which is a simple technique for microbial growth and production of metabolites. This bioprocess does not require complex equipment, which results in lower production costs (5).
Furthermore, *Rhodotorula glutinis*, pigment yeast has been used for microbial production of βcarotene (6) at a relatively high growth rate (5). The treatment of the microorganism with 2-(4-chlorophenylthio)-triethylamine been shown to cause the accumulation of lycopene (7).

The objective of this study was to select the best support (polyurethane foam or sugarcane bagasse) for fermentation and to chose the more suitable bioreactor type (column or tray) for carotenoids production by SSF.

Materials and methods

Rhodotorula glutinis YB-252 was obtained from the Department of Agriculture of United State of America (USDA). The polyurethane foam (PUF) was provided by the company Johnson Controls

Automotriz Mexico (Saltillo, Coahuila) y the sugarcane bagasse (SB) was obtained from local market. The bioreactors were employed in plastic columns and trays. ().

The material was prepared as follows: milled, passed through a sieve number 20, washed with hot water (80 °C) and subsequently with cold water (20 °C), this treatment was repeated three times. Next, the supports were dried in an oven at 60 °C for 46 h.

The critical humidity point (CHP) was estimated by adding 1 g of sample to a thermobalance at 120 °C for 60 min.

Water absorption index (WAI) was determined. The sample (2.5 g) was suspended in 30 mL of distilled water. Was stirred for 1 min and centrifuged at 3000 g and 25 °C for 10 min. The supernatant was discarded, and the WAI was calculated from the weight of the remaining gel and expressed as g gel/ g dry weight.

The packing density (PD) was determined by measuring the volume occupied by each of the carriers in a column 3 cm in diameter.

Carotenoids production was carried out in column and tray bioreactors containing 3 g of support and 7 mL of yeast and malt extract medium. The inoculum was prepared on yeast and malt extract medium (3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, 10 g/L glucose) at a concentration of $1.8x10^6$ cells/mL and each of the impregnated supports in a ratio of 7 mL of inoculum per 3 grams of support, to have moisture of 70 %. Bioreactors were incubated at 30 °C for 7 days.

Carotenoids extraction from yeast biomass was performed using dimethyl sulfoxide (DMSO). The support and the biomass were suspended in 10 mL of DMSO. The suspension was agitated on a rotatory shaker operated at 250 rpm at 40 °C for 2 h and was then filtered. Total carotenoid concentrations were quantified spectroscopically at an optical density of 480 nm.

All analyzes were performed in triplicate. The results were analyzed with a compare means test of Tukey, using statistical software SAS 9.0 with a value of p= 0.05.

Results and discussion

CHP is a point which the water in system is no longer free to be chemically bound to the material and cannot be used by the microorganisms for metabolic functions. The SB and the PUF presented CHP of 26 and 40 %, respectively. These values are within the recommended value. Mussatto*et al*. (2009) report a CHP for PUF of 40 %, while Mata-Gomez (2009) reported 35 %, the results obtained in this study are similar to those obtained by these authors.
WAI, an indicator of the sample ability to absorb water, depends on the availability of hydrophilic groups which bind water molecules, and on the gel forming capacity of macromolecules (8). Among the materials tested, PUF showed higher WAI than SB, as shown in Table 1. Mussatto*et al*. (2009) report a value for the PUF of 12.2 g/g dry weight, this difference from the results of this work can be attributed to the particle size that these authors used. Packing density is shown in Table 1.

Table1. Values of CHP (%), WAI (g/g dry weight) and PD (g/mL) of different material evaluated.

Material	CHP (%)	WAI (g/g dry weight)	PD (g/mL)
PUF	$40^{a}\pm1.4$	$7.34^{a}\pm 0.22$	$0.098^{b}\pm0.006$
SB	$26^{b}\pm1.7$	$6.16^{b}\pm 0.34$	$0.150^{a}\pm0.001$

Carotenoids production in SSF had the highest values at 72 h, with a yield of 2.41 mg/kg using PUF as support and column bioreactor, while the lowest yield (0.3 mg/kg) was obtained using SB and trays (Figure 1). These differences may be due to the packing density, which is lower for PUF and possibly support composition.

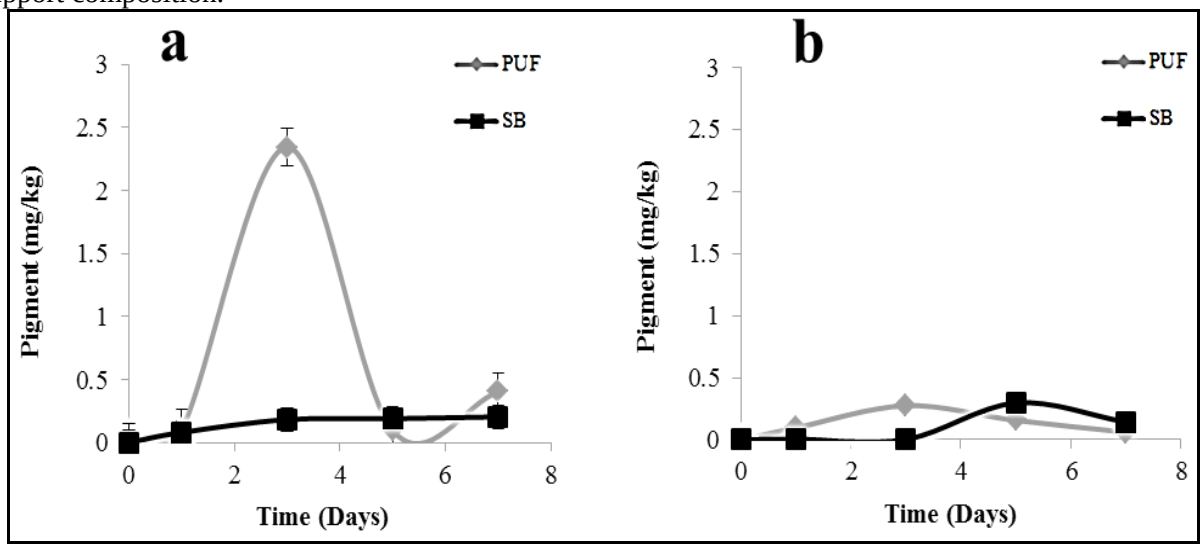

Figure1. Pigment produced (mg/kg) using column (a) and tray (b) as production unit.

Malisorn and Suntornsuk (2009) reported a production of β-carotene with *R. glutinis* in continuous culture fermentation using radish brine; these authors reported a pigment yield of 0.092 mg/L. Furthermore, Roadjanakamolson and Suntornsuk (2010) produced β-carotene from *R. glutinis* using rice bran as support and obtained a yield of 1.65 mg/kg. The results obtained in this study are higher than those reported by other researchers.

According to the obtained results, we conclude that carotenoids production on SSF is better when PUF and column bioreactor are used.

Acknowledgements

Hernández, A. Y., thanks to the Mexican Council for Science and Technology (CONACYT) for the post-graduate scholarship.

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Identification And Characterization Of Peptidoglycan Hydrolases Of Lactic Acid Bacteria Isolated From Cotija Cheese

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Introduction

Cotija cheese is a Mexican artisanal-made dairy product manufactured in farmhouses in the mountains between the states of Michoacan and Jalisco. It is produced from whole raw milk. Clotting is made with commercial chymosin, after curd cutting and whey draining, dry salting is accomplished by paste kneading. No thermal step, neither starter cultures are used in the manufacturing process (1). After a three-month ripening stage, it becomes a characteristic hard, crumbly, salted and smelly cheese. Moreover, there are changes in microbial populations, as the decrease in coliform bacterial counts (1, 2).This effect could be caused by physicochemical factors such as the loss of humidity during ripening; but it may also be the consequence of growth of lactic acid bacteria (LAB), like enterococci, and the production of antibacterial compounds (2).The Enterococci group is foundinseveral spontaneous fermented products (3), and it is one of the most abundant bacteria in artisanal cheeses (4), due to the fact of its ubiquity and its tolerance to the typical salt and acid concentrations found in cheese (5).In many artisanal cheeses the presence of LAB plays an important role in the fermentation process that occurs during ripening, because they are involved in lactic acid production and are relevant in ripening and during flavor development (6).Furthermore, LAB may play a part on the selection of the microorganisms that can survive in advanced stages of ripening and that could contribute to diminish or inhibit the growth of pathogen bacteria (7) by producing compoundssuch as organic acids, bacteriocins or peptidoglycan hydrolases (PGH)(8).PGH are enzymes that hydrolyze glucosidic or peptide bonds in peptidoglycan, the bacterial cell wall main component; in consequence PGH have different roles in growth, turnover, and cell lysis. Depending on the bond that they hydrolyze, PGH are classified into *N*-acetylmuramidases, *N*-acetylglucosaminidases, *N*-acetylmuramoyl-L-alanine amidases and endopeptidases (9).Before in our group, an *Enterococcus faecalis* strain fromartisanal Cotijacheese that produces extracellular proteins with antibacterial activity against *Staphylococcus aureus*and *Escherichia coli*, was isolated. The aim of this work was to identify, clone, express and characterize the extracellular proteins responsible for such activity.

Methods and Materials

The *Enterococcus faecalis* strain was activated in 20-ml MRS broth(Oxoid), and incubated at 37 ± 2 °C with 250 rpm shaking for 24 h (Incubator Shaker Innova 4000, New Brunswick Scientific). Afterwards, the supernatant was collected by centrifugation at 10,000 *g* for 30 min at 4 °C (Beckman model J2-MC centrifuge). The pH was adjusted to 7.0 using 1 N NaOH and the supernatant was then filtered using a 0.22 µm cutoff sterile membrane (Durapore Membrane Filters HVLP, Millipore). The protein in the supernatant was precipitated using ammonium sulfate (40% w/v) and dialyzed against water. Afterwards, proteins were precipitated with 10% w/v trichloroacetic acid (TCA)and finally suspended in 50 µl phosphate buffer, this partially purified protein sample was used for SDS-PAGE and zymograms.

Ten-percent polyacrylamide SDS gels were prepared according to Laemmli (10). Thirty-microliter samples were mixed with 8 µl loading buffer and were boiled for 5 min before loading polyacrylamide gels. Electrophoresis was run in a Mini-PROTEAN III cell unit (Bio-Rad) at 80 V for 1.5 h and stained with G-250 Coomassie blue (Bio-Rad). For zymograms, renaturing SDS-PAGE electrophoresis was

performed using 10% (w/v) polyacrylamide separating gels containing 0.2% w/v cells of *Micrococcus lysodeikticus* ATCC 4698 (Sigma-Aldrich),*E. coli* (DH5α, Invitrogen) or *S. aureus*as substrate.Following electrophoresis, gels were incubated in renaturing buffer (100 mM, Tris-HCl pH 8.0, 1% v/v Triton X-100) and stained with 0.01% w/v KOH and 0.1% w/v methylene blue for 30 min and then destained in distilled water (11). The protein bands of interest were excised from the Coomassie-stained SDS geland sent foridentification by LC-MS/MS (Department of Biochemistry, Faculty of Medicine, UNAM). Protein identification was performed with the MASCOT search algorithm. Searches were conducted using the Firmicutes (Gram-positive bacteria) subset of the NCBInr database.
Two of these proteins (expected molecular weight 54 and 72 kDa) were cloned and expressed for its characterization using the pET system (Novagen). *E. faecalis* genomic DNA was extracted using the Fast ID Genomic DNA Extraction Kit (Genetic ID NA, Inc.). Genes encoding for the proteins of interest were amplified using specific primers and*Pfu* DNA polymerase. The PCR products and the pET-22b(+) were then digested with the enzymes *Xho*I and *Nco*I (Fermentas) and ligated using the T4 DNA Ligase (Fermentas).BL21(DE3) and BL21(DE3)pLysS competent cells (Novagen) were transformed with the construct for 54-kDa and 72-kDa proteins, respectively. Clones containing inserted fragments were selected and analyzed by sequencing (Macrogen, Seoul, Korea). Positive transformants were grown overnight at 37°C in LB medium containing 100 µg/ml ampicillin for BL21(DE3) and 34 µg/ml in addition for BL21(DE3)pLysS. The culture was then inoculated into fresh LB medium (1:100 dilution) containing antibiotics and grown at 37°C to an A_{600} of 0.6–0.8. After induction with IPTG, the culture medium was collected by centrifugation, concentrated by lyophilization and dialyzed against water using a 50 kDa cutoff dialysis tube (Spectra/Por7, Spectrum) with slight stirring, at 4 °C.The concentrates were analyzed bySDS-PAGE and zymograms.The recombinant enzymatic activity was evaluated by thermal stability at 37, 50, 60, 70, 80 and 90°C during 30 and 60 min.The effect of pH was evaluated at 5, 6, 7 and 8 and ionic strength at NaCl concentrations of 0, 1, 10, 50, 100, 200, 300, 500 and 1000 mM.

Results and Discussion

Results from SDS-PAGE and zymography showed a 95-kDa band that was active against the three indicator microorganisms (Fig. 1). This band wasanalyzed by LC-MS/MS and was identified by MASCOT and protein BLAST.It contained peptides matching two different proteins. The first was a putative *N*-acetylmuramoyl-L-alanine amidase from *E.faecalis* V583 with a theoretical molecular mass of 54 kDa; peptides derived from the trypsin digestion showed a 46% coverage of the complete sequence, with 100% identity. This protein has a domain that belongs to the lysozyme superfamily, with a highly conserved region of *N*-acetylglucosaminidases. In the carboxyl terminus it has a conserved domain of cysteine, histidine-dependent amidohydrolases/peptidase (CHAP). It has been reported that CHAP domains are associated to other catalytic domains in PGH, which allows an enzyme to have several hydrolytic activities. This variety of domains present in PGH, suggest that they can act synergistically to hydrolyze different substrates in a multifunctional way (12). The second protein was another enzyme, namely autolysin, from the same *E. faecalis* strain, with a theoretical molecular mass of 77 kDa; peptides obtained from the digestion of the band had a 5% coverage of the protein sequence with an identity value of 100%. It also belongs to the lysozyme superfamily and it has a highly conserved *N*-acetylglucosaminidase region with six Lysin motif regions (LysM), which are small domains involved in peptidoglycan binding. The comparison of both *N*-acetylglucosaminidase sequences shows that, although they are different, their catalytic region is conserved. Only the 77-kDa enzyme has been identified and characterized as an *N*-acetylglucosaminidase with an active form of 72 kDa; its specificity, catalytic domain and peptidoglycan binding motifs (LysM) have been analyzed (13, 14). The 54-kDa protein is a new peptidoglycan hydrolase not previously reported.

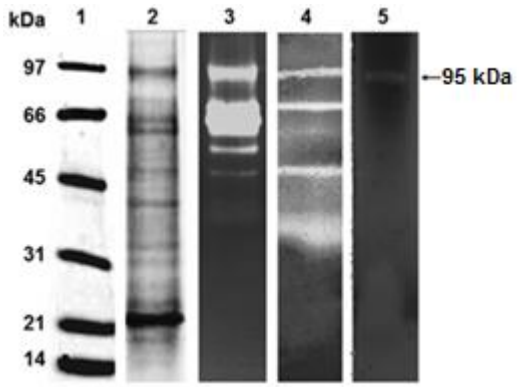

Fig. 1.Protein profile and lytic activity after precipitation with TCA. Lane 1, high molecular weight marker (Bio-Rad); lane 2, Coomassie stained protein profile (SDS-PAGE 10%). Zymograms against: lane 3, *M. lysodeikticus*; lane 4, *S. aureus*; lane 5, *E. coli*. Total protein per well 25 μg.

Both enzymes were cloned and expressed in *E. coli*, but with a molecular mass higher than expected. The 54-kDa protein had a molecular mass of 80 kDa, and the 72-kDa protein of 86 kDa (Fig. 2). Although the pET-22b(+) vector directs the protein to periplasm, the mayor activity was found in the extracellular medium. This may be because the recombinant enzymes are PGH and they can hydrolyze the *E. coli* peptidoglycan, causing its lysis and releasing its content to the medium.

The characterization of the recombinant enzymes was performed with the extracellular concentratedextract. The 80-kDa protein had an optimum pH value of 6 (Fig. 3A), it retained 50% of activity with a 90°C 30 min-treatment (Fig. 4A), and in NaCl 300 mM (Fig. 5A). The 86-kDa protein had an optimum pH between 6 and 7 (Fig. 3B), it retained 50% of activity after a 70°C 30 min-treatment (Fig. 4B), and in NaCl 500 mM (Fig. 5B).

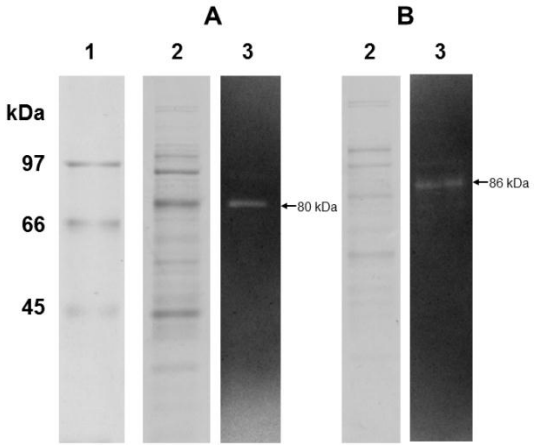

Fig. 2.Protein profile and lytic activity of extracellular extracts of both clones.A) 54-kDa clone, B) 72-kDa clone. Lane 1, high molecular weight marker (Bio-Rad); lanes 2, Coomassie stained protein profile (SDS-PAGE 10%). Lanes 3, zymograms against*M. lysodeikticus*. Total protein per well 25 μg.

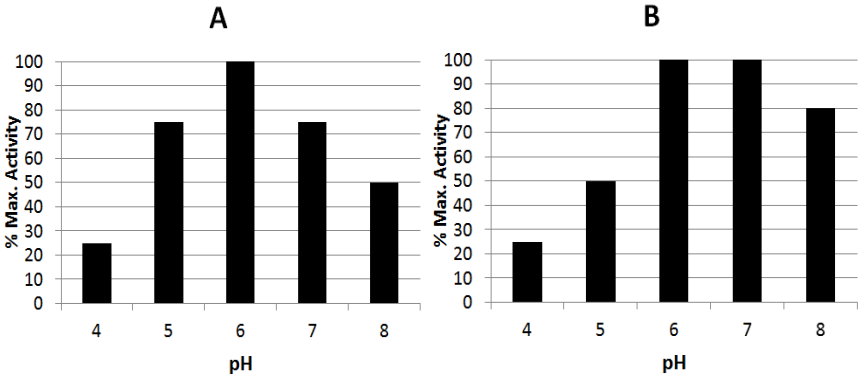

Fig. 3.pH activity profile of:A) 80-kDa protein, B) 86-kDa protein. Enzymatic activity was assayed onzymograms against *M. lysodeikticus*.

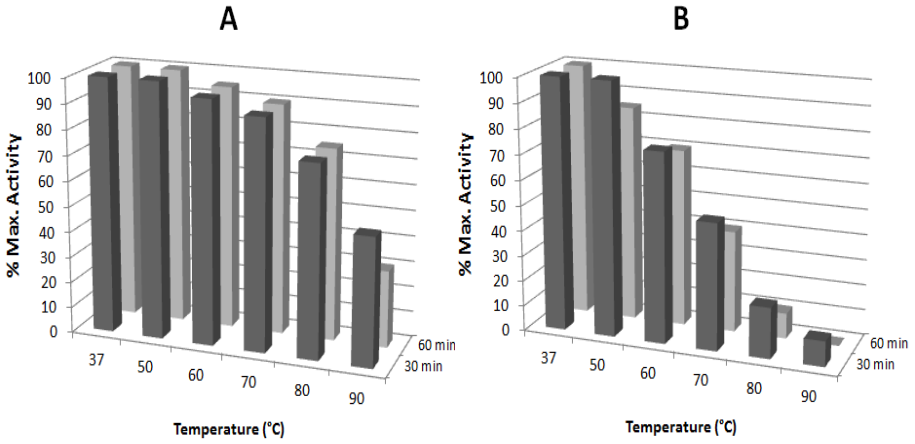

Fig. 4. Temperatura stabilityof: A) 80-kDa protein, B) 86-kDa protein. The enzymes were incubated at different temperatures for 30 or 60 min. Lyticactivity values are expressedrelative to the control (37°C).

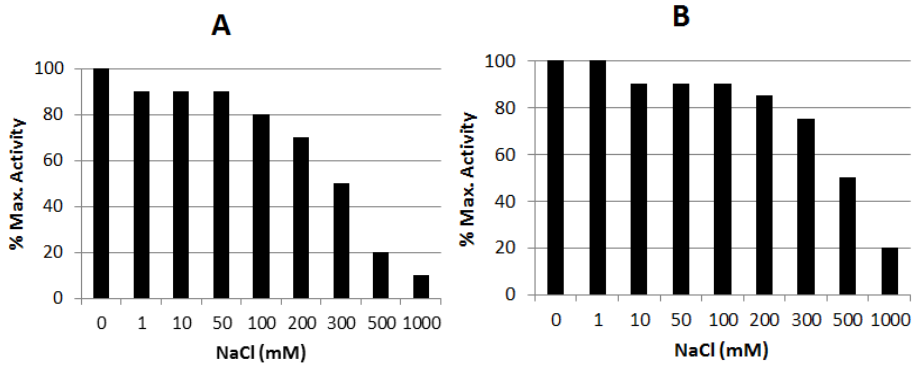

Fig. 5.Influence of NaClconcentration on activity of: A) 80-kDa B) 86-kDa protein. Lyticactivity values are expressedrelative to the control (0mM).

The 86-kDa protein showed its maximum activity at a similar pH value reported by Eckert *et al* (14) and decrease rapidly at acid values. Both recombinant enzymes showed high thermal stability compared with other PGH that lost up to 50% of activity with 60°C/30 min-treatmentsand are even considered thermoresistant (15).

Due to the fact that the enzymes characterized in this work showed lytic activity against microorganisms important in public health,and that they are stable to temperature, active at pH values and NaCl concentrations found in food, they have a potential biotechnological use as biocontrol agents.

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Pathogen Inhibition Profile And Safety Evaluation Of Enterococci Isolated From Cotija Cheese

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Introduction

The genus Enterococcus belongs to a group of microorganisms known as lactic acid bacteria (LAB). This genus consists of non-spore-forming, Gram-positive low G/C content (<50%), catalase-negative bacteria that are grouped as individual cocci, in pairs or short chains, and produce lactic acid as the major end product of glucose fermentation. *Enterococcus* spp. are part of the intestinal microbiota in different animal species, a complex ecosystem in which a delicate balance exists between the intestinal microbiota and the host. In this system, *Enterococcus faecalis* and (to a lesser extent) *Enterococcus faecium* are the dominant species, which is believed to play a key role in the microbial balance. For that reason they have been recently used as probiotics. Moreover, they are involved in spontaneous fermentation of many foods due to their ubiquity and their tolerance to sodium chloride and nitrate. This tolerance allows them to survive and to multiply during fermentation (1). A considerable number of strains belonging to different species of the genus *Enterococcus* display many interesting biochemical and biotechnological properties such as proteolytic, lipolytic, esterolytic and other enzymatic activities, citrate utilization and bacteriocin production (2). However, their presence in food is controversial, because they are also considered undesirable microorganisms, indicators of fecal contamination and responsible of food spoilage (3). This controversy has been emphasized due to the clinic relevance that enterococci have acquired in the last decade, because they have become the third cause of hospital diseases, causing urinary tract infections, bacteremia and endocarditis (4).

On the other hand, Cotija cheese is a farmhouse product, from which we have isolated LAB. Some *Enterococcus* spp. strains showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, where the antibacterial effect was influenced by the culture medium. The aim of this research was to determine their antibacterial activity against microorganisms important in food safety and to elucidate their possible pathogenic potential.

Methods and materials

Six *Enterococcus* strains (strains A and D, *E. faecalis*; strains B, C, E and G, *E. faecium)*, isolated previously, were cultured in MRS broth for 12 h at 37 °C. Cell–free supernatants were obtained by centrifugation at 10,000 g for 15 min at 4°C. Supernatants were adjusted with concentrated NaOH to pH 7, filtered (0.22 mm membrane cutoff) and concentrated by lyophilization.

An agar well diffusion assay was used for detection of antagonistic activity. BHI agar (1%) plates were overlaid with 10 mL of molten BHI agar (0.8%) inoculated with a culture of 8 h (20µL) of indicator strain. Wells 9 mm in diameter were made in the agar plates. Supernatants (filtered, neutralized and concentrated) were placed into each well. The plates were then incubated aerobically for 24 h at 37°C and were subsequently examined for zones of inhibition (10 mm clear or larger zones around the well were scored as positive inhibition). The indicators used were *Escherichia coli* DH5α, *Staphylococcus aureus* ATCC 6558, *Bacillus cereus* CFQ-B-230, *Bacillus subtilis* ATCC 6633, *Lactobacillus paracasei* CFQ-B-90, *Listeria monocytogenes* CFQ-B-103, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 14028, and *Streptococcus pyogenes* CFQ-B-218 were obtained from Faculty of Chemistry (UNAM) culture collection, *Enterococcus faecium* E and *Enterococcus faecalis* A (isolated

previously). We used Nisaplin ® as positive inhibition from Gram positive and Kanamycin from Gram negative bacteria.
SDS-PAGE and zymograms were performed for protein analysis. SDS-PAGE consisted of Tris-Tricine SDS-10% gels (5), stained with commassie blue G250 and was run in a Mini-PROTEAN III cell unit (BIO-RAD) at 80 V for 4 h. The bacteriolytic proteins were identified by zymography against *Micrococcus lysodeikticus* (0.2% w/v) and SDS-PAGE (6); gels were incubated 18h at 37°C and stained with methylene blue. The supernatants (neutralized, filtrated) were precipitated with trichloroacetic acid (TCA) (10% v/v) before analyzed by SDS-PAGE. The molecular masses of the bands that showed lytic activity were determined using high molecular weight marker. The intensity of each band was calculated using a densitometer AlphaDigiDoc 1000 software (BIO-RAD).
Besides, several populations of enterococci were isolated from 12 Cotija artisanal chesses in EVA broth and Kanamycin plates, and the DNA was extracted using the FAST-ID kit (Genetic-ID) and they were analyzed by multiplex PCR looking for virulence factors, namely aggregation substance *(asa)*, enterococcal surface protein (*esp)* and cytolysin A *(cylA)* (7). Analyses were performed to test: hemolysis activity, on sheep blood agar (5%); adherence to Caco-2 and HT-29 cells incubated during 3h with the strains (8); and ability to form biofilms on polystyrene microplates (Abs 620nm) (8). Resistance to vancomycin was evaluated as well.

Results and discussion

Inhibitory activity against bacterial growth

Inhibitory spectrum is shown in Table 1 and is reported in specific activity units (mm inhibition/ mg protein). Only four Gram-positive species tested were inhibited by the partially purified extract. *Yersinia enterocolitica* was the most strongly inhibited Gram-negative specie, whereas there was no effect on *Ps. aeruginosa*. Previous research indicates that enterococci produce antibacterial compounds of proteinic origin: bacteriocins and peptidoglycan hidrolases (PGHs), which have the ability to inhibit Gram-positive bacterial growth, some of them of extreme importance in food safety (1).
Bacteriocins kill closely phylogenetically related bacteria by various mechanisms such as inhibiting cell wall synthesis, permeabilizing the target cell membrane, etc. (9). However, none of the extracts was able to inhibit *Lactobacillus* or enterococci growth. For this reason we analyzed their electrophoretic prolife and bacteriolytic bands, looking for their probability to be PGH, which in turn can exert antibacterial activity. PGHs hydrolyse glycosidic bonds or peptides found in the peptidoglycan, the main component of bacterial cell wall, producing cell lysis (10).

Table 1 Antibacterial spectrum

	Gram-positive				**Gram-negative**		
Target microorganism	*L. monocytogenes*	*S. pyogenes*	*B. cereus*	*S.aureus*	*S. enterica* Typhimurium	*E. coli*	*Y. enterocolitica*
Partially purified extract	mm inhibition/ mg protein						
Strain A	0	14.25	18.82	48.52	13.58	0	13.11
Strain B	0	18.81	11.55	23.43	8.46	32.54	17.22
Strain C	7.62	0	22.18	29.90	11.56	29.51	15.65
Strain D	9.68	11.01	20.98	30.20	14.09	0	21.42
Strain E	0.00	0	13.25	41.61	5.02	11.15	14.15
Strain G	0.00	0	14.44	57.72	6.38	0	7.94

Results showed that all strains isolated from Cotija cheese had a similar electrophoretic profile with predominant bands at 60-85 kDa (Fig 1A). A 75-kDa band was detected in B, C, D, G strains and a 85-

kDa band in A, G strains with bacteriolytic activity (Fig 1B). Some PHGs have been reported in enterococci and their molecular weights are approximately 72 kDa (11), consequently this group of enzymes could be responsible for the antibacterial activity observed.

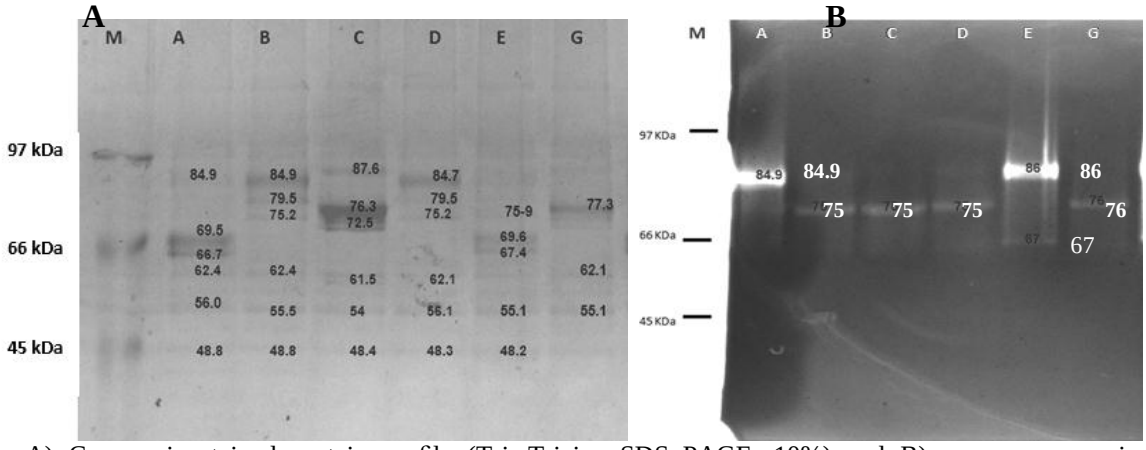

Fig 1. A) Coomassie stained protein profile (Tris-Tricine SDS–PAGE, 10%) and B) zymograms agains *M. lisodeytikus*. The supernatants were precipitated with TCA (10%). *M:* high molecular weight marker*;* A: *Enterococcus faecalis*; B: *Enterococcus faecium*; C: *Enterococcus faecium*; D: *Enterococcus faecalis*; E: *Enterococcus faecium*, G: *Enterococcus faecium.*

Virulence factor screening

For the safety evaluation of bacterial strains, the European Food Safety Authority has proposed a new system to be used in food applications, the "Qualified Presumption of Safety" (QPS), which requires conducting a thorough investigation on biosafety to discard the existence of virulence determinants in such strains, including the presence of hemolysins/cytolysins and the examination of antibiotic (8). Therefore, we have investigated the presence of relevant virulence traits in *Enterococcus spp.* strains isolated from Cotija cheese. We decided analyzed 12 cheeses from different farm-houses to evaluate the influence of enterococci in their safety. In this experiments, the six strains previously characterized and another one (*Enterococcus faecalis* –strain I) were included. As for the cheeses, in order to have a wider screening of the presence of virulence traits, we decided to work with *Enterococcus* populations isolated from the cheese samples in a selective media (EVA broth), instead of pure strains.

Through multiplex-PCR we detected the presence of the *asa* gene in three strains (A, B and I) and seven populations. In order to complete the study, in-vitro studies on the capability of adhesion to human epithelial cells derived from colon adenocarcinoma (Caco-2 line) and colon carcinoma (HT-29 line) were also performed. All the strains and populations, who had the *asa* gene, had the ability to adhere to Caco-2 and HT-29 cell lines (Fig 2). Although the capability of adhesion to epithelial cells is characteristic to virulent strains, it is also a criterion to define a probiotic behavior. Currently research also focuses, rather than merely on the simple adhesion of putative probiotics, on its interference with pathogen adhesion (8). Consequently, we must evaluate this characteristic in the future.

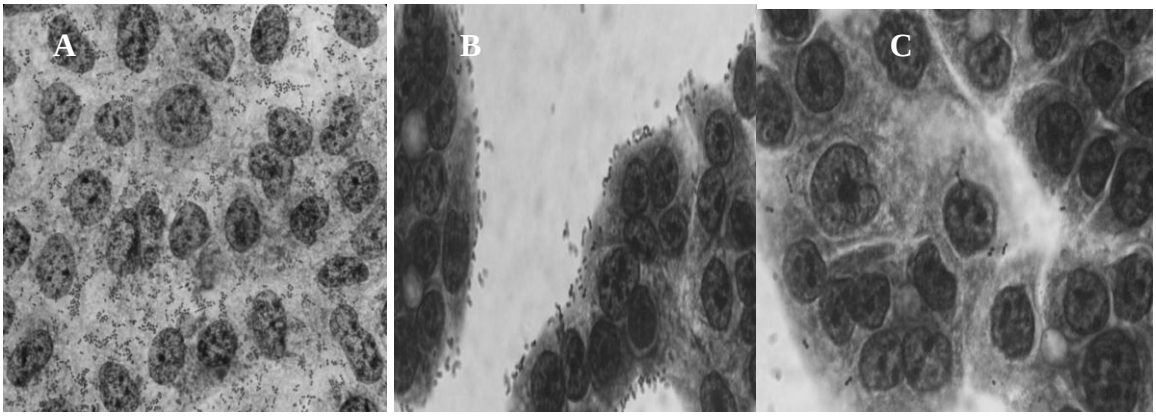

Fig 2. A) Example of adhesion of *Enterococcus faecalis* A to Caco-2 cells. Several bacteria around of the cell and widespread of the citoplasm. B) Example of adhesion of *Enterococcus faecalis* I to HT-29 cells. Bacteria only around of the cells. C) Example od adhesion of one population to HT-29 cells.

Biofilm production is controversial. It may be considered beneficial due to the fact that biofilms prevent colonization by undesirable microorganisms, when covering epithelial receptors. However, this trait has been strongly correlated with the presence of a putative virulence factor, the surface protein Esp, which promotes primary attachment and biofilm formation on abiotic surfaces (8). Two of the isolated populations and strain B showed amplification for the *esp* gene. However, eleven populations and all the strains isolated were able to form biofilms, but only four populations and the strains A and E formed a well-structurated one (D.O. >0.22). Therefore, others genes could be involved in the development of this structure.

Among the examined traits, β-hemolysis is one of the most determinant factors in virulence. Nosocomial *Enterococcus* strains show the capability to infect host cells due to the presence of this attribute. *Cyl A* gene was not detected in none of the populations and isolated strains. Thus, β-hemolytic activity was not observed. Moreover none were resistant to two different concentrations of vancomycin (20µg/mL and 256µg/mL). It has been proposed that the thin line that separates the dual lifestyle of enterococci (as commensals or pathogens) is the result of an orchestrated interaction between processes involving the sequential action of colonization and potential virulence factors that govern adaptation of bacterial cell physiology during the infection, as well as the host response to the bacterium and its products (12).

Enterococci are common bacteria in artisanal fermented food products, in which they could contribute to the development of sensory properties. Moreover, their production of antibacterial compounds contributes to food safety. Although some genes encoding virulence factors had been found in enterococci strains isolated from food, they have not been linked so far as the direct cause of clinical infections (13).

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Production Of Pectate Lyase By *Colletotrichum* spp. From Mango Ataulfo As A Mechanism Of Pathogenicity

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Introduction

Mango has short time storage principally due to the susceptibility to the attack by phytopathogens. The principal mango disease is anthracnose caused by *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk (anamorph: *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. In Penz., *C. gloeosporioides* Penz. var. *minor*. J.H. Simmonds (2) and *C. acutatum* J.H. Simmonds . Losses in farming and postharvest caused by anthracnose are estimated between 15 and 50% (4).

Differentiating between *Colletotrichum* species responsible for anthracnose disease is important for understand the epidemiology of this disease and developing better control strategies (3).

The aim of this work was to characterize *Colletotrichum* spp. and to determine the production of pectate lyase (PL) as a virulence factor in the pathogenicity process.

Methods and materials

Fungal isolates

Mango fruits cv. "Ataulfo" of different sizes and displaying symptoms of anthracnose as well as inflorescences were collected to identify the pathogens involved in this disease. They were obtained from four orchards from Aticama and Miramar, Mpio. San Blas, Nayarit.

Colletotrichum spp. samples were isolated within 18–24 h of harvesting fruits with symptoms, by excising a small piece of necrotic pericarp with healthy tissue margins. The fruits had previously been disinfected in 1% sodium hypochlorite for 3 min and rinsed three times with sterile distilled water. Tissue samples were placed on Petri plates containing potato dextrose agar (PDA) and incubated at room temperature (25°C) for 7 days until mycelia development. Then, monoconidial cultures were inoculated into water agar (WA). One single germinated conidia, was transferred with a sterile needle to PDA amended with 10% tartaric acid at 14 mL L^{-1}. All single-conidium isolates obtained were used for further characterization and pathogenicity test. In a number of cases, more than one isolate was obtained from each sample (Table 1).

Morphological and growth characteristics

The description of Mordue (4) was used as a reference to identify the fungi at the genus level, and *C. acutatum* and *C. gloeosporioides* were identified according to Sutton's key (7). Small portions of approximately 5 mm 2 plugs of agar containing mycelia from each culture of *Colletotrichum* spp. were transferred onto Petri plates containing PDA to study morphological characteristics. The plates were incubated at about 24°C with a 12 h light - dark cycle for 7 days. The colony diameters (mm) were recorded every day for 1 week to determine the growth rates. The lengths and widths of at least 50 conidia were measured, and the colony colour was recorded for each isolate. The size, shape and colour of the conidial masses and other key characteristics of each structure were also scored.

Genomic DNA extraction

Each isolate was cultured on PDA at room temperature for 10 days. After this time, the mycelium was scraped from the surface of the plate and crushed with a mortar in 1 mL of lysis solution (2% Triton-X

100, 1% SDS, 100 mmol L^{-1} NaCl, 10 mmol L^{-1} Tris-HCl, pH 8.0) containing 10 µL of proteinase K (10 mg mL^{-1}). Subsequently, 600 µL of the sample was transferred to a 2.0 mL Eppendorf tube for the DNA pellet of each tube was suspended in 50 µL of TE buffer (10 mmol L^{-1} Tris-HCl, pH 8,1 mmol L^{-1} EDTA). The quality of the DNA was verified by electrophoresis in a 1.0% agarose gel in 1X TAE buffer (Tris Acetate-EDTA) run at 87 V cm^{-1} for 1 h. The gel was stained with gel RED, and the bands were visualized under a Gel Doc 2000 UV transilluminator (Bio-Rad). The DNA concentration was quantified using a Nanodrop spectrophotometer (Thermo Scientific), and the samples were diluted to 20 ng μL^{-1} for PCR reactions.

PCR amplification of ribosomal RNA genes

The primers, ITS5 and NL4 (5'-GGTCCGTGTTTCAAGACGG- 3'), were used to amplify a fragment of 1100 bp that included the ITS region and approximately 600 bp of the conserved domain in the 28S rRNA gene. A PCR master mix was prepared in a final volume of 25 µL containing 1x Taq DNA polymerase buffer, 2 mmol L^{-1} MgCl2, 0.8 µmol L^{-1} deoxynucleoside triphosphates (0.2 $\mu molL^{-1}$ each), 100 ng DNA, 20 pmol of each primer, and 2 units of GoTaq Flexi DNA (Promega). PCR amplifications were performed with an initial denaturation at 95°C for 4 min; 38 cycles of denaturation at 95°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 1:30 min; followed by a final extension at 75°C for 10 min. All PCR reactions were carried out in a Peltier Thermal Cycler C1000 (Bio-Rad), and the PCR products were verified by loading 5 µL in a 1.2% agarose electrophoresis gel, which was stained as described above. The remaining amplified PCR products were purified using a QIAquick PCR purification kit (Qiagen) following the manufacturer's instructions. To ensure the absence of misreading, the PCR products were sequenced in both directions usingan Applied Biosystems model 3730XL automated DNA sequencing system (Applied BioSystems).

Sensitivity to benomyl

PDA Petri plates were simultaneously inoculated with a conidial suspension of each isolate as described above in order to determine the sensitivity of isolates to benomyl (4). Filter paper disks (1 cm in diameter), previously saturated with 100 µl of 0, 300, 600, or 1,200 µg/ml of benomyl and air-dried, were placed on the surface of inoculated PDA plates. The plates were incubated for 3 days at 25°C, the radius of the inhibition zone was recorded for each fungicide concentration evaluated, and plates were photographed. Three replications of each isolate for each fungicide concentration were evaluated, and the experiment was performed twice. The factorial experiment was evaluated using general linear model procedures and means were separated using Tukey's studentized range test provided in the statistical algorithms of SAS version 6.04 (SAS Institute) (4).

Pectate lyase activity

PL were assayed as described by Collmer et al. (2). PL activity were conducted with 5 µg of protein, which was on the linear portion of the curve for 5 min in a spectrophotometer (UV-vis 6405,, Janway)) at 232 nm. PL activity was expressed as the accumulated absorption change of 1 µmol of the 4,5-unsaturated galacturonide product of polygalacturonic acid (Fluka) for 10 minutes, at 37ºC. The molar extinction coefficient for the unsaturated product at 232 nm is 4,600 per M per cm. One unit of PL is 1 µmol of 4,5-unsaturated products from, respectively, for 10 min under the conditions of the assay.

The substrate stock solution was prepared by adding Tris HCl 50mM, pH 8.5, $CaCl_2$ and desionizaded water and 0.2% polygalacturonic acid.Reaction mixtures consisted of 950 µl of substrate solution and 50 µl of protein extract concentrated media,followed by incubation for 10 min at 37ºC. The absorbance was read at 235nm.

Results and discussion

Fungal isolates, morphological and growth characteristics

Six isolates showing macroscopic characteristics vary from grey to salmon, sometimes showing luxuriant orange conidial masses with grey or white bottom were obtained.According to Sutton (1992), the isolates

M3V, SA, GAQ37, F1 and A71NBcorrespond to*C. gloeosporioides*(13.4–24x4.0–5.9)and the isolate DG correspond to*C. acutatum* (11.8–19.1x3.2–8.5) (Fig 1 and Table 1).

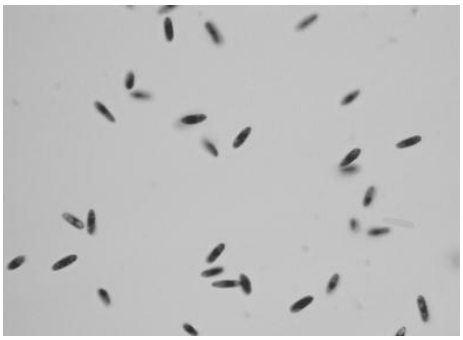

Figure 1. Isolates conidia GAQ37

Isolates	Length	Width
M2V	10.39	2.75
SA	12.00	2.77
F1	11.00	3.34
A71 NB	11.24	3.21
DG	16.14	4.02
GAQ37	9.78	3.40

Table 1. Conidia measures of *Colletotrichum* spp.isolates

Growth rates

*C. gloeosporioides*generally present higher amounts of growth rates than *C. acutatum.* Acording to this, the isolates M2V and DG probably correspond to *C. acutatum* and SA, F1 y GAQ37 *C. gloeosporioides*but this classification it is not definitive.

Table 2. Growth ratesof *Colletotrichum* spp.isolates

Isolate (anamorph)	Mean growth rate mm Day^{-1}
M2V	0.1948
SA	0.2239
F1	0.2047
A71 NB	0.1990
DG	0.1871
GAQ37	0.2109

Molecular identification

According to TB6 PCR amplification M2V correspond to *C. gloeosporioides* with 99% homology to the AJ409292.1 by genBank (Table 3). The isolate DG correspond to *C. ignotum* with 98% homology to GU99470 by genBank (Table 4).

Table 3. Identification M2V isolate after sequencing the TB6 gene

Isolate	Sequence 547bp	% Homology
M2V	ACATTTCTGGCGAGCACGGCCTCGACAGCAATGGA GTGTATGTCATGCCCCTTATCTGGCCACATTGATGG TTGACCGCTAAACTCGAACAGCTACAACGGCACCTC TGAGCTCCAGCTCGAGCGCATGAGCGTCTACTTCAA CGAAGTTTGTTACCTTATAGCCCCCAGAGTGCAAGA TAAACATATTGACGAGTACTGACCTTCGCTCCTACCC AGGCTTCCGGCAACAAGTACGTGCCCCGTGCCGTCC TCGTCGATTTGGAGCCCGGTACCATGGACGCCGTCC GTGCCGGTCCTTTCGGCCAGCTCTTCCGCCCCGACA ACTTCGTCTTCGGCCAGTCTGGTGCCGGCAACAACT GGGCCAAGGGTCACTACACCGAGGGTGCCGAGCTC GTCGACCAGGTTCTCGATGTTGTCCGCCGCGAGGCT GAGGGCTGGCGACTGC	99% *Colletotrichum gloeosporioides* 99% *Colletotrichum ignotum*

Table 4. Identification DG isolate after sequencing the TB6 gene

Isolate	Sequence 436bp	% Homology
DG	TTTCTGGCGAGCACGGCCTCGACAGCAATGGA GTGTATGTTATGCCCCTTATCTGGCCACATTGG TGGTTGACCGCTAAACTCGAACAGCTACAATG GCACCTCTGAGCTCCAGCTCGAGCGCATGAGC GTCTACTTCAACGAAGTTTGTTACCTTATAGCCC CCAGAGTGCAAGATAAACATATTGACGAGTAC TGACTTTCGCTCCTACCCAGGCCTCCGGCAACA AGTACGTGCCGCGTGCCGTTCTCGTCGATTTGG AGCCCGGTACCATGGACGCCGTCCGTGCCGGT CCTTTCGGCCAGCTCTTCCGCCCCGACAACTTC GTCTTCGGCCAGTCTGGTGCCGGCAACAACTG GGCCAAGGGTCACTACACCGAGGGTGCAGAG CTCGTCGACCAGGTTCTCGATGTTGTCCGCCGC GAGGCTGAGGGCTG	98% *Colletotrichum ignotum* 98% *Colletotrichum gloeosporioides*

Sensitivity to benomyl

Colletotrichum strains were classified as *C. gloeosporioides* or *C. acutatum* according to their sensibility to benomyl. The isolate DG was sensible to benomyland classified as *C. gloeosporioides*. The isolates M2V, SA, GAQ37, F1 and A71 NBdid not show sensibility to benomyl and they were classified as*C. acutatum*(Results not showed).

Table 5.Enzymatic activity of Pectate Lyase obtained from*Colletotrichum* spp. isolates in presence and absence of mango cells cv "Ataulfo" as inductor.

Isolates	Without INDUCTOR (U/L)	With mango cells as INDUCTOR (U/L)
D G	387.69[a]	416.13[a]
F1	102.7[a]	271.6[a]
M2V	234.7[a]	412.1[a]
M3V	241.0[a]	483.4[b]
SA	238.0[a]	410.96[b]

Different letters show significant differences by LSD with$p<0.05$.

Pectate lyase activity

It was observed a higher amount of PL activity in assays performed in the isolates cultured in M3S liquid medium with presence of mango cells comparing with those cultured in absence of mango cells. The isolates M3V and SA showed a difference between absence and presence of mango cells; then it is inferred a mechanism of PL induction by components of mango cells ($p<0.05$) (Table 5).

The electroblotting from the polyacrylamide gels to 14% showed in Figure 2 wherein the bands are observed more intense blue color with the presence of the inductor, the molecular weight marker is located in lane 1 from left to right the pectate lyase 40kD weights so bands that are between carbonic anhydrase (45.7kD) and soybean trypsin inhibitor (32.5kD).

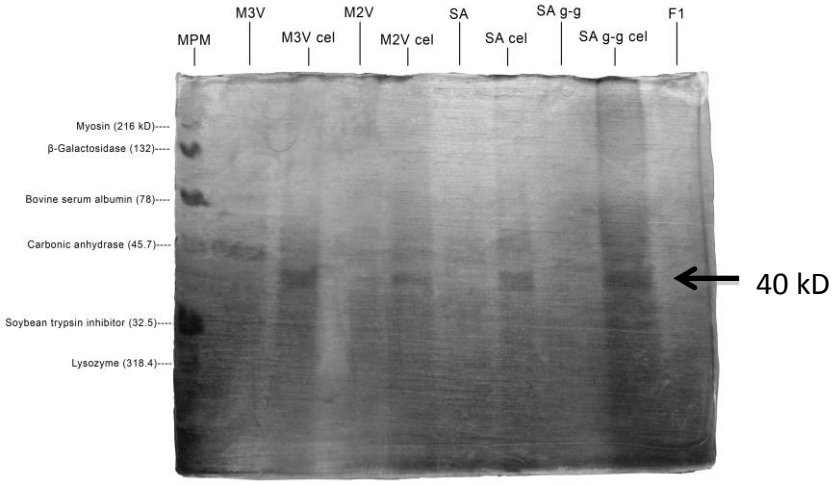

Figure 2. Western blot of extracts from *Colletotrichum* spp. isolates culture with and without mango cells.

Conclusion

The morphological and physiological characteristics of Colletotrichum isolates are not definitive to classify them in the species of *gloeosporioides* or *acutatum*, but the molecular criteria are. PL enzyme is involved in the process of pathogenecity of *Colletotrichum* spp. cv "Ataulfo", however, other enzymes may be involved. The identification and characterization of *Colletotrichum* spp. responsible for anthracnose in Nayarit will contribute to design better monitoring methods in pre and postharvest.

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Interaction Among *Colletotrichum gloeosporioides*, Mango Fruit And *Pichiaguilliermondii* In The Production Of Glucanase And Chitinase

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Introduction

The mango is the main fruit grown in theNayarit State, Mexico, however, there are economic losses due to diseases, being the anthracnose(causes by *Colletotrichum gloeosporioides*), one of the most important problems[1]. A variety of chemicals areused to combat this problem, nevertheless, they are not selective, and they may leave toxic residues in fruitscausing diseases to humans and environmental pollution. This has led a trend of importing countries torestrict the use of these products, so that environmentally friendly alternatives have been sought for diseasecontrol, being the biological control an excellent alternative to it. Also, biological control reduces the risks tothe health of the producers, and it is friendly to the environment, then, their practice promotes sustainabilityof resources[2, 3].

An example of an effective microorganism for biological control of anthracnose is the yeast *Pichiaguilliermondii*, which showed in preliminary studies, an excellent effectiveness in reducing the incidence of thedisease, besides presenting different mechanisms of action for biocontrol, however, it is necessarydetermine if there is some interaction among this organism, *C. gloeosporioides* and the plant to help incontrolling the pathogen.

Methods and materials

Harvest and preparation of the fruit

"Ataulfo" mangoes were harvested at physiological maturity and with no apparent damage from 20 year-old-trees cultivated using organic practices. The fruits were disinfected with 1.5% sodium hypochlorite for two min, washed with tap water, allowed to dry at room temperature (28 °C) and were finally placed in a plastic container [4].

Obtaining microorganims

The microorganisms (yeast and mold) were obtained from the CIBNOR-Instituto Tecnológico de Tepic Collection, which were previously obtained from commercial organic orchards of the municipality of San Blas, Nayarit, Mexico.

Secretion of hydrolytic enzymes

One swipe of the fungal pathogen *C. gloeosporioides* was inoculated in 50 mL of Potato Dextrose Broth (PDB). The culture was maintained under conditions of moderate aeration and agitation at 25 °C for 72 h until the appearance of mycelial growth. The culture was sterilised at 121 °C for 20 min and then was centrifuged at 1400 g for 10 min. The precipitate was washed twice with 50 mM phosphate solution (pH 6.7). Finally, it was dissolved in 50 mL of the same solution and was subsequently used to stimulate the production of hydrolytic enzymes in yeast [5, 6, 7].

For *in vitro* testing, the yeasts were cultured for 72 h at 28 °C in yeast glucose extract broth in the presence and absence of 10% V/V sterile mycelium. The obtained cultures were centrifuged at 1400 g for 10 min and filtered through a 0.20 µm nitrocellulose membrane. The supernatants were recovered for the enzymatic analysis of β-1, 3-glucanase and chitinase[5, 6, 7]. To test the induction of these enzymes *in vivo*, two wounds were made in five mango fruits (3 cm by 3 cm above and below the centre of the fruit), and 100 µL of yeast suspensions of $1x10^7$ cells/mL were inoculated in the wounds. Additionally, a second treatment consisted of inoculating 100 µL of sterile mycelium of the pathogen in these fruits. The fruits

were then incubated at 28 °C and high relative humidity (above 90%) for 72 h. In the case of β-1, 3-glucanase, the wounds were washed with 250 µL of 50 mM sodium acetate buffer (pH 5.0). The solutions obtained during the washing of the wounds were centrifuged at 1400 g for 10 min and filtered through a nitrocellulose membrane of 0.45 µm. The supernatant or enzymatic extract was recovered, and the enzyme β-1, 3-glucanase activity was determined. The same procedure was performed to quantify the activity of the chitinase enzyme (five replicates for each treatment) using tris-HCl (pH 7.5) as buffer solutions. It had controls which consisted of mangoes to which the wounds were performed above, with the presence and absence of sterile mycelium of the pathogen.

Determination of enzyme activity

The β-1, 3-glucanase enzyme activity was obtained using the technique described by Castoria et al. [5]. The activity was quantified according to the nmol of reduced sugars that were released per mg of protein per min (mU/mg). Chitinase activity was determined using the technique described by Wu et al. [8] and was estimated as the nmol of p-nitrophenol released per mg of protein per min (mU/mg). Finally, protein quantification was performed using a protein assay kit (Biorad, Ref 500-0006) based on the Bradford method (1976).The different activities were reported as mU per mg of protein (mU/mg).

Results and discussions

In all treatments performed found enzyme activity of β-1, 3-glucanase (Figure 1) either *in vitro*, in control fruit and the tests with the yeast *P. guillermondii* under conditions *in vitro* and *in vivo*, in the presence and absence of sterile mycelium of *C. gloeosporioides*.

Significant differences in glucanase activity were found when treatments were performed with and without the addition of mycelium (figure 1), that for the case of control fruits and "*in vivo"* test ($p<0.05$), this situation is consistent with the findings reported by some authors [9,10], who hypothesised that yeast-pathogen interactions or plant-pathogen or yeast-plant are due to a signal recognition system between the yeast cells and the fungal hyphae however, there were no differences in "*in vitro*" treatments with and without the addition of sterile mycelium, which indicates that glucanase enzyme is constitutive and are the result of normal yeast metabolism [9].

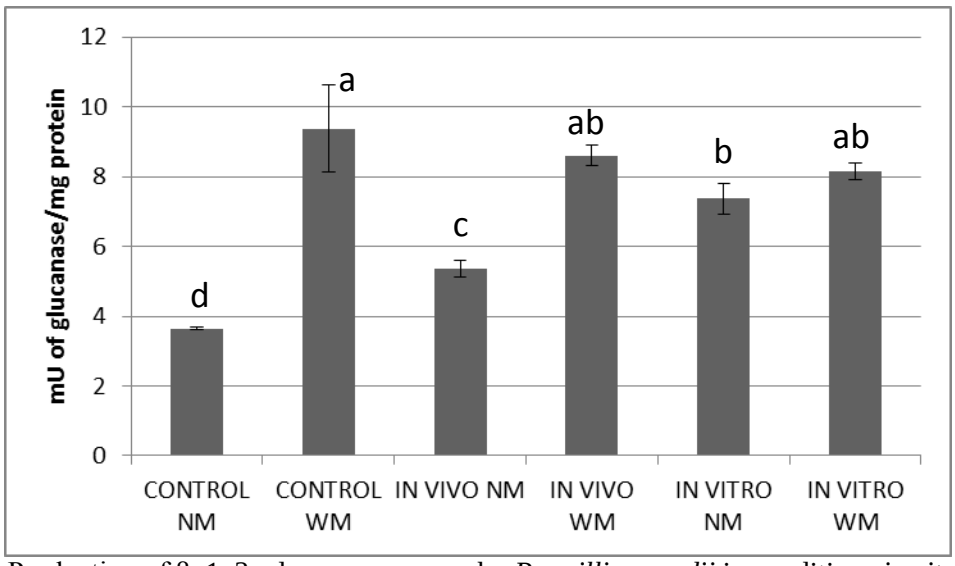

Figure 1. Production of β–1, 3–glucanaseenzyme by *P. guilliermondii* in conditions *in vitro*, *in vivo* and their control, with mycelium (WM) and without mycelium (NM)

Also, it was found a significant increase in "*in vivo*" treatment without mycelium compared with control treatment without mycelium (from 3.65 mU/mg to 5.36 mU/mg), that significant increase can be attributed to an induction in enzyme activity by the yeast, however, to this same treatments but with the addition of

mycelium was no found a significant increase, this can be due to there was not recognition between the yeast and the mold which is observed in "*in vitro*" and "*in vivo*" treatments.
As glucanase enzyme, all treatments showed chitinase enzyme activity (figure 2), however, only control fruit treatments showed a significant increase in the chitinase activity with la addition of sterile mycelium of phytopathogen ($p<0.05$).

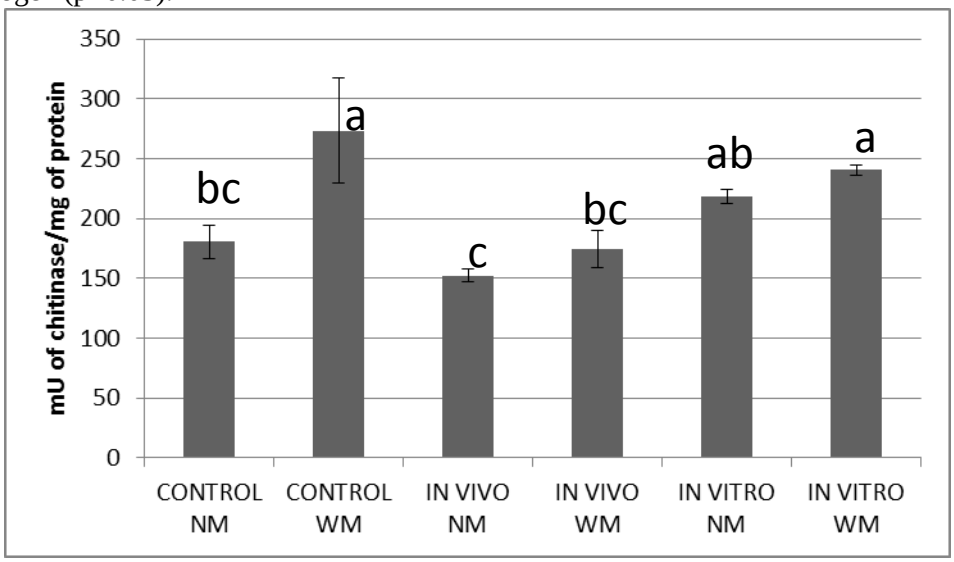

Figure 2. Production of chitinaseenzyme by *P. guilliermondii* in conditions *in vitro*, *in vivo* and their control, with mycelium (WM) and without mycelium (NM)

Either "*in vitro*" and "*in vivo*" treatments notes an increase in chitinase activity with the addition of sterile mycelium although those were no significant ($p<0.05$), this indicates that there was no induction either mycelium to yeast or yeast to fruit, but there was recognition of the pathogen by the fruit.
In the case of "*in vivo*" treatment, there was no statistically significance differences between without mycelium treatment compared with control treatment without mycelium, however, there was a decrease in the chitinase enzyme activity in "*in vivo*" treatment with mycelium compared with control treatment with mycelium (from 273.17 mU/mg to 174.53 mU/mg), this can be due to production of chitinase inhibitors in order to prevent their cell wall hydrolysis,some microorganisms have been reported toproduce chitinase inhibitors as allosamidin, styloguanidine, L-DOPA, and cyclo(L-arginyl-D-propyl) [Rosas and Casadevall, 2001; Iketa et al., 2003; Omura and Shiomi, 2007).

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Antimicrobial Activity Against *Escherichia coli* And *Listeria monocytogenes* Of The Extracts Obtained From Three Mexican Pepper (*Capsicum*)

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Introduction

Consumer´s needs for food with less synthetic food additives have increased the demand for natural products, such as substances used to extend its shelf life by inhibit the growth of bacteria, yeast and mould.

For years, studies have been made to preserve food qualities for longer time. In the same way, it has been discovered vegetable origin substances to counteract food decomposition due to microorganisms. These substances contain elements that works as defense mechanisms like enzymes, proteins, vitamins and phenolics compounds and have broad antibacterial spectrum, antiviral, antioxidant and antifungal properties; but above all, they are very important because bacteria does not generated resistance against them (1,2).

Other studies have demonstrated that phenolics compounds from different plants have their impact over membrane resistance by interfering in the use of amino acids to initiate the germination process (3).

One of this plant species is pepper (*Capsicum anuum*), which have been used for long time as vegetable and spice in several dishes. Besides, they present a great deal of active components, such as a large group of substances called capsaicinoids, responsible of its peculiar taste and the burning sensation in the mouth, finding capsaicin and dihydrocapsaicin in greater percentage and being also, the most pungent of all (4).

Also total phenolic compounds found in pepper probed that they contribute to reduce the risk of degenerative diseases, such as phenolic compounds, which also have antioxidative properties (5). The aim of this paper was to evaluate the inhibitory effect of methanolic extracts obtained from different parts (pericarp, seeds and whole pepper) of three pepper species; Poblano (*Capsicum annum var. anuum*), Serrano (*Capsicum anuum L. acuminatum*) and Habanero (*Capsicum chinense*) against *Escherichia coli* and *Listeria monocytogenes* and its relation with capsaicinoids and total phenolic compounds.

Materials and methods

Materials

Three species of pepper fruits (Capsicum) unripe were used; Poblano pepper (*Capsicum annum var. annum*), Serrano pepper (*Capsicum annum L. acuminatum*) and Habanero pepper (*Capsicum chinense*) and purchased from a local market of Puebla, México.

Pepper fruits were sliced in approximately 1 cm cubes and placed in a food dehydrator (Excalibur, 4900-220/240V, EUA) with an air flow of 2 m/S and at 65°C for 48 h until reached 5% of water content.

Extract preparation

Methanol (Omnichem ®) was used to obtain pepper extracts in a adaptation of the method used by Dorantes *et al*. (6) in which they used instead isopropilic alcohol. Approximately 70 ml of solvent was added to 3 g of sample and refluxed for 3 h. The solution was passed through large pore filter paper. Activated carbon (15% p/p) was added to the filtrate and filtered again. Solvent was evaporated and recaptured by simple distillation. Extracts was kept at -4°C until used

Capsaicinoids content

Spectrophotometric method to determined capsaicinoids proposed by Xu et al. (7) was used. Distilled water (1 mL) was added to 100 μL of extract. Then, 2 mL of a 0.5M solution of HCl was added along with 1 mL of a solution containing 0.5 M of $NaNO_2$ and 0.025 M of $NaMoO_4$ and mixed. The solution is let settled for about 15 min and then 2 mL of NaOH 0.1 M was added. After 30 min absorbance at 420 nm was measured with a Spectrophotometer UV-Vis (Unico, 2800H, EEUU).

Standard curve was made using 0, 0.2, 0.4, 0.5, 1.0, 2.0, 3.0 y 4.0 mL of a vainillyn and ethyl acetate solution (0, 0.010, 0.020, 0.025, 0.050, 0.100, 0.150 and 0.200 mg of capsaicinoids respectively).

Determination of phenolic content

This method has previously been used by Ornelas-Paz et al. (8). 0.5 mL of 50% of Folin-Ciocalteu reagent and 8.5 mL of deionised water was added to 1 mL of the extract. After incubated at room temperature for 10 min, the solution was mixed with 1.5 ml of 20% of sodium carbonate solution and again incubated at room temperature for 60 min. Finally the absorbance of the solution was determined at 750 nm with an UV-Vis Spectrofotometer using Gallic acid as a standard reference. Total phenolic content was expressed as gallic acid equivalents (mg g.a./L extract).

Strains and growth conditions

Two strains of bacteria, *Escherichia coli* and *Listeria monocytogenes*, were obtained from the Microbiology Laboratory of Universidad de las Américas Puebla at 4°C until use.

Both *E. coli* and *L. monocytogenes* were inoculated in nutritive broth (BD Bioxon) for 24 h at 37°C.

Evaluation of antimicrobial activity

The Kirby-Bauer method to evaluate the antimicrobial activity was reported before by Coliveti *et al*. (9). Impregnated filter paper discs with each one of the extract were placed in Petri dish previously inoculated with 50 μL of bacteria broth. Tripticase soy agar (BD Bioxon), was cultured with *L. monocytogenes* and *E. coli*. Petri dishes were incubated for 48 hrs at 37°C, for *E.coli* and *L. monocytogenes*. Inhibition halos were then measured with a Vernier rule; being lower the antimicrobial activity if this measure is small and vice versa.

Statistical analysis

The data obtained for yield of both capsaicins and phenolics compounds were analyzed with Minitab v. 15 (Minitab Inc., EE.UU) by ANOVA with a 95% level of significance.

As well as the yield, antimicrobial activity was also analyzed by two way ANOVA and with Tukey's comparation of means with a 95% level of significance.

Results and discussions

Capsaicinoids content

Method proposed by Xu et al. (7) using vanillin as derivate of capsaicin. Capsaicinoids concentration was calculated, for each extract, using the following equation (Equation 1).

$$\text{Absorbance}=25.789(\text{mg capsaicinoids})-0.0623 \qquad \text{Eq. 1}$$

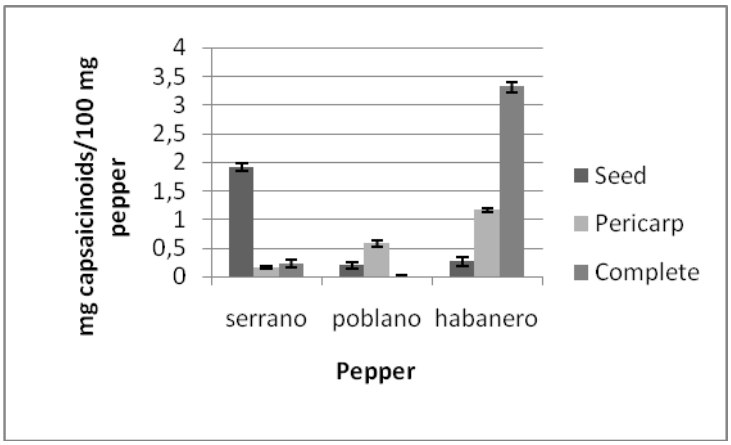

Figure 1. Capsaicinoids quantification from three *Capsicum* species extracts

Results obtained from the quantification of capsaicinoides are shown in Figure 1. An analysis of variance (ANOVA) showed that Habanero pepper extracts (*Capsicum chinense*) had a significantly ($p<0.05$) greater content of these compounds (0.27-3.32 mg of capsaicinoids/100 mg pepper). The aforementioned coincide with the results of Kurian and Starks (10), where they observed that content of capsaicin and D-hydrocapsaicin were higher in Habanero pepper extracts than in other pepper species. Poblano pepper extracts had the lowest concentration of capsaicin ranged from 0.01-0.58 mg capsaicinoids/100 mg pepper.

It was observed that complete pepper extracts had the highest capsaicinoids content ($p<0.05$) (0.01-3.32 mg capsaicin/100 g pepper).These could be due to capsaicinoids synthesis take place in placental tissue and therefore presents an elevated capsaicinoids concentration (11). In the same way pericarp extracts showed the lowest concentration of capsaicinoids ranged from 0.16-1.16 mg capsaicinoids/100 g pepper; this also was observed in the work of Greger (12) who found that pericarp presents the lowest content of capsaicin and D-hydrocapsaicin in pepper .

Determination of total phenolic content

The total phenolic content were determined by Folin-Ciocalteu colorimetric method using galic acid as control and was calculated using **Equation 2**.

$$\text{Absorbance} = 0.1143(\text{mg galic acid/mL}) - 0.0977 \qquad \textbf{Eq. 2}$$

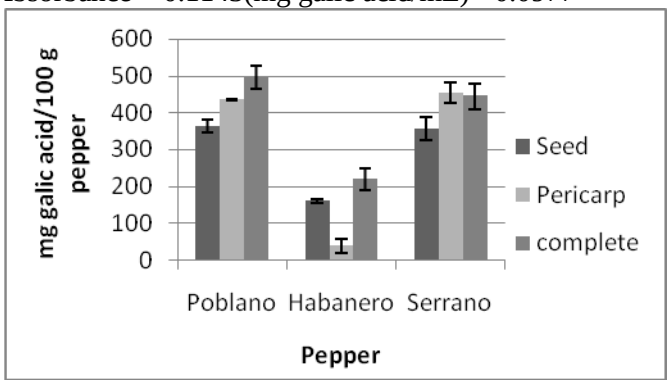

Figure 2. Total phenolic content from three *Capsicum* species extracts

The results given by one way ANOVA, showed that Habanero pepper had the highest concentration of phenolics compounds regardless of the part of the fruit (**Figure 2**).

These results were in agreement with those observed by Antonius *et al*. (13) in which they compared the phenolic content of four Capsicum species and realized that *Capsicum chinense* had the highest concentration of phenolic compounds.
It was observed a significative difference ($p<0.05$) between the three pepper species specially Serrano and Poblano pepper which presents the highest concentration of total phenolic compounds (356.76-453.85 and 363.87-496.6 mg galic/100 g pepper respectively) being these probably because it has been probed that Capsicum species area a good source of phenolic compounds such as vitamin C and carotenoids (14).
It was also observed that complete pepper extracts had the highest concentration of phenolic compounds (219.4-496.6 mg galic acid/100 g pepper). The reduction and augmentation of phenolics, it is due to the quantity of seeds used in the elaboration of the extracts, and this have direct relation because it has been probed that seeds are rich in phenolics and also antioxidative compounds (15).

Evaluation of antimicrobial activity
In **Figure 3** are the observed results from the inhibitory effect for three pepper species extracts against Escherichia coli. A greater inhibitory effect is observed as the measured diameter increase.

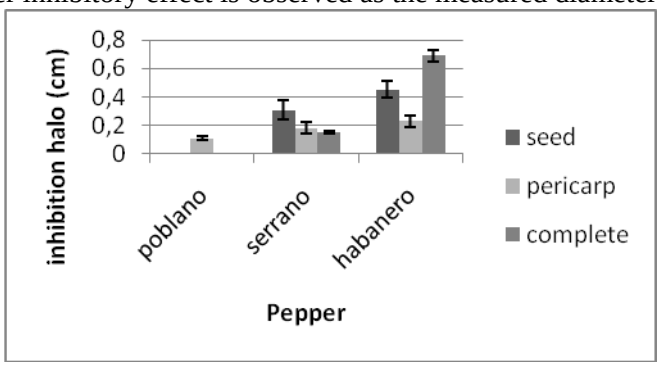

Figure 3. Inhibitory effect of three Capsicum species extracts against *Escherichia coli*

Habanero pepper present significantly ($P<0.05$) higher growth inhibition (0.23-0.69 cm) for *E. coli* (Figure 3). In similar studies, it has been probed that ethanolic extracts of green *Capsicum chinense* had some inhibitory effect due to pepper active compounds soluble in polar solvents (9) such as capsaicin.
It can also been observed that complete pepper extracts showed an elevated antimicrobial effect (0.0-0.69 cm). These results are probably observed due to the greater quantity of capsaicinoids in extracts obtained from Habanero pepper pericarp (Figure 1).

The results obtained from the evaluation of inhibitory effect of pepper extracts against *L. monocytogenes* are presented in Figure 4.
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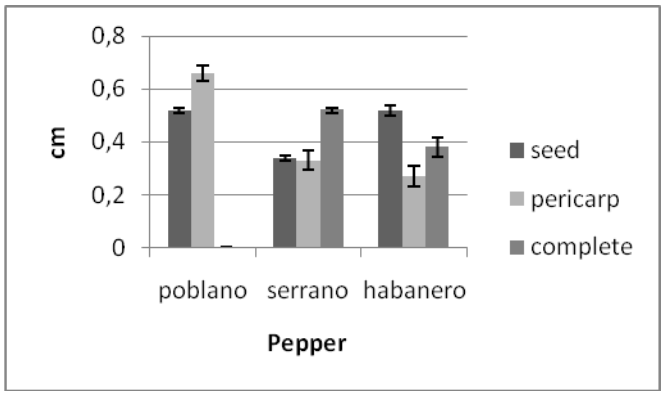

Figure 4. Inhibitory effect of three Capsicum species extracts against *Listeria monocytogenes*

Based in the results observed from the evaluation of the inhibitory effect of pepper extracts against *L. monocytogenes*, it was observed that there wasn't a significative difference ($p>0.05$) of the inhibitory effect between all the pepper extracts against *L. monocytogenes*. However, most of the pepper extracts was observed to inhibit growth of this bacteria.

In the same way, seed extracts showed the greater diameters of inhibition halos against this bacteria.

Similar results were found in a study made by Davidson (16) in which it was concluded that grampositive bacteria are more sensitive to poliphenolics components than gram negative bacteria. Also Dorantes *et al*. (6) observed a great growth inhibition of *L. monocytogenes* when Capsicum extracts were evaluated.

It can been seen that capsaicinoids (present in greater quantity in the most pungent peppers species) have its antimicrobial effect only with some bacterial strains such as *E.coli* however other active compounds like phenolics also presents antimicrobial activity.

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Corn Use For The Preparation Of A Culture Medium For Penicillin G Production By The Fermentation Of The Microorganism *Penicillium chrysogenum*Under Stress Conditions

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Introduction

Antibiotics are molecules with antimicrobialactivity that include several chemical compounds. They are secondary metabolites, produced generally after the growing phase of organisms. The fist known antibiotic was penicillin (1).

Penicillin refers to a group of antibiotics β-lactam used in the treatment of bacterial infections caused by gram-negative organisms (2). All penicillins have a basic structure, but differ in the nature of the side chain. The chemical characteristics that identify them are: amide ring (beta-lactamic ring) fused with a thiazoldinering and one side chain (3). The form in which penicillin acts is by inhibiting the formation of the bacterial cell wall. The β-lactam ring blocks the bacterial enzyme transpeptidase, which connects long polymers of sugars that form the cell wall, provoking cell rupture (4).

Alexander Fleming discovered penicillin, in September 1928, when he found a fungus in one of his plates by accident (5). Fleming grew the fungus and demonstrated that the liquid from the microorganism inhibited the proliferation of bacteria (4).

The commercial production of penicillin began in 1941 with the strain *Penicillium notatum*on the surface of a liquid medium with an incubation period of 6 to 12 days, followed by solvent extraction after separating the mycelium (1). However, the production was not enough, so new strains were investigated to produce higher yields of the metabolite (6).

A new strain identified as*Penicillium chrysogenum*was discovered in Illinois, producing about 200 times more penicillin than*Penicillium notatum*. Scientists tried to increase the amount produced by inducing mutations and created a mutant that made more than 1000 times the amount *P. notatum*produced. At this time, scientists began growing the fungus in fermentation tanks (4).

In 1943 the new strain with improved characteristics was introduced for commercial production, *Penicillium chrysogenum*(6). Mass production of penicillin began and by the end of the Second World War, many companies like Merck, Squibb and Pfizer manufactured the antibiotic (4). In 1945 Fleming won the Nobel Price in Physiology and Medicine along with Florey and Chain (4).

Materials and Methods

Equipment and Material:

- 3kg white corn - 4 blenders - Water bath - Electric grill with stirring - Plastic bags - Petri Dishes - Inoculation loop - Cotton - Potentiometer Oaklon - 2 glass bottles (1L) - Burette 250 ml - Sterile syringes 10 ml	- Filter paper discs Vernier - Hose clamps - Refractometer - Plastic wash bottle - Spatula - Lighter - Funnel - Thermometer - Syringe 50 ml - Oil vacuum pump - Air pump - Mangueras	- Autoclave - Analytical balance OHAUS - CentrifugeBiofuge Primo R Thermo Scientific - Incubator - Microwave - Laminar flow hood - Fume hood - HPLC Agilent Series 1200 - Reactor filters	- 0.45 micrasfilters, 2 cm diameter - 2 ml vials - HPLC C18 Column (15cmx4.6mm) - 2 Probetas (1L y 100 ml) - Sterile gauze - Bunsen burner - 5 Pipettes10 ml - Micropipettes 1 ml - Filter paper - Gloves	- New Brunswick BioFlo/CelliGen 115Fermentator - 2 Erlenmeyer flasks 500 ml - 1 Erlenmeyer flask 1L - 3 Erlenmeyer flasks 2L - 4 Beakers 500 ml - 36 falcon tubes 15 ml

Reagents and microorganisms:

- Peptone - Yeast Extract - Hydrated glucose - Bacteriological agar - Distilled water - Penicillin G Sigma standard - Acetonitrile HPLC - Ammonium acetate Acyl acetate	- Butyl acetate - Phosphate buffer - Sodium bicarbonate solution - Glucose - Sodium phenylacetate - Calcium carbonate Inorganic salts Ethylendiaminotetracetic acid	- Acetic acid - K_2HPO_4 - NaH_2PO_4 - $MgSO_4$ $7H_2O$ - $CaCl_2$ - $ZnSO_4$ $7H_2O$ - $MnSO_4$ $4H_2O$ - $CuSO_4$ $5H_2O$	- $FeSO_4$ $7H_2O$ - Na_2SO_4 - $(NH_4)_2SO_4$ - Hcl 1N - NaOH1N - NaCl - Sacarose - *Penicillium chrysogenum*

Methods:

Cornsteep liquor preparation: White corn was cleaned of foreign matter and different grains, then rinsed with water (500 corn/ 500 ml water in containers of 2 L) at 35 °C during 5 min with manual stirring. All the corn was transferred to the autoclave with distilled water at 45 °C for 20 min. Using blenders, corn was mashed (200 g corn/ 200 ml distilled water at 45 °C) during 5 min. The resulting solution was filtered and was stored same as the solid residues obtained.

Medium preparation: PW medium [500 ml PM1 and 500 ml Czapek- 0.7M KCl, agar 2 % (w/v)]. PM1medium [5 g Bacto-peptone, 5g lactose, 4g NaCl, 1 g cornsteep, 60 mg K_2HPO_4, 50 mg MgSO4*$7H_2O$, 3 mg $FeCl_3$*$6H_2O$ and 1 mg $CuSO_4$*$5H_2O$. Adjust pH to 5.5 with H_3PO_4, distilled water until 1 l and autoclave]. Czapek medium [30 g sucrose, 2g $NaNO_3$, 0.5g K_2HPO_4, 0.5 g $MgSO_4$*$7H_2O$ and 0.01 g $FeSO_4$*$7H_2O$. Adjust pH to 5.5 with H_3PO_4, add distilled water to 1l and autoclaved]. MCFP medium [55 g lactose, 35 g cornsteep, 10 g $CaCO_3$, 3 g $MgSO_4$*$7H_2O$, 7 g K_2HPO_4 and sodium phenylacetate 40% (w/v). Adjust pH to 6.8, distilled water until 1l and autoclave]. MCIP medium [20 g sucrose, 20 g cornsteep, 5g $CaCO_3$ and 10 g yeast extract. Adjust pH to 5.7, distilled water until 1l and autoclave]

Sterilization: Applied for bioreactor (fermentation medium inside) and different mediums on flask (PM1, MCIP, MCFP, Czepek) at 121° C for 20 min.

Harvesting spores: *Penicillium chrysogenum* (InstitutoPolitécnicoNacional) was seeded by extension on PW agar, incubated during 10 days at 25 °C. Six Petri dishes were prepared (3 plates seeded in 3 planes and 3 plates with chopped technique). The spores were collected in sterile H_2O with 0.1 % Tween 80. The suspension was homogenized and filtered through sterile gauze to quantify the concentration of spores in a Neubauer chamber.

Reactivation of inoculum in liquid medium: In a 250 ml flask containing 100 ml of medium MCIP, $10x10^8$ spores were inoculated. Incubation was at 25 °C, for 36 h and 250 rpm (Note: agitation was performed with a grill magnetic stirring and the temperature was controlled from the laboratory atmosphere).

Fermentation: Two reactors (BioFlo®/CelliGen® 115) were filled with 2 L of MCFP medium. Calibration was performed and after sterilization, each reactor was inoculated with 34 ml of fermentative medium. The fermenters had the same process conditions except for oxygenation, the reactor # 1 was 100 % OD and reactor # 2 was 0.2% OD. The other process parameters were as it follows: 132 h, pH 6.5, 250 rpm and 25 ° C. During the fermentation 10 ml sample was taken every 12 hours. The samples were stored at 4 º C.

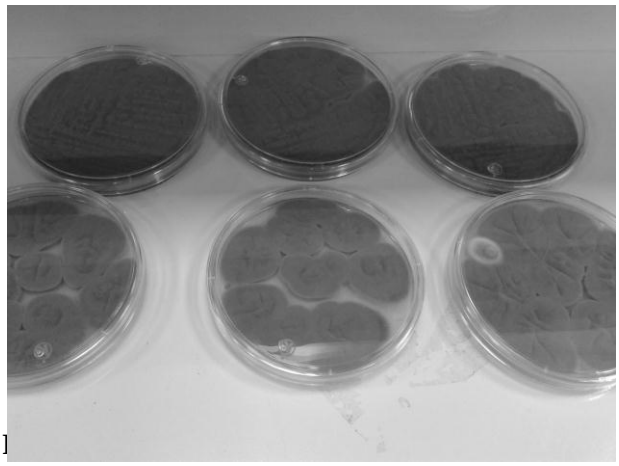
Penicillin extraction: 5 ml of each sample were put into 10 ml Pyrex screw cap centrifuge tubes, vortex and mixed with 400 µl of 10% acetic acid aqueous solution, then centrifuged at 3500 rpm for 10 min at 4 °C. The clear supernatant phase was taken by a syringe, avoiding taking the upper fat layer, and filtered through a 0.45 µm nylon filter. The filtered extract was put into 2 ml auto sampler tubes and stored at 4 º C.

Analysis by High Performance Liquid Chromatography (HPLC): Column C18, 15 cm x 4.6 mm, Mobile phase: 75:25 (Acetonitrile and Amonium acetate pH 4.5 (10mM), both filtered using a vacuum oil filter

with Whatman paper of 0.45 µm). Flow rate: 1 mL/min, Column temp.: 35 °C, Detector: UV at 220 nm, Injection: 10 µL, Sample: 100 µg/mL.
A calibration curve was performed, previous to the sample analysis, with known concentrations of penicillin in order to obtain the concentration in each sample.
On the sixth day, both bioreactores were turned off and their content was stored in plastic bags and stored at -10°C.

Results and Discussion

Reactivation of the microorganism *Penicillium chrysogenum*in a solid medium produced excellent results (see Figure 1). The microorganism grew as expected in the totality of the surface area of the Petri dish. Despite the perfect results obtained, one of the six plates was contaminated by another fungi species and was discarded.



solid media

After the solid media reactivation, the microorganism was maintained in a liquid medium. It was not possible to do a spore count during this step of the process; therefore the growth was not evaluated in liquid media. After the fermentation (132 hours) samples taken every 12 hours were obtained and frozen.
The HPLC results showed a maximum concentration of 10.801µg/ml at 80 hours in the bioreactor with oxygen. The penicillin production in this bioreactor increased over time, suddenly decreasing in the final sample. It can be appreciated in Figures 2 and 3 that penicillin production started until sample number five. This is because the first hours of fermentation, both bioreactors were functioning under anaerobic conditions. It was until after the fourth sample was taken that the oxygen was introduced into one of the bioreactors. This supports the fact that oxygen was essential for the production of the metabolite.
On the other hand, the bioreactor in anaerobic conditions did not present any concentration of penicillin during the total amount of fermentation time.

Sample	Area	Concentration	Sample	Area	Concentration
1-1	0	0	2-1	0	0
1-2	0	0	2-2	0	0
1-3	0	0	2-3	0	0
1-4	0	0	2-4	0	0
1-5	20.05	3.05779339	2-5	0	0
1-6	35.4	6.993589908	2-6	0	0
1-7	41.55	8.570472552	2-7	0	0
1-8	41.55	8.570472552	2-8	0	0
1-9	50.25	10.80118459	2-9	0	0
1-10	49.05	10.49350017	2-10	0	0
1-11	25.1	4.352631984	2-11	0	0

Figure 2: HPLC results. The first and fourth columns show the sample number (the first number refers to the bioreactor, 1 for aerobic and 2 for anaerobic conditions; and the second number refers to the sample taken). The second and fifth columns show the area under the curve at 2.6 minutes. The third and sixth columns show the final result of the concentration of penicillin in each sample.

The expected result was to find penicillin production under the anaerobic condition since it stresses the microorganism and triggers the metabolite production. The obtained results might be due to the death of the microorganism in anaerobic conditions, showing that a small amount of oxygen is necessary for the fungus to stay alive and to be able to produce the antibiotic.

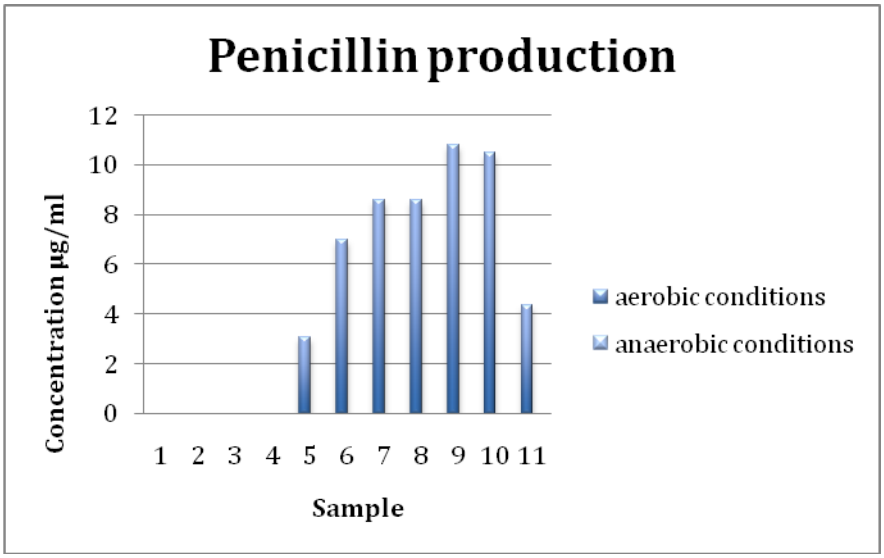


Figure 3: HPLC results. This graph represents the concentration of penicillin present in each sample. Sample 1 was taken at time zero before fermentation started.

The bioreactor with penicillin production might have had the amount of oxygen necessary for the microorganism to stay alive but under stress conditions, therefore, producing penicillin.

Nowadays, fermentation processes for penicillin production reach less than half of the maximum theoretical production possible, this is an indicator of the need to maintain an elevated production during the entire process, being 15 units/mgh the highest production reached (1). Optimization of fermentation process and factors related to fermentation are of great importance in the area of biotechnology, since they allow the achievement of a better penicillin production; that is why the manipulation of the different variables that affect the whole process play a key role and allow a better production of the desired metabolite, in this particular case, penicillin (7).

The substrate used in the fermentation process is a key factor in the production of the metabolite of interest (8). One of the most important factors that affect the fungus growth and development is the media concentration. Corn provides the necessary nutrients for the microorganism to grow adequately, and when combined with other ingredients, the media presents the conditions for the fungus to grow and produce penicillin under the right circumstances.

Using a defined and controlled media will result in a different production than the one obtained from a traditional culture with an undefined media, such as corn liquor. The selection of the appropriate starter media for penicillin production will be based in the perspective and in the search for favorable factors in the process.

Biotechnological processes of penicillin production, even though being relatively standardized, still have different variables that can be modified, considering the desired process conditions and the required final product. Investigation in the penicillin production process by the microorganism *Penicillium* spp. is essential because of the great importance of this antibiotic and its derivatives in medical applications.

It was concluded that the use of corn in media preparation for *Penicillium chrysogenum* fermentation is successful and produces microorganism growth. It would be important to continue this investigation to set the range of dissolved oxygen necessary to obtain the maximum yield of penicillin using homemade corn-steep liquor as mayor nitrogen source.

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Alginate Encapsulation And Activity In Model Food Systems Of The Bacteriocin Produced By The *Pediococcus acidilactici* ITV 26

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Abstract
Industrialised food stuffs often require addition of substances for its preservation. Bacteriocins produced by lactic acid bacteria are natural bioactive compounds that can be used as food preservatives, but the addition of bioactive ingredients to foods presents challenges to conserve its bioactivity during processing and storage of stuffs. The bacteriocin activity can be reduced by physicochemical effects, as the pH, of the different foods ingredients, as sugars, proteins, lipids and salts. This work report the study of the activity and stability of the bacteriocin produced by the *Pediococcus acidilactici* ITV 26 encapsulated in calcium alginate beads to protect its bioactivity. The bioactivity was evaluated measuring the antilisterial activity of the encapsulated bacteriocin, on model food systems. *P. acidilactici* ITV 26 was cultivated on MRS broth and incubated at 37° C for 18 h for the production of bacteriocin. The agar diffusion assay was utilizated for evaluation of antimicrobial activity. *Listeria innocua* AST-062 was used a sensitive indicator strain. The stability was determined in food models of glucose and albumin to 2.0, 5.0 and 10 % (p/v). The crude extract of bacteriocin (BCE) was encapsulated by extrusion in calcium alginate to 1% The bacteriocin release from calcium alginate beads was evaluated in order to determine the protein concentration in the beads. The experimental release results were analysed by ANOVA. An activity significant difference ($\alpha > 0.05$) between the food models with glucose to 5 % and 2 % compared against the model with glucose to 10 % was observed. The albumin to 2 % was significant different with regard to albumin to 5 and 10 %. It was observed a main stability of the bacteriocin when it was contacted with glucose at 10 % and albumin at 5 %, remaining 87.5 % of it is original activity in both treatments.
Key words: *Pediococcus acidilactici*, *Listeria innocua*, bacteriocins, encapsulation, alginate

Introduction
The bacteriocins produced by lactic acid bacteria used as food preservatives could be susceptible to reduce its activity by effect of the different food components pH, sugars, proteins, lipids, and salts. An alternative is to use calcium alginate to encapsulate them, protect their activity and increase its stability, showing an effect against pathogenic and spoilage bacteria, such as *Listeria monocytogenes*, which can survive in conditions that are usually adverse to many other bacteria [1]. So, the developments of functional foods through the addition of bioactive compounds hold many technological challenges, being the microencapsulation an interesting proposal to consider. The encapsulation is defined as a coating technology for protection and transport of bioactive compounds, such as antioxidants, fatty acids, enzymes, plant sterols, minerals, probiotics, protein, peptide, etc., and can release their content controlled under specific conditions. This technology is of interest in the pharmaceutical industry (release of drugs and vaccines). Used as a tool to improve the release of these compounds and deliver them in the gastrointestinal tract. However, they also have relevance in the food industry for the production of foods requiring the addition of functional ingredients. The choice of materials and encapsulation technology, are based on the active agent to encapsulate and their possible application, as it has been reported in studies with the aim of increasing the viability of probiotic bacteria, during food processing and their release in the gastrointestinal tract [2,3].

Methods and materials
Strains and conditions of cultivation

P. acidilactici ITV 26 preserved by freeze-drying, cultivated in the Man Rogosa and Sharpe (MRS) broth and incubated at 37 ° C for 18 hours.The strain was preserved in glycerol to 40% (v/v) at - 20 ° C. The sensitive strain was *Listeria innocua* 062 AST, cultivated in Luria Bertani (LB) broth at 37 ° C for 18 hours. It was stored in glycerol to 40% (v/v) at - 20 ° C

Determination of antimicrobial activity

The bacteriocin production in *P. acidilactici* ITV 26 was examined in cell free supernatant and the inhibitory activity in terms of arbitrary units (AU) (reciprocal of the highest dilution at which inhibitory activity was still observed) was determined [4] using spot on lawn assay. The 10 µL of cell free supernatant was spotted in double dilution on a lawn of indicator organism.
The lawn of the indicator strain was prepared by pouring the LB agar seeded with the indicator organism. Plates were preincubated at 4 ° C and incubated for 18 h at 37° C growth temperature. Growth inhibition was visually detected by observing growth free clear inhibitory zones.
Models of food were prepared a solution of bovine serum albumin 2, 5 and 10% (w/v) glucose solution to 2, 5 and 10% (w/v). Both solutions were processed with sodium phosphate (pH 6.5).

Production and purification of the bacteriocin

P. acidilactici ITV 26 cultivated in MRS broth and incubated at 37 ° C for 18 hours. The extraction and purification of the bacteriocin,was carried out by the method of adsorption-desorption cells [5]. *P. acidilactici* ITV 26 was incubated at 37 ° C for 18 hours, the culturewas heated at 72 ° C for 30 minutes to kill the cells after its pH adjusted to 5.5 and incubated at 4 ° C, The cells were harvested by centrifugation at 12, 000 x g at 4 ° C for 20 minutes. The cell-free supernatant was obtained (crude extract of bacteriocin) was adjusted to pH 6.8 with NaOH to 5% (w/v) and filtered through a Millipore membrane (0.22 µm). Stored under sterile conditions, it kept frozen until the determination of its activity. After the cells were had been washed twice with 5mM sodium phosphate (pH 6.5). After the cells had been washed with sodium phosphate (pH 6.5). Cells suspension was then centrifuged at 12, 000 x g at 4 ° C for 20 minutes. Thesupernatant obtained was preserved by freeze. The cells were then resuspended in NaCl 100 mMand the pH of the mixture was adjusted to 2.0 for the desorption of the bacteriocin. The mixture was kept at 4° C for 2 hours by mixing with stirrer cells suspensions were then centrifuged at 12, 000 x g at 4 ° C for 20 minutes and the supernants were ultracentrifuged in membranesof 3 kD (Amicon Ultra 15 Millipore) and then freeze-dried.

Encapsulation

The encapsulation of the bacteriocin was carried out by the technique of ionic gelling and extrusion [6]. The purified bacteriocin, mixed with sodium alginate to 1% (w/v). The mixture was extruded by asyringe of insulin (30 G x 13 mm) in a solution of $CaCl_2$ 0.3 M.
The capsules were filtered through Whatman paper, stored under sterile conditions and planted in wells over LB agar plates inoculated with the sensitive strain. The Plates were preincubated at 4 ° C and incubated at 37 ° C for 12 hours. They were later observed to determine the presence of inhibition zone. (Figure 2)

Determination of protein

Content of protein in different samples of bacteriocin were determined by the Bradford method [7], using as a standard protein bovine serum albumin (BSA).

Results

Antimicrobial activity of bacteriocin

The activity of the bacteriocin expressed in Units of Activity per ml, as well as in Units of Activity per milligram of protein, crude extract and purified samples, shown in table 1.

Table 1 Bacteriocin activity in crude extract and purified

Sample	Activity (UA/ml)	Activity (UA/mg)
Purified Bacteriocin BP	6,600	22,405
Extract crude of Bacteriocin ECB	2,333	11,017

Activity of the bacteriocin encapsulated in models of food

The recovery of the activity from the bacteriocin encapsulated in calcium alginate, was 87.5% in formulated treatments with albumin to 10% and 5%, as well as treatments with glucose at 10 and 5% compared to the recovery of theactivity observed in treatments with albumin and glucose at concentration of 2%, were recovered the 54.0% of the original activity.(Figure 1)The experimental release results were analysed by ANOVA.An activity significant difference ($\alpha > 0.05$) between the food models with glucose to 5 % and 2 % compared against the model with glucose to 10 % was observed. The albumin to 2 % was significant different with regard to albumin to 5 and 10 %.

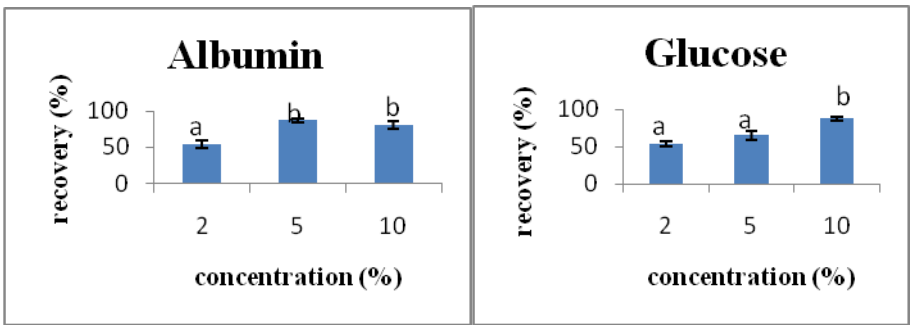

Figure 1. Recovery of the activity from the bacteriocin encapsulated in food systems models.
[ab]different letters indicate significant differences between treatments ($\alpha < 0.05$)

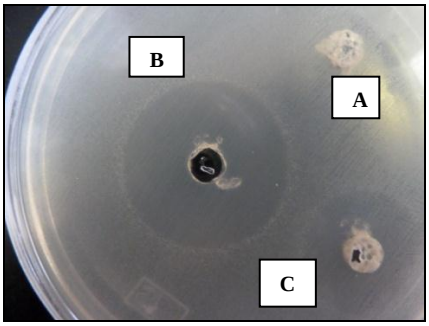

Figure 2.Inhibitory zones of the capsules containing bacteriocin. A Alone capsules; B Free Bacteriocin;C Capsules containing bacteriocin. Plates were incubated at 37° C for 18 h

Discussion

The samples of bacteriocin purified by the cell adsorption-desorption method, presented one activity greater compared to the activity observed in samples of bacteriocin in crude extract, which has been reported in the case of Pediocin PA-1/AcH purified by this method, which is based on the affinity between the molecules of bacteriocin and the cell surface on function of pH. Reporting for this bacteriocin an activity of 800 AU/ml and 3,276800 UA/ml in crude extract samples and samples obtained after the process of precipitation and dialysis respectively [8]. The bacteriocin encapsulated in calcium alginate inhibited the growth of the indicator strain, observing inhibitory zones. This suggests that the bacteriocin

activity was stable after the encapsulation process, even after being in contact with the models of food at different concentrations. These results are consistent with those reported for other bacteriocins as it studies on the activity of the bacteriocin synthesized by *Enterococcus faecium* CRL 1385 encapsulated by ionic gelling on cells of *Listeria*monocytogenes 99/287. Demonstrating the inhibited the growth of the pathogen in two logarithmic cycles, on solid and liquid medium [9]. On the other hand, it has been reported the effect of milk on the activity of nisin encapsulated in phosphatidylcholine nanovesicles, demonstrating nisin inhibited the growth of*L. monocytogenes* ATCC 7644. In another study, found that the bacteriocin from *Bacillus licheniformis* p40 encapsulated in nanovesicles phosphatidylcholine also inhibited the growth of *L. monocytogenes* ATCC 7644 [10-12]. According to the results obtained in the many investigations, encapsulated antimicrobial peptides can be considered a potential alternative for application as food additives.

Conclusions

The albumin and glucose at minimal concentration were the models that caused the greater decrease in the activity of bacteriocin.

The adsorption cell purification method allowed obtaining purified bacteriocin, which will be used in subsequent studies.The encapsulation of the bacteriocin from *P. acidilactici* ITV 26 in calcium alginate is viable method to stabilize the antimicrobial activity, protecting it from the effect of the ingredients of a foodstuff.

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Estimation Of The Shelf Life Of Pellets From Different Formulations Under Extreme Temperature And Humidity Conditions

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Introduction

Pellets or third generation snacks products are considered as semi or half-products used to manufacture snacks that can be stored and then expanded by frying or by using hot air. They require less storage space, present high stability during storage; better production flexibility and the costs for their distribution are lower. These products are made by an extrusion process by which a set of mixed ingredients are forced through an opening in a perforated plate or die with a designspecific to the food [1].

Despite the various advantages pellets represent to the food industry, their shelf life can be affected by different factors after storage. The shelf life of a food is the length of time that is given before it is considered unsuitable for sale, use or consumption. In other words it is the period of time that food products can be stored, during which their defined quality remains acceptable under expected (or specified) conditions of distribution, storage and display [2].

Prediction of the shelf life of food products may be performed by two ways. The first consists in subjecting the product to a certain extreme condition during different storage times and evaluate their quality by sensory analysis and then extrapolate the results (usually empirical) to normal storage conditions. The other option is to use a design based on the principles of chemical kinetics and determine the actual temperature dependence of various quality attributes, because it strongly affects the reaction rates [3].

The aim of this study was to estimate the shelf life of pellets manufactured by a company from Hidalgo (Mexico). The study was accomplished by placing five different pellet formulations in packaging paper/polypropylene and polypropylene/polyester and stored in a chamber food stability at 25°C, 20% and 50°C, 89% relative humidity (RH) for 36 days. The parameters monitored were peroxide index measured spectrophotometrically and moisture content through increase in weight.

Methods and materials

Five different pellet formulations belonging to the same production lot were provided by a company from Hidalgo, Mexico. Samples were placed in two different packaging: paper/polypropylene and polypropylene/polyester, after which they were stored in a food chamber stability at 25°C, 25% RH and 50°C, 89% RH for 36 days.

The initial microbial load: coliforms, aerobic mesophilic bacteria, yeasts and molds were analyzed according to established techniques in the Mexican Official Standards [6,7,8,9].

The increase in weight of the pellets was monitored to assess the effect of moisture as well as the peroxide index for which a chloroform/acetic acid (2 : 3) extraction was carried out for each sample. Peroxides present in the samples react with Fe^{2+} oxidizing it to Fe^{3+}. Fe^{2+} excess was measured on the spectrophotometer at 512 nm and a correction wavelength of 700 nm.

Results and discussion

For the microbiological analysis, the numbers of mesophilic bacteria, total coliforms and molds and yeasts were less than 10 colony forming units per gram for all the samples studied.

After 36 day of storage, an increase in the weight gain of all the samples was observed due to the effect of relative humidity. It was also observed an increase in the peroxide index of the samples, mainly under extreme conditions of 50°C and 89% RH in both packaging assayed.

In order to observe the behavior of samples regarding the gain in weight and the increase in peroxide values, results obtained for these determinations were plotted (Figures 1, 2, 3, 4 and 5). Afterwards, the kinetics of degradation was determined by adjusting the pattern of the reaction order. The results indicated that the zero-order model showed the best fit to the physicochemical changes occurred to the pellets. Labuza has reported that deterioration reactions of food products generally present a kinetic of either zero or first-order [5].

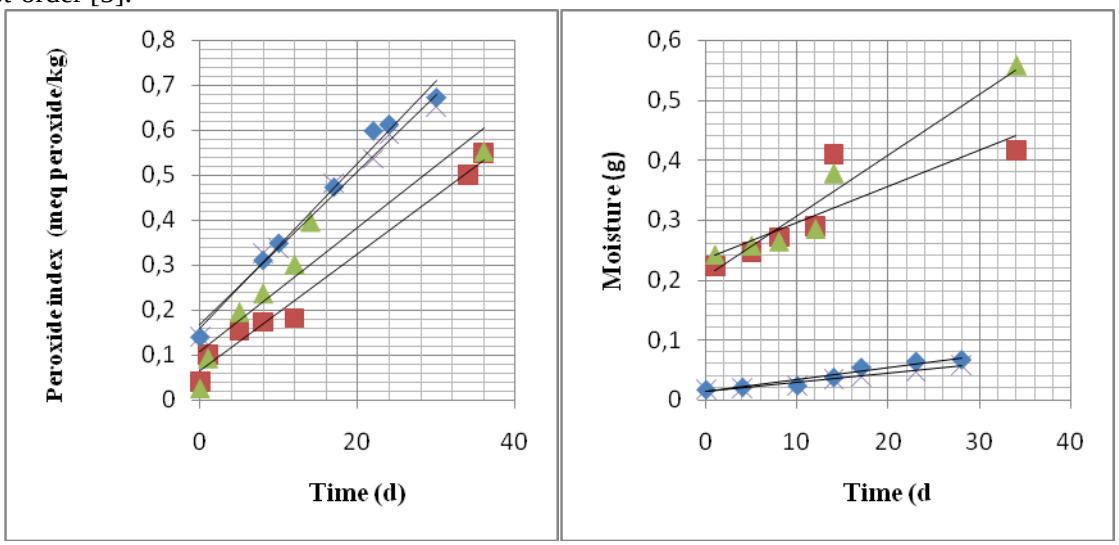

Figure1. Sample P1: A) Peroxide B) Humidity as a function of the time, in packaging ◆ polypropylene/polyester × paper/polypropylene (25° C, 25% HR) y ▲ polypropylene/polyester ■ paper/polypropylene (50 °C, 89 % de HR)

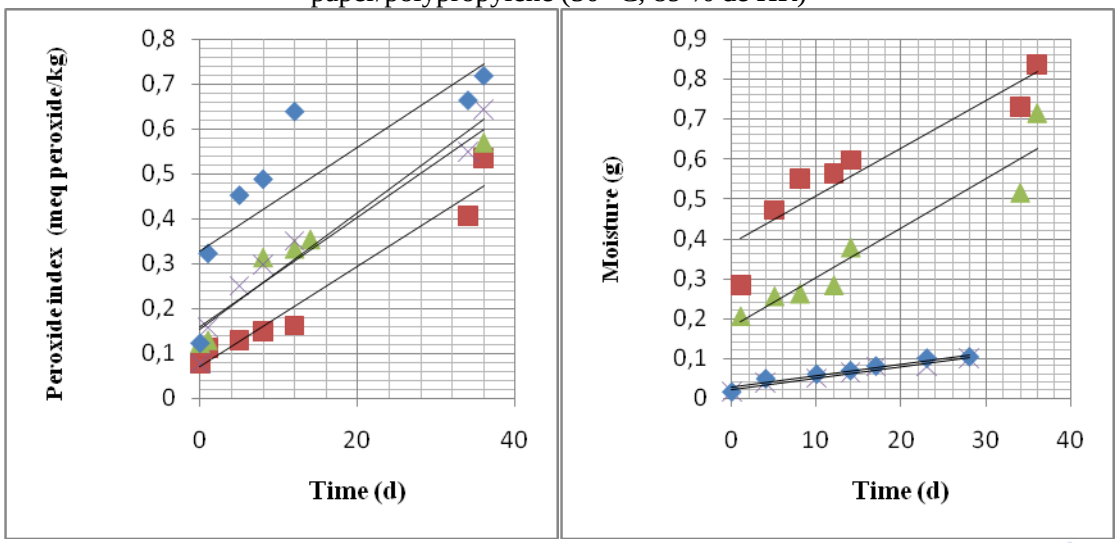

Figure 2. Sample P2: A) Peroxide B) Humidity as a function of the time, in packaging ◆ polypropylene/polyester × paper/polypropylene (25° C, 25% HR) y ▲ polypropylene/polyester ■ paper/polypropylene (50 °C, 89 % de HR)

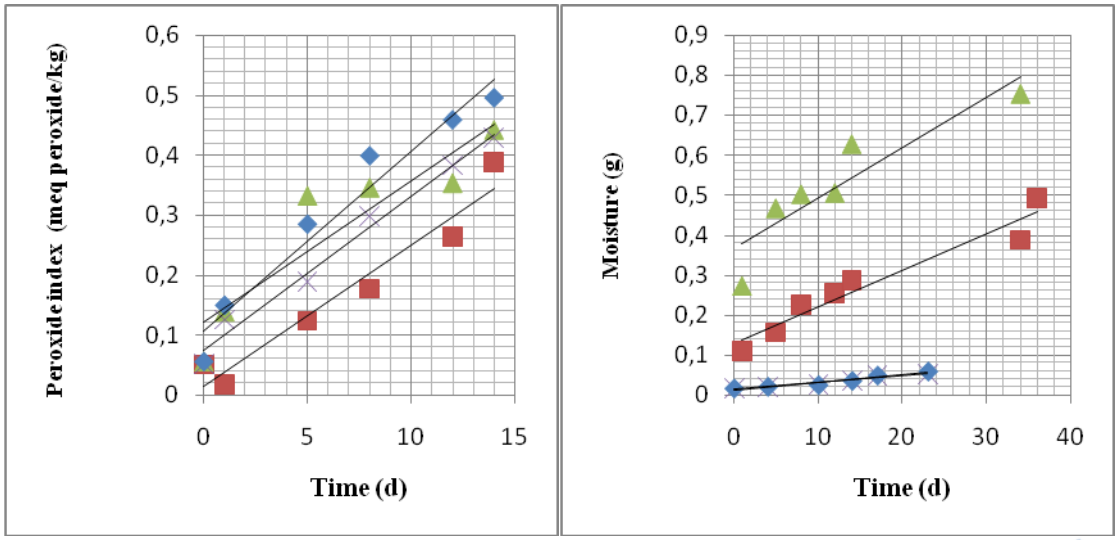

Figure 3. Sample PL3: A) Peroxide B) Humidity as a function of the time, in packaging ◆ polypropylene/polyester × paper/polypropylene (25° C, 25% HR) y ▲ polypropylene/polyester ■ paper/polypropylene (50 °C, 89 % de HR)

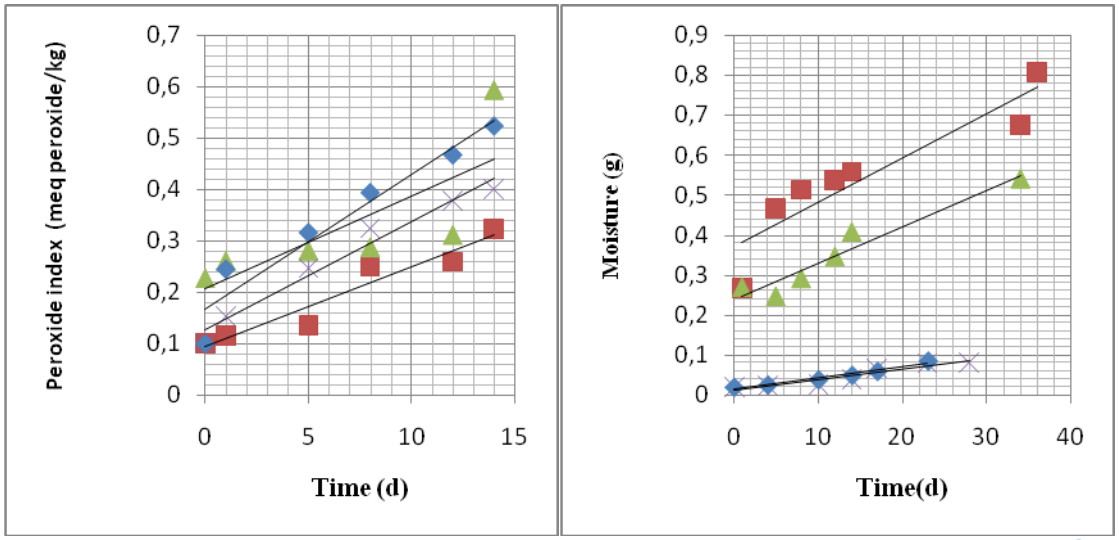

Figure 4. Sample PL4: A) Peroxide B) Humidity as a function of the time, in packaging ◆ polypropylene/polyester × paper/polypropylene (25° C, 25% HR) y ▲ polypropylene/polyester ■ paper/polypropylene (50 °C, 89 % de HR)

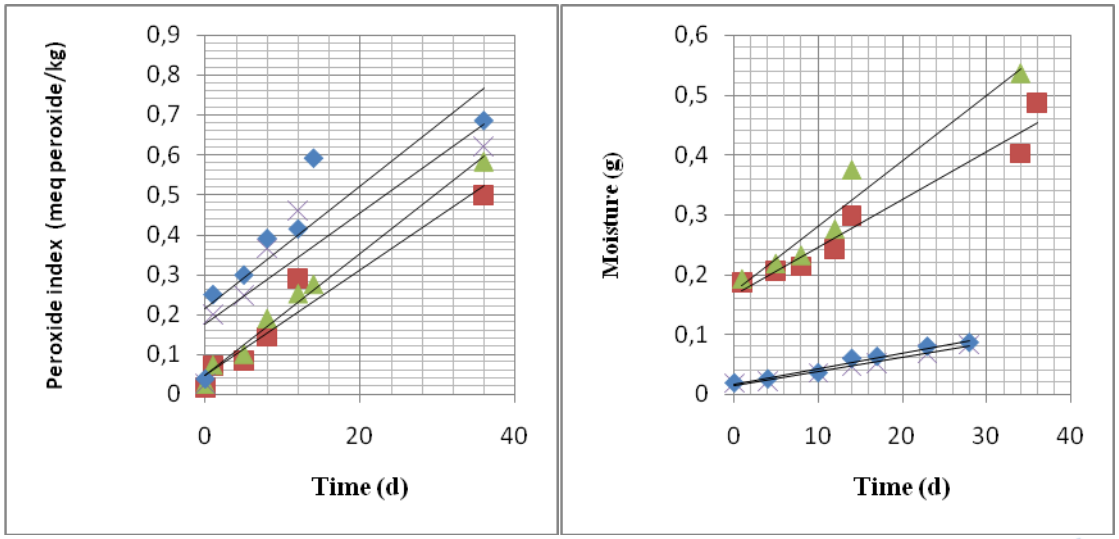

Figure 5. Sample PL5: A) Peroxide B) Humidity as a function of the time, in packaging ◆ polypropylene/polyester × paper/polypropylene (25° C, 25% HR) y ▲ polypropylene/polyester ■ paper/polypropylene (50 °C, 89 % de HR)

Based on the Arrhenius equation for a zero order kinetic, the shelf life of the 5 formulations of pellets analyzed was estimated taking into account the effect of relative humidity on the increase of peroxide values and weight gain under extreme conditions. A value of 10 meq peroxide/kg sample was considered as critical according to the Argentinean Alimentary Codex, which has set it as the maximum limit for food consumption [3]. The critical value of the increase of moisture content in each sample was different because of the difference in their composition, shape and size. For example, P1 and PL5 critical value were 0.5918 and 0.5768 g of water respectively, while samples P2, PL3 and PL4 presented higher values (0.7302, 0.6752 and 0.7638 respectively). However at all these values samples showed significant defects after frying due to its high water content.

The shelf life of samples estimated on the basis of the monitored parameters under the conditions of this study is shown in Table 1.

Table 1. Shelf life estimation of pellets at 25°C, 25% RH and 50°C, 89% RH

Sample	Packaging	Shelf life (days) Moisture content		Shel life (years) Peroxide index	
		25° C 25% RH	50 °C 89 % RH	25° C 25% RH	50 °C 89 % RH
P1	Polypropylene/polyester	289	38	1.46	1.95
	Paper/Polypropylene	385	58	1.58	2.06
P2	Polypropylene/polyester	234	28	2.05	2.18
	Paper/Polypropylene	252	44	2.28	2.4
PL3	Polypropylene/polyester	348	24	0.9	1.13
	Paper/Polypropylene	389	59	1.05	1.14
PL4	Polypropylene/polyester	267	34	1.02	1.48
	Paper/Polypropylene	298	58	1.28	1.72
PL5	Polypropylene/polyester	221	37	1.74	1.78
	Paper/Polypropylene	234	51	1.92	2.07

The shelf life of the pellets studied was considerably diminished when subjected to extreme conditions of

relative humidity, so it is recommended to keep them in a cool and dry place, to preserve their desirable properties for a longer time, although for a longer period of storage, the peroxides value slowly increases.

Results obtained suggest that the packaging made of paper and polypropylene form a barrier that do not avoid but reduces significantly the passage of light and oxygen which promote the lipid oxidation. On the contrary, polyester packaging has pores which permit the passage of oxygen.

Modeling the different types of deterioration in a food product is an alternative to generate quantitative tools to predict the loss of quality. The composition, packaging material and storage conditions are the main factors that determine the physicochemical properties of a product and its shelf life. The results obtained in this study indicate that the use of an adequate packaging can significantly reduce degradation reactions such as oxidation and the effect of relative humidity preserving the physicochemical properties and prolonging the shelf life of pellets used to manufacture snacks.

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Antimicrobial Activity Of *Rosmarinus Officinalis* On Methicillin-Resistant *Staphylococcus aureus,* Susceptible *Staphylococcus aureus* And *Salmonella typhi*

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MRSA is a serious problem in food industry, in nosocomial infections as in Animal production. Ever since last century, humans have used plants as an alternative for infections and possible contaminations. The objective of this work is to assess the activity of Rosemary (*Rosmarinus officinalis*) on MRSA (Methicillin-Resistant *Staphylococcus aureus*) and other strains. The culture was done based in the McFarland standard and Standards Guidelines for Disk Diffusion Susceptibility with the variant of Kirby-Bauer method; instead of using a disc in the Mueller-Hinton agar, a hole was made in the center of the petri dish and it was inoculated with three repetitions. The strains were: MRSA (ATCC BAA-1552 and 33592), susceptible *Staphylococcus aureus,* and *Salmonella typhi.* The strains were inoculated in the entire dish with a sterile swab. More experiments were made to the CA-MRSA to obtain the Minimum inhibitory concentrations (MIC). The ethanolic extract was from the leaves and stem previously washed with water and soap. It was filtered and put on the hole of the petri dish previously inoculated with the bacterial strains. Bacterial strains were incubated at 37ºC to the fungus at room temperature for 18 hours. These strains were measured with an average in the inhibition zone of 24mm, 29mm and 22mm to CA-MRSA, HA-MRSA and susceptible *S. aureus* respectively and for *S. typhi* there was no zone, but it had a bacteriostatic effect. It was compared with Kanamicyn ($50mgmL^{-1}$), which had obtained 24mm of inhibition zone. The MBC to CA-MRSA was of $0.17gmL^{-1}$ and the MIC to MRSA was of $10mgml^{-1}$. In conclusion, Rosemary has a potential antimicrobial activity and the next step is to recognize and identify who acts, where it interacts, and how is the activity to know the type of activity of gram-positive, gram-negative and acid-fast bacteria, and to obtain a new antimicrobial to use as treatment in infected human or animals, in the food industry or as sanitizer.
Keywords: *Rosmarinus officinalis*, Antimicrobial, MIC, MRSA.

Introduction

Rosemary is a very important plant used in Mexico because of its multiplex effects, as Anti-inflammatory, antioxidant, antiseptic, antifungal, control of Ectoparasites, etc., in humans and animals. In the early years, many kinds of bacteria have gained resistance against many antibiotics and people sometimes appeal to natural medicine that has been used all around the world for many years, and the use of medicinal plants is an option that people take. MRSA is a serious problem in the food industry, nosocomial infections as well as in Animal production. Ever since last century, humans have used plants as an alternative for infections and possible contaminations.

Staphylococcus aureus, commonly called *"Staph aureus",* is a common cause of foodborne illnesses, this bacterium produces toxins that cause the illness and the bacteria can be present in air, dust, sewage, water, milk, food or in food equipment, environmental surfaces, medical equipment, humans and animals. People can contract the illness by eating food that has been contaminated with *Staph aureus*, usually because the food has not been kept hot enough or cold enough. When contaminated food is left out more than two hours at room temperature, *Staph aureus* begins to grow and will produce a toxin. The toxin produced by staph bacteria is very heat-stable and it is not easily destroyed by heat. Depending on the strain, *S. aureus* is capable of secreting several toxins that are categorized into three groups, associated with specific diseases: 1) Pyrogenic toxin superantigens (PTSAgs), 2) Exfoliative toxins, implicated in staphylococcal scalded-skin syndrome and 3) Staphylococcal toxins that act on cell membranes and include alpha-toxin,

betatoxin, delta-toxin and several bicomponent toxins like Panton-Valentine leukocidin (PVL) that is associated with severe necrotizing pneumonia in children.

Methicillin-Resistant S. aureus (MRSA) is now a bacterium object of multiple studies due to the acquisition of resistance to antibiotics in recent years and it is a worldwide growing problem. MRSA has a proportion of isolates that was increased from 35.9% in 1992 to 64.4% in 2003 for hospitals, it has been reported in many species, not only in humans: pigs, dogs, horses, cats, cattle, sheep, rabbits, rats, guinea pigs, a chinchilla, a bat, a seal, dolphins, a walrus, an elephant, poultry, pigeons, parrots and turtles. For that, the preventing MRSA infection is a high priority for CDC. The federal government uses several complementary systems to track drug-resistant infections and drug-resistant bacteria, including MRSA in humans as well as in animals and food. The vast majority of the estimated 94,000 life-threatening MRSA infections annually are associated with medical care (3).

Many naturally occurring compounds have been tested in an extensive battery of strains. Polyphenols to tannins, which are the most abundant compounds in a plant, have important effects on human metabolism and as antimicrobial agents. It has been shown that rosemary essential oil is able to growth inhibition such as *L. monocytogenes, Mycobacterium smegmatis, Staphylococcus spp., C. albucans* and *C. tropicalis* among many others (4, 6).

It has been reported that *R. officinalis* has effect on *Staphylococcus aureus*, in tests with RP-HPLC techniques that investigate the anti-QS activity through quantification of δ-hemolysin (2). Some tests were expanded with Rosemary to also include its essential oil and four of the major oil constituents, oleoresin, encapsulated oil, and antioxidant extract of rosemary against *Lysteria monocytogenes* (10). It also has been shown that *R. officinalis* essential oil, coated nanoparticles ((Fe3O4/oleic acid:CHCl3 0.33% (w/v)) strongly inhibited the adherence ability and biofilm development on catheter surface of the *C. albicans* and *C. tropicalis* (1).

In this work, we demonstrated that Rosemary has an important effect on Methicillin-resistant *Staphylococcus aureus* (CA-MRSA and HA-MRSA), susceptible *S. aureus* y *Salmonella typhi*.

Methodology

Plant material was collected from Salinas Victoria, Nuevo León, and fresh leaves were used for the extraction, where 5g of leaves were macerated in 30ml of EtOH 70% and filtered with Whatman # 1 paper in absence of light. The EtOH extract was stored at -20ºC with a final concentration of 160mgml-1. Strains of Methicillin-resistant *Staphylococcus aureus* (ATCC HA-33592 and ATCC CA-BAA 1556), susceptible *Staphylococcus aureus* "CCIV4" from a Cow of the Agronomy College, in Marin, N.L., *and S. typhi* from Agronomy College of the Laboratory of Environmental Remediation and Analysis of Soil, Water and Plants, were grown with the McFarland Standard to create inoculums densities of 1.5x108 cfu/ml in Nutrient Broth. This same procedure applied for the next experiments. For the Minimum bactericidal concentration (MBC), culture was done based in the McFarland standard and Standards Guidelines for Disk Diffusion Susceptibility with the variant of Kirby-Bauer method; instead of using a disc in the Mueller-Hinton agar, a hole was made in the center of the petri dish and it was inoculated with three repetitions. The strains were inoculated in the entire dish with a sterile swab and the concentration of the EtOH extract was poured in the hole, waiting 15 minutes to spread in the agar and incubated at 37ºC. For the Minimum Inhibitory Concentration (MIC) in petri dish, the procedure was like the one of MBC but instead of big concentrations, they were used minimum concentrations in dilution 1:2 with a starting concentration of 16mgml-1. It was incubated at 37ºC. And for Minimum Inhibitory Concentration (MIC) in tubes of nutrient broth, they were used minimum concentrations in dilution 1:2 starting with a concentration of 80mgml-1 incubated too at 37ºC for 20h.

Comparison of the Effect of R. officinalis versus Kanamycin

16mg of vegetal material was compared against 1.5mg of Kanamycin for give a bactericidal equivalent.

Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. The data were subjected to one-way analysis of variance (ANOVA), and differences between samples were

determined by Tukey's tests using the SPSS 17.0 software. A significant difference was considered to be $p < 0.05$.

Results and Discussion:

Rosemary presents an important inhibition, more than Sue Chao et al. (2008) (6) reported, with a diameter inhibition of 26mm by an essential oil of Rosemary; here with an EtOH extract and a final concentration of 16mgml-1 it has an average of 27.2mm, 26mm and 20.60mm to CA-MRSA, HA-MRSA and sensible *S. aureus*, meanwhile to *S. typhi* it only has a bacteriostatic effect. It can be an option due to the fact that sometimes the extraction of essential oils is expensive. It is required .16gml-1 of Rosemary extract for the inhibition with an average in the diameter zone inhibition between 20-27mm, which is similar to the one of *Nase Jarrar et*. al reported with a diameter zone of inhibition between 16-23mm with essential oil. It's important to highlight that HA-MRSA and CCIV 4 have an important resistance that CA-MRSA does not present. In Table 1, we show the zones of growth inhibition with their concentrations.

***Table 1**. Zones of growth inhibition (mm) showing antibacterial activity, well diameter 6.0mm. (MBC).*

mg/ml	Average (mm)		
	CA-1552	*HA-33592*	*CCIV4*
0.8	*9.5*	*6.0*	*8.0*
1.2	10.3	9.0	10.5
3.2	14.3	13.0	11.5
4.8	17.3	15.0	14.0
6.4	17.3	17.0	15.0
8	18.7	19.0	15.5
9.6	20.0	18.0	16.5
11.2	20.0	18.5	16.5
12.8	21.0	20.0	18.0
14.4	22.3	21.5	18.5
16	27.2	26	20.60
SE	***± 2.3768***	***± 2.339***	***± 1.9477***

In Figure 1, the inhibition zone between Kanamycin (K) [1.5mgml-1] and Rosemary (R) [4.8gml-1] was of 23mm and 21mm respectively. All the negative controls with EtOH 70% for the tests in petri dishes, were correct. For the MIC tests, the result was of 10mgml-1. There is a comparative between an EtOH and aqueous extract where the aqueous extract had no effect (Figure 3). In other experiment, with 16mgml-1 in a mixture with 5ml of sterilized agar before solidifying and after inoculated we demonstrated that there is no growth of CA-MRSA (Figure 2).

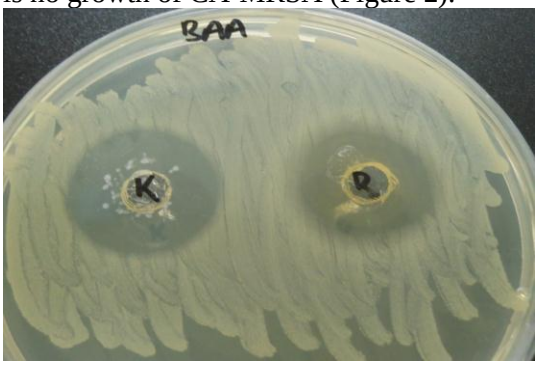

***Figure 1.** The inhibition zone comparation between Kanamycin (K) 1.5mgml-1 with 23mm and Rosemary (R) 4.8mgml-1 with 21mm.*

Conclusion

In Mexico, rosemary is very used in gastronomy because of its strong and delicious flavor, and also in natural medicine, because of its use in many treatments like Headaches, for anemia, depression, menstrual problems, strengthen memory, painful indigestion, rheumatism, twisting, wound healing, alopecia, etc.

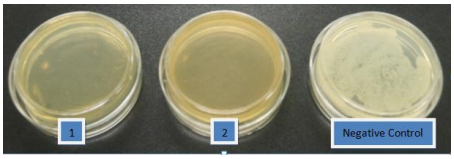

Figure 2. Growth inhibition in Muller-Hinton agar added with *Rosmarinus oficinalis* extract.

In this paper, we showed that Rosemary has an important effect *in vitro*, such bactericidal and bacteriostatic, but most important on CA-MRSA and HA-MRSA that produces important infections around the world. We can see how the Strain CCIV4 presents some particular resistance like the CA-MRSA. However, HA-MRSA Strain has a very important sensibility to rosemary with a diameter inhibition of 15-35mm with a concentration of .17gmL^{-1} that can prevent the growth of this bacterium. It show that it can been uses in hospital as a measure to clean like complement in the hospital surface or catheters because it also has an effect on fungus, and it can be corroborated with other reports. This can be used to give a use as antimicrobial or a combination with other treatments because it has a wide spectrum, against important food microorganism like *Lysteria monocytogenes* (gram-positive) (42) and *E. coli* (gram-negative). The use of Rosemary can be a support in treatments against MRSA too as we showed in this report. 10mgml-1 of EtOH extract of Rosemary to have effect. However, it must be studied even more to understand the kind of compound or compounds that the activity has against the bacterial and their use in different ways.

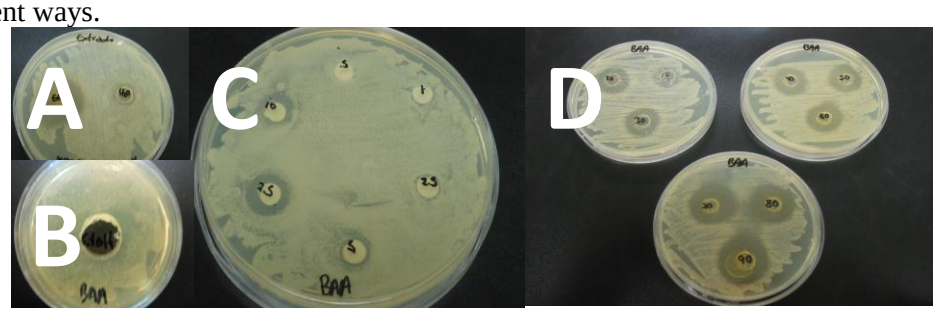

Figure 3. A); The comparison between the EtOH and aqueous extract where the aqueous extract doesn't have effect. B); Only EtOH 70% in the hole where doesn't have effect. C y D);

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Combination Of Bacteriocin 29 With Nitrites To Control Of *Brochothrix thermosphacta* InVaccum Packaged Sausage

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Introduction

The loss of food quality is generated by deteriorating microorganisms and pathogens. Different processes are used for their control or elimination, in order to obtain healthier, nutritional and sensory acceptablefoods(1).The chemical preservatives, likenitrate and nitrite,may not by the consumers, as they could generate carcinogenic compounds (2). Therefore, consumers preferminimally processedfoods freed of chemical additives in their formulation.

On the other hand, bacteriocins are antimicrobial peptides and considered natural preservatives as they are inactivated by proteinases during their transit into the intestinal tract (3). The use of bacteriocins in foods is acceptable only asa barrier mechanism, so it is necessary to conduct studies to find out their behavior in combination with other chemical preservatives to ensure the microbiological quality of foods. It was found "in vitro" that the combination of sodium nitrite and the bacteriocin 29, produced by *Enterococcus faecium* MXVK29, inhibits the growth of *Brochothrixthermosphacta*, *Listeriainnocua* and *Escherichia coli* (4). The aim of this study was to evaluate the combined effect of sodium nitrite and bacteriocininto a vacuum packed meat sausage inoculated with *Brochothrixthermosphacta*.

Methods and materials

Bacteriocin extraction:

Enterococcus faecium MXVK29 was reactivated in CGB medium. The bacteriocincrude extract was obtained from a 16 h culture after heating (70˚ C for 30 minutes) and pH adjusting to 6.5 by using 10M NaOH. Cells were removed by centrifugation (3100 × g for 20 min at 4˚ C) and the supernatant containing the bacteriocin was freeze-dried (0.005 atm and -70˚ C). Then, thefreeze-dried extract was dissolved in sterile distilled water (500mg/mL). Finally the solution was sterilized by filtration (sterile 0.22 µm pore sizeDurapore® Membrane Filters; GV, Ireland)and stored at -80 ˚C(5).

Meat model:

The meat emulsion was formulated with 49.95% pork, pork fat (lard) 14.98%, 24.97% crushed ice, wheat flour 7.0%, 2.5% NaCl, 0.2% sodium erythorbate and sodium phosphate 0.4% and50 µg/g of sodium nitrite. The meat batter was stuffed in a cellophane sleeve and subjected to a thermal process (72 °C for 30 minutes) followed by thermal shock (0 °C for 5 minutes).

The cellophane cover was removed aseptically and the bacteriocin was applied as a coating, in concentrations of 3,125 mg / mL and 6.25mg/mL, the sausages were left to dry into a laminar flow cabinet at 25 °C for 2 minutes. Then, sausages were inoculated with *B. thermosphacta*NCIB-10018 previously reactivated in TSB medium (5 log CFU / mL). Finally, sausages werevacuum-packed (0.005 atm Torrey® Vaccum System EVD16, México) and stored under refrigeration at 10 °C to simulate refrigeration abuse conditions.

Six treatments were performed using a chessboard array (Table 1): 1 with NaNO2 (50 ppm), 2 with the bacteriocin (3.125 and 6.25 mg / mL), 2 combinations of bacteriocin and nitrates (3,125 mg / mL bacteriocin + 50 ppm nitrites and 6.25 mg / mL bacteriocin + 50 ppm sodium nitrite) and a negative control (without antimicrobials).

Table 1. Nomenclature for treatments applied in the model.

Code		TS	TBI3	TBI6	TSN	TNBI3	TNBI6
Combination	$NaNO_2$	0 mg/mL	0 mg/mL	0 mg/mL	0.05 mg/mL	0.05 mg/mL	0.05 mg/mL
	Bacteriocin	0 mg/mL	3.125 mg/mL	6.25 mg/mL	0 mg/mL	3.125 mg/mL	6.25 mg/mL

Microbiological analysis was performed on days 1, 3, 7, 14 and 30 for aerobic mesophilic (agar standard account, BD Bioxon ®), lactic acid bacteria (LAB, APT agar BD Difco ®) and *Brochothrixthermosphacta* (STAA agar base BD Difco ®, supplemented with cyclohexamide, thallium acetate and streptomycin sulfate).

Results and discussion

Figure 1 shows the growth of aerobic and facultative anaerobes microorganisms. The treatment TS (corresponds to the control) as no antimicrobials were added and was not inoculated. The initial microbial concentration was acceptable according tothe NOM-213-SSA1-2002 (6), its limit is 60 000 CFU/ mL (4.8 log CFU / mL). This treatment presents a biphasic growth curve (7) with a decrease in growth at day 7 as anaerobes and facultative aerobes can survive into anaerobic conditions. However, an increase in the population was observed after day 14(8). The TSN treatment shows inhibition for nitrite against microorganisms, this chemical preservative under acid conditions produced HNO2, which also is catalyzed by the presence of sodium chloride and erythorbate. The nitrogen oxides attack the amino dehydrogenase system of microbial cells (9). The treatment TNBI3 with nitrites and bacteriocin [3.125 mg/mL] were less efficient than the use of one antimicrobial due to their similarity in their inhibitory mechanism. Nitrites could produce stress in microorganisms, and the bacteriocin could be degraded by proteolytic enzymes produced by *B.thermosphacta* (10).The treatment TNBI6 with 6.25 mg /mL of bacteriocin, shows a similar growth to TNBI3, but unlike this, TNBI6 decreases microbial load initial.

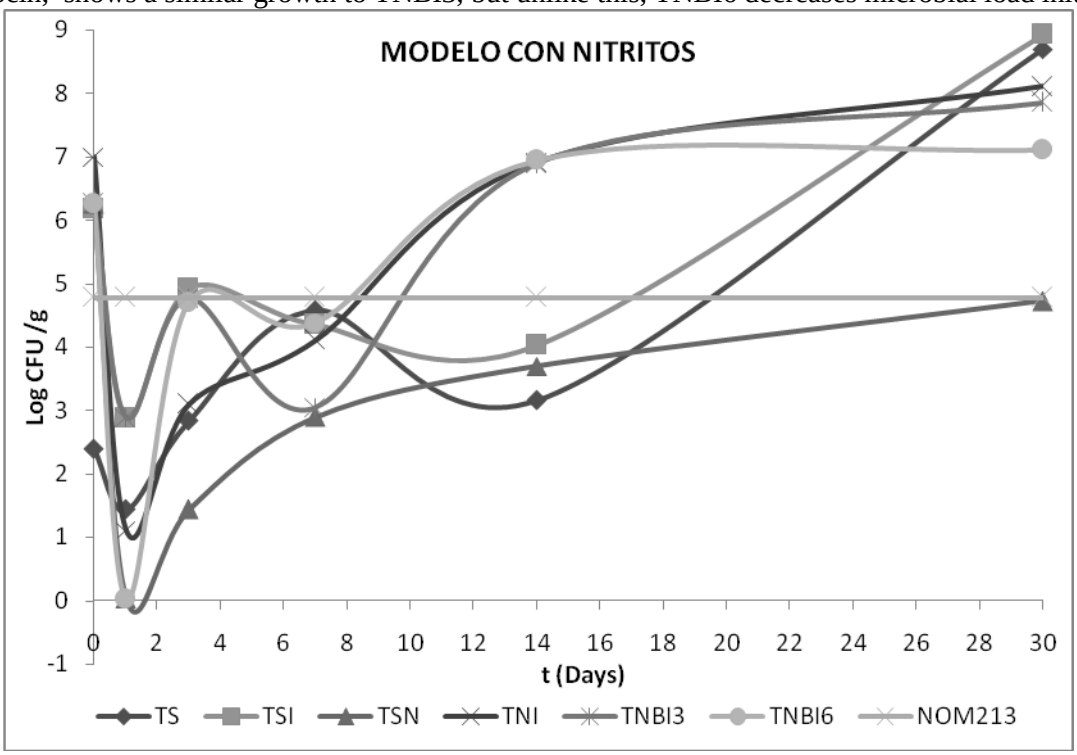

Figure 1.Variation of aerobic bacteria population on meat model.

Figure 2 presents growth of LAB. The TS treatment presents a typical growth curve that could be considered acceptable and microbially stable until day 20, as data did not exceed the NOM213 limit. By comparing this treatment with its counterpart from the standard account (Figure 1) it is clearly shown that the amount of viable of LAB cells is lower than the standard viable count.The TSI treatment followed a similar trend of that for TS, but TSI treatment isn't very effective against LAB in the meat model. Initially TNI did not show effect against LAB, since these bacteria reach the first 7 days the microbiological limit; after day 7 little inhibition was observed against LAB. This inhibition could be by the action of nitrites present in treatment in combination with a pH reductition.

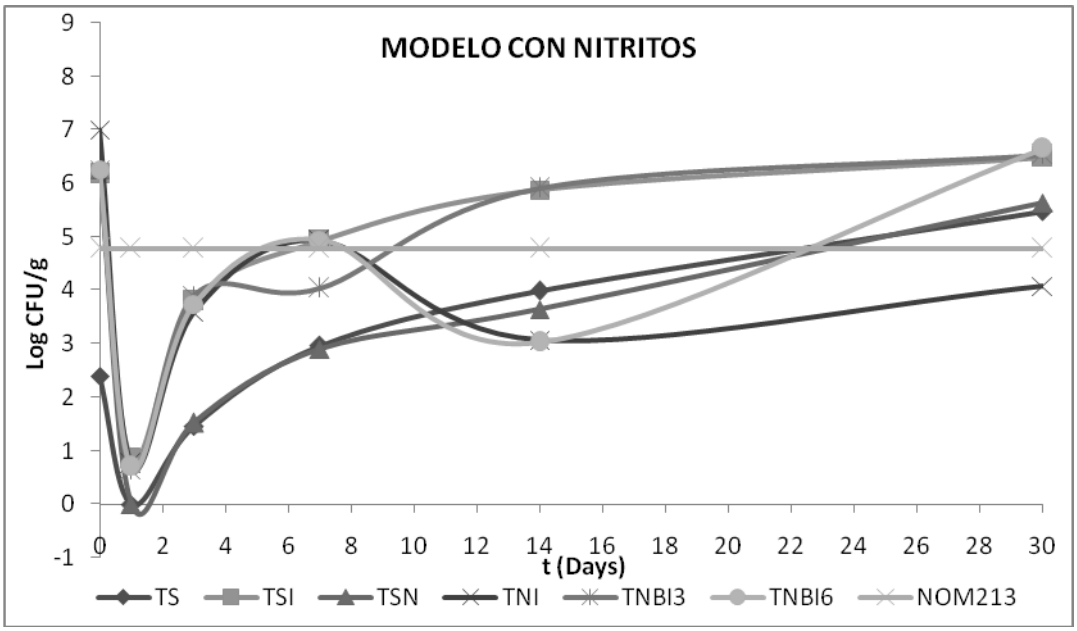

Figure 2.Variation of LAB on meat model.

Figure 3 presents the *B. thermosphacta*growth. The TS treatment shows a growth curve acceptable and microbiologically stable. It is recommended that the bacteria population does not exceed 5 log cfu /mL, because after this point the aromas and flavors developed by *B. thermosphacta* in meat are perceptible by consumers (11). The TSI treatment had a higher microbial population reaching the suggested limit in 3 days. The TNI treatment keeps the meat model below the suggested limits until day 10. The treatment TNBI6 presents very similar trends to TNI, showing an inhibition of *B. thermosphacta,* until day 14.

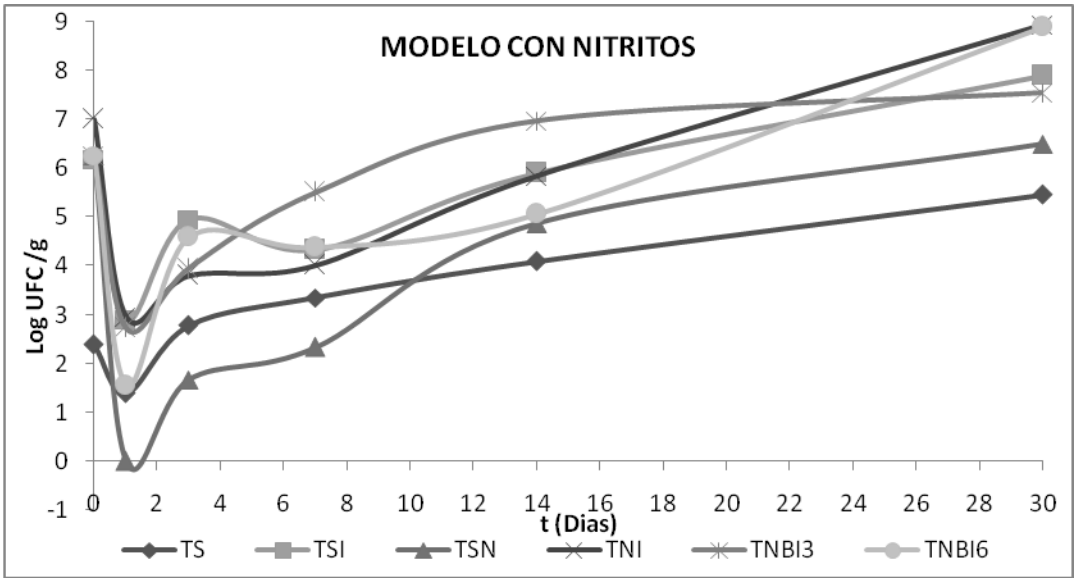

Figure 3.Variation of *B. thermospacta* on meat model.

Conclusion

None of the combinations have microbiological acceptable conditions after 7 days of storage. The combination with the best microbiological stability of the meat model for 7 days of storage was 6.25 mg/g of bacteriocin with 50 µg/g of sodium nitrite, unlike the control (3 days of storage). This combination has a synergistic effect against *B.thermosphacta* in a meat emulsion with temperature abuse, indicating that the bacteriocin could be used as part of hurdle technology. Also the reduction of nitrites into sausages (100 µg/g maximum permissible limits) could reduce the formation of nitrosamines and carcinogens compounds in the final product, but more studies need to be performed.

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Arsenic In Water And Its Effect On Goat's Milk Quality In The Comarca Lagunera, Mexico

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Introduction

The Comarca Lagunera is a region located in northern Mexico formed of the states of Coahuila and Durango. This region consists of sixteen municipalities, eleven of the state of Durango and five of state of Coahuila (see figure 1). The Comarca Lagunera is between latitudes 25 and 27 degrees latitude north and between 103 and 104 degrees longitude west of Greenwich, with a height of 1129 m above sea level [(7)].

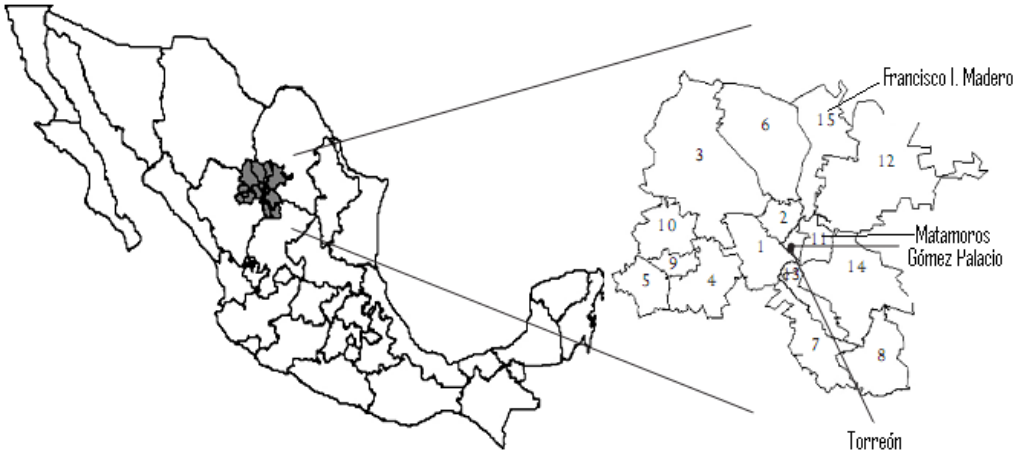

Figure 1. Map from Comarca Lagunera. Image modified from Garcia and Mora, 2008

Investigations show that in this region there is heavy metal poisoning caused by lead, cadmium and arsenic principally, which are highly harmful elements for health [(20)].Heavy metals have become a current topic in the field both in environmental and public health. The damage they cause is sometimes so severe and so absent of symptoms, the environmental and health authorities around the world devote much attention to minimize public exposure to these toxic elements [(2)].

In relation to arsenic, given the weather conditions of the Comarca Lagunera, which is characterized by a very dry place with an annual precipitation of about 250-350 mm, it is necessary to extract groundwater at great depths, and is known that this region has an abundant substrate of arsenic in this water, as mentioned Rosas and collaborators (1999). Generally, heavy metals remain in the groundwater and soil, but in certain areas of your level increase and tend to accumulate to toxic levels in human tissues and animal derived food, water and contaminated soils [(1)]. Arsenic is very toxic and causes damage to the neurological system, the cardiovascular system and is linked to various types of cancers such as skin. Chronic arsenic poisoning

may manifest itself by the appearance of sores and look lepros [2]. Moreover, one of the major concerns about safety and water quality is due to the potential presence of chemical contaminants. These may include organic and inorganic chemicals and heavy metals from industrial sources, agricultural and urban runoff [10].

Also, Comarca Lagunera is characterized by having and maintaining milk productivity since the forties represents one of the major dairy regions of Mexico and still is to date [4]. This region has also benefited from the production of caprine milk. A study in the Region has determined that goat milk production is cost effective. In 2002 it had a total of 476, 494 heads exploited to obtain milk with a production of 71 million liters per year that make production of goat milk a very important activity, as it manages to obtain indices of return to the 120%, where 70% of revenues come from traditional producer milk sales and the rest from selling the goats [16].

The goat is characterized as a dairy animal, the adequate management of their milk is one of the best strategies to alleviate famine and fighting undernutrition in developing countries [5]. In addition to that goat milk for their various properties and characteristics of high efficiency, represents an interesting commercial alternative today [6]. The goat milk is a dynamic and growing industry that is essential to the welfare of hundreds of millions of people worldwide and is an important part of the economy in many countries, so it is important to know its properties nutrition [19].

The milk is a defined mixture of substances: lactose, fatty acids, casein, among other components [14]. Below are the constituents of goat milk highlighting its nutritional qualities. In general, it is estimated that approximately goat milk contains 194 mg of calcium, 270 mg of phosphorus, 50 mg of sodium and 204 mg of potassium per 100g [9]. Goat milk favorably meets the needs of thiamine (B1), riboflavin (B2), pantothenic acid (B5). The main carbohydrate found in milk is lactose. The content of this disaccharide in goat milk ranges from 3.8% to 5.12%. From lactose goat milk is characterized by its higher digestibility compared to cow's milk, since it has less intolerance to this [3].Regarding with proteins, goat's milk contains about 5.2 grams of nitrogen per kilogram, which was converted into 33.2 g of protein [13]. Respect to the content of fat, medium chain triglycerides are present in the milk of goats, have six to fourteen carbon atoms, these fatty acids are caproic (C6), caprylic (C8) and capric (C10) is by this that take their name from which mostly appear in 15% in the composition of goat milk. Overall, goat milk differs from cow for the following beneficial properties: better digestibility, buffering capacity, high nutritional value and medicinal qualities [8,13].

The macronutrient and micronutrient composition of goat milk depends on main factors of production are: race, genetic characteristics of animal reproduction and animal health, environmental factors, time of lactation, socio-economic status, type of diet supplied to them, including water which is the most important single component for livestock, that is involved directly in every physiological process. Water is a means of transport of nutrients, waste materials, hormones and other chemical messengers, as well as to the food through the gastrointestinal tract. In this sense the amount and type of fiber, protein level, particle size, the addition of vegetable fats or oils and concentrated forage ratio are major players involved nutritional scale on the production and quality of milk in reality, the relationship between these factors may be narrow and complex [3,11, 19].

It is known that goat's milk has very diverse physiochemical characteristics. Cow's milk is slightly acidic, while the goat is almost alkaline (pH 6.7), due to its higher protein content and different combinations of its phosphates. So this milk is used in people with heartburn. As their density ranges from 1.026 to 1.042, which variation largely explains the different fat content in milk from goat, and on which are also involved in the non-fat solids content. The freezing point of goat milk is next to -0.590 ° C, lower than that of beef (-0.540 ° C) as a result of the higher content of that solute [3, 11,17]. Improving the hygienic quality of raw goat milk produced on farms, is an essential condition for increasing the competitiveness of

dairy farms. For its part, the consumer requires that the different processes of production, conservation, storage, transport and processing of raw milk are kept sanitary quality levels and applying measures to ensure food security [18]. Therefore, in the present research the objective was determining whether the water quality at various levels of arsenic has effects on the quality parameters of milk.

Methods and materials

During the months of July and August 2012 was conducted using water samples and goat milk in twelve ejidos in the municipalities of Matamoros, Francisco I. Madero and Gómez Palacio de Coahuila and Durango, with a total of 18 water samples and 18 samples of milk. To collect samples of water and milk were used clean polyethylene containers that were previously treated with 10% HNO_3 and deionized water. In the case of the water samples were collected from 250 mL, were added concentrated HNO_3 suprapure degree, while closed air chamber, identified and transported in a container with ice to the laboratory, the samples were preserved at 4 ° C until analysis. Milk samples were taken 150 mL, while closed air chamber, were identified and transported in a container of ice to the laboratory and preserved samples at 4 ° C until analysis.

For the physicochemical analysis of the water samples were measured pH and electrical conductivity (EC) with Orion pH meter model 710A, nitrates analysis was performed using the technique of mineral nitrogen, where an aliquot of 20 mL in a tube distillation takes a distiller Labconco. Are added to the distillation tube magnesium oxide 0.3 g was allowed to distill until a 60 mL sample in the beaker which was previously added 20 ml of boric acid. After the alloy was added to the tube 0.3g Devarda distillation is distilled out in another beaker containing 20 mL of boric acid. After obtaining the two distillates were titrated with 0.005N hydrochloric acid and then calculate the nitrate content.

Analysis of the goat milk quality parameters it was used the equipment MilkoScope where were measured: fat, density, lactose, solids-not-fat, protein, water, freezing point and total solids. The water samples are one phase, colorless, odorless and transparent, directly they were analyzed by atomic absorption spectrophotometry, without digestion. When you started analyzing the samples were exposed to room temperature for an hour and then proceeded to quantify arsenic using an atomic absorption spectrophotometer with graphite furnace AAnalyst Perkin Elmer model 700 with a Perkin Elmer autosampler AS800, using software Datamanagemente WinLab32 Atomic Absorption. For use arsenic reading the hollow cathode lamp As, at a wavelength of 193.7 nm. We performed a calibration curve with the standard arsenic 100 $\mu g \cdot L^{-1}$, was used HNO_3 as pure white supragrade and as modifier of matrix palladium / magnesium. Analysis of data from statistical test was used Kruskal-Wallis with SAS 9.2 statistical program (2009).

Results and discussion

Based on the results of arsenic in water, the samples were classified into four levels: Low (<25 $\mu g \cdot L^{-1}$), Medium (25-50 $\mu g \cdot L^{-1}$), High (50 – 100 $\mu g \cdot L^{-1}$) and Very High (> 100 $\mu g \cdot L^{-1}$), where differences were significant (Pr <0.05) between them. Arsenic concentrations, pH, electrical conductivity and nitrate obtained in the water samples of the ejidos of the Comarca Lagunera are shown in Table 1, where it can be seen that the highest concentrations of arsenic were found in the ejidos of Francisco I. Madero ie El Venado and Charcos de Risa their concentrations are exceeding 10 and 20 times the concentration allowed in accordance with standard NOM-127-SSA1-1994 of water for human use and consumption sets the maximum permissible limit of 0.025 $\mu g \cdot L^{-1}$ for arsenic. Benito Juárez presents a significantly higher concentration, be urgent action in these three locations. Also in the stratum of higher concentrations of arsenic are statistically higher pH and EC, there is a significant correlation between pH and EC.

Table 1. Analyses of water samples

Sampling sites	Concentration of Arsenic ($\mu g \cdot L^{-1}$)	pH	EC (mS·cm)	Nitrates ($mg \cdot L^{-1}$)
El Cariño, Gómez Palacio	12,44	7.252	0.62	2.927
El Consuelo, Matamoros	15.82	7.545	0.44	0.546
Corona, Matamoros	17.26	7.587	0.48	1.092
La Esperanza, Matamoros	18.05	7.686	0.45	1.456
Benito Juárez, Matamoros	18.51	7.529	0.48	1.274
San Felipe, Gómez Palacio	18.92	6.865	0.37	0.77
Atalaya, Matamoros	40.11	7.550	0.38	0.364
Solima, Matamoros	47.47	7.567	0.37	0.546
El Venado, Francisco I. Madero	63.19	7.289	1.38	0.924
Morelos, Gómez Palacio	65,15	7.223	1.91	10.197
Charcos de Risa, Francisco I. Madero	242.30	9.427	5.2	0.462
Benito Juárez, Franciso I. Madero	1332.40	7.548	2.2	1.078

The quality parameters of the milk that were measured are shown in Table 2, in which only significant difference in the density of fat and the samples in the different groups. The fat had a significant correlation coefficient with density. Also significantly correlated with the density of lactose, solids-not-fat, protein, water, freezing and total solids.

Table 2. Quality parameters of milk

Stratum	Concentration	Fat	Density	Lactose	Non fatty solids	Proteins	Water	Freezing point	Total solids	Percentage acidity
B	<25	3.304	45	4.708	8.597	3.161	0.935	-0,547	0,702	0,191
M	25-50	6.521	40.664	4.563	8.333	3.076	1.725	-0,546	0,681	0,201
A	50-100	5.446	40.449	4.298	7.847	2.868	6.498	-0,501	0,601	0,19
MA	>100	3.283	45.428	4.78	8.703	3.201	1.633	-0,545	0,713	0,168

Finally, no effect was found in any of the variables for arsenic concentration in milk quality, however the water quality is being affected by the high content of arsenic in some ejidos of the Laguna District, very important case study because water is an essential element for livestock, because this involved in every physiological process, which includes the synthesis of milk so the water must have certain quality parameters to not pose a risk to the public health. It is suggested the analysis of arsenic in milk to determine the transfer this metalloid to milk.

Conclusions

Water quality is affected by the high content of arsenic, therefore, it is important to further study this situation, since water is essential for cattle, and is involved in every physiological process including milk synthesis.

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Screening Of Lactic Acid Bacteria As A Potential Source For The Production Of Novel Antimicrobial Compound Phenyllactic Acid

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Abstract

In this study, 15 Lactobacilli strains including 7 *L. casei*, 2 *L. rhamnosus*; 2 *L. fermentum*; 1 *L. acidophillus*; 1 *L. sakei*; and 1 *L. reuteri* species were tested for their ability to produce phenyllactic acid (PLA). All *Lactobacillus* strains were grown in Man-Rogosa-Sharpe (MRS) broth at 37°C for 72 h. High-performance liquid chromatography (HPLC) was used to quantify PLA production in the cell-free culture supernatant. With the exception of *Lactobacillus casei* L9 and *Lactobacillus plantarum* NRRL B-4497, PLA production was observed in 13 of the 15 LAB strains used, in the range of 0.020 to 0.275 mM, where *Lactobacillus casei* 21/1 and *Lactobacillus rhamnosus* 13075 achieved the highest concentrations (0.275±0.065 and 0.242±0.054 mM, respectively). Moreover, variable PLA production levels were obtained between strains of the same species ($p < 0.05$). Growth and PLA production curves for *L. casei* 21/1 and *L. rhamnosus* 13075 indicated that highest PLA concentration is achieved during the stationary growth phase.
(Keywords: Antimicrobial; Lactic Acid Bacteria; Phenyllactic Acid)

Introduction

Phenyllactic acid (PLA) is a novel antimicrobial compound produced by some lactic acid bacteria (LAB). This compound is an organic acid produced as a by-product during phenylalanine metabolism and is able to inhibit the growth of some fungi and both Gram-positive and Gram-negative bacteria, including *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli*.[1-3] PLA potential as an antimicrobial agent in food applications has been demonstrated in bakery, flour, cereals and dairy products, in which LAB may also contribute to the development of desirable sensory attributes.[4,5] While PLA can be produced by a wide range of *Lactobacillus* species, its production is rather low.[3] Thus, the search for new PLA-producing strains is highly relevant for developing of new food applications. In this study, 15 Lactobacilli strains belonging to 6 species were screened in order to assess their ability to produce PLA and identify high producers.

Methods and materials

Bacterial strains. Lactobacillus plantarum NRRL B-442; *Lactobacillus casei* NRRL B-1922, 21/1, 7R1, ATCC334, DPC3968, 12A, and L9; *Lactobacillus sakei* NRRL B-1917; *Lactobacillus rhamnosus* NRRL B-442 and 13075; *Lactobacillus fermentum* NRRL B-1932 and ATCC 11976; *Lactobacillus reuteri* NRRL 14171; and *Lactobacillus acidophilus* ATCC 4796 were tested for their ability to produce PLA.
PLA production by LAB. All Lactobacillus strains were grown in 20 mL of sterile de Man-Rogosa-Sharpe (MRS) broth at 37°C for 72 h. Cell-free culture supernatant was obtained by centrifugation (12000 rpm, 10 min) and sterile filtration (0.45-µm pore-size Millipore filter). The culture supernatant was adjusted to

pH 2.0 with formic acid (10 M), extracted three times with 20 mL of ethyl acetate; the extract was dried with Na2SO4, and concentrated in a rotary evaporator. The dried residue was reconstituted with 5 mL of 2.5 mM H_2SO_4 and analyzed in an HPLC system with an Aminex HPX-87H ion exclusion column (300 mm × 7.8 mm) at 50°C, using 2.5 mM H_2SO_4 as mobile phase and a UV monitor at 210 nm. Selected strains showing the highest PLA production were further characterized by their cell growth-PLA production curves. Cell growth was determined by the standard pour plate dilution method, where colony-forming units (CFU) were counted after incubation at 37°C for 48 h.

Results and discussion

All LAB strains acidified the medium, leading to pH values ranging from 3.60 (*L. casei* 21/1) to 4.56 (*L. sakei* NRRL B-1917). With the exception of *L. casei* L9 and *L. plantarum* NRRL B-4497, PLA production was observed in 13 of the 15 LAB strains used, in the range of 0.020 to 0.275 mM, where *L. casei* 21/1 and *L. rhamnosus* 13075 achieved the highest PLA concentrations (0.275±0.065 and 0.242±0.054 mM, respectively) (Figure 1). In addition, variable PLA production levels were obtained between strains of the same species ($p < 0.05$). PLA production levels found in this study are comparable to those reported by Valerio *et al*.[5] (from 0.02 to 0.37 mM) during the screening of 21 LAB, including different Lactobacilli strains of *L. plantarum*, *L. rhamnosus* and *L. acidophilus*.

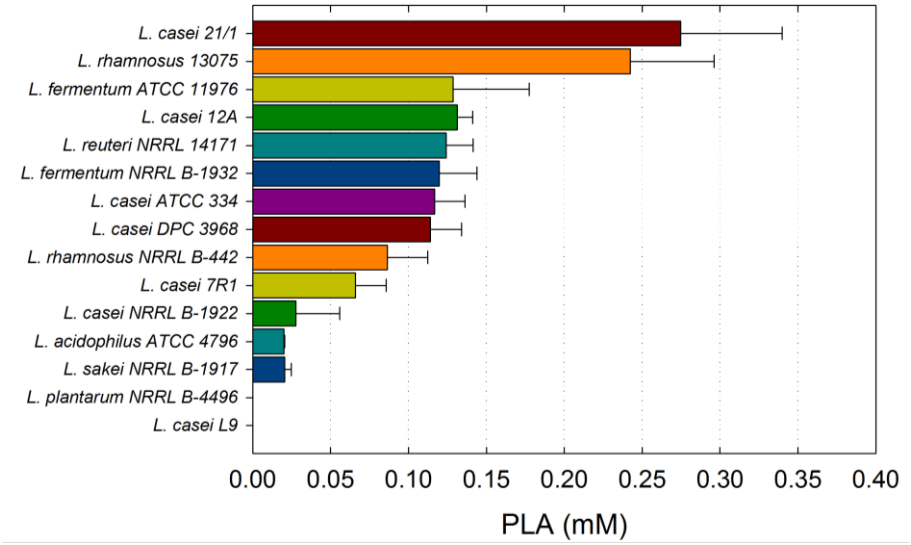

Figure 1. PLA production in MRS broth (37°C, 72 h).

Growth and PLA production curves for *L. casei* 21/1 and *L. rhamnosus* 13075 are shown in Figures 2 and 3, respectively. PLA appeared sooner during *L. rhamnosus* 13075 fermentation; however, in both cases, the highest PLA concentration was achieved during the stationary growth phase. A similar result was described by Vermeulen *et al*.[6] during the kinect analysis of growth rate, substrate and PLA levels of *L. sanfranciscensis* DSM20451 and *L. plantarum* TMW1.468.

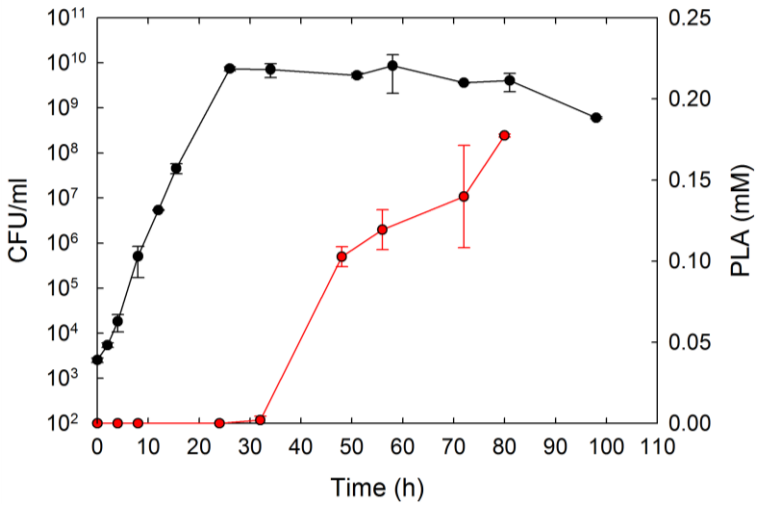

Figure 2. Growth (black) and PLA production (red) curves of *Lactobacillus casei* 21/1 (37°C).

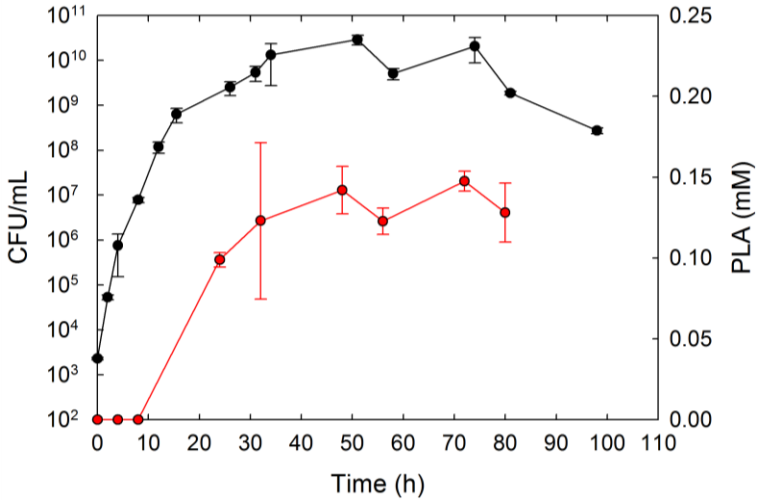

Figure 3. Growth (black) and PLA production (red) curves of *Lactobacillus rhamnosus* 13075 (37°C).

Conclusions

Five out of 6 tested species were able to produce quantifiable PLA levels during the study. Growth conditions for high PLA producers could be further investigated in order to improve metabolite production. PLA-producing strains may be applied to the development of new foods, while also contributing to the quality and safety of the end product.

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Growth Kinetics And Microencapsulation Characteristics Of Three Probiotic Bacteria

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Introduction

Probiotics have different mechanisms to protect the body such as acting as mechanical barriers or stimulating the production of IgA and short chain fatty acids within the gut (1). They have been used to treat and prevent several illnesses such as diarrhea, cancer and irritable bowel syndrome (2, 3). In order to have these benefits, it is necessary to consume between 10^8 – 10^9 CFU/day to ensure their bioavailability and functional action. This is equivalent to consume 100g of a commercial product that contains 10^6 – 10^8 CFU/g (4-6). Bacterial strains from the genus *Lactobacillus*, *Bifidobacterium,* and *Bacillus* have been the most studied and used to prepare ready-to-eat foods; while *Lactobacillus acidophilus (*LA) and *Lactobacillus casei* (LC) have been extensively used to prepare refrigerated foodstuffs, *Bacillus coagulans* (BC) has been recently introduced in non-refrigerated commodities due its thermotolerance (7). However, the bio-availability of *LA*, LC and non-sporulated BC to formulate certain food products has been challenging for many years. Microencapsulation (ME) helps to improve the odds of survival of these bacteria, because it protects them from harsh conditions such as high temperature, pH or salinity during the preparation of a final food product and its gastrointestinal passage.

ME has been used to pack solids, liquids or gaseous material in miniature sealed capsules that can release their content at a controlled rate and under specific conditions (8). There are many ME techniques commonly used to immobilize bacteria for the food and pharmaceutical industry (9), and many coating materials (10-12) have been used as coating materials such as gelling polysaccharides (e.g. starch, cellulose, alginate, pectin, carrageenans and chitosan), proteins (e.g. soy, whey, casein, gelatin and β-lactoglobulin) and some lipids (e.g. waxes), all extensively studied. Those used to ME *Lactobacillus sp.* and *Bifidobacterium sp.* includes ionic polysaccharides (e.g. alginate), microbial exo polysaccharides (e.g. gellan gum) and milk proteins (13, 14). However, LC and BC strains microencapsulated (ME) have not been as studied as LA and so is their comparative growth kinetics. Beside this and despite the wide variety of dairy products with probiotic bacteria is huge in México, baked products prepare with them do not exist, since the temperature is a key factor for availability (within food) and bioavailability (within the gut). Based on these facts, the aim of this study was to evaluate the growth kinetics & fermentative metabolism of LA, LC and BC and the characteristics & thermal resistance of their ME preparations of LA and BC.

Methods and materials

Growth conditions and kinetics. Inoculums (100 µL) per strain (LA, LC and BC) were cultured in Man, Rogosa y Sharpe (MRS) agar (160 mL) and then incubated at 37°C/48h with agitation (150rpm). Aliquots (16 mL) were collected every hour within the first 12h and at 24, 36 and 48h, and kept at 4oC to restrict bacterial growth for further analysis. Bacterial growth was firstly measured by optical density (600 nm) and serial dilutions from each aliquot/strain were also assayed by plate method (Log_{10} CFU/mL). Lactate production and glucose consumption were evaluated by standard enzymatic techniques (lactate and glucose oxidases).

ME. Cells were then immobilized by extrusion in $CaCl_2$ (1M) using sodium alginate (Alg, 1%) and low methoxyl pectin (LMP, 2%) as coating materials. Pre washed cell suspensions (3%) plus coating materials were added drop wise (at a 10mL/min flow rate using a peristaltic pump) into the $CaCl_2$ solution, and left for 30 min for complete hardening. Microcapsules were further filtered and washed under aseptic conditions.

Size & Shape. To explore the morphology of microcapsules, image captures were taken with a Carl Zeiss microscope and processed with the infinity analyze program. Measurements were preformed along and across using the 5x lens.

Thermotolerance. Viability test were made before and after Microencapsulation by serial dilutions and counting plate method, incubated at 37 ^{o}C/24-48 hours. Thermal resistance was evaluated at 48, 60 and 98°C/30min, followed by serial dilutions and counting plate method.

Results and discussion

At 48h, *LA* and *BC* were at log-phase while *LC* at stationary-phase of growth (**Fig. 1a, b**), reaching 10^9-10^{10} UFC/mL. From 24-48h, the growth of *LA* (**Fig. 1c**) and *C* (**Fig. 1d**) was directly proportional to lactate production ($R^2 \geq 0.95$).

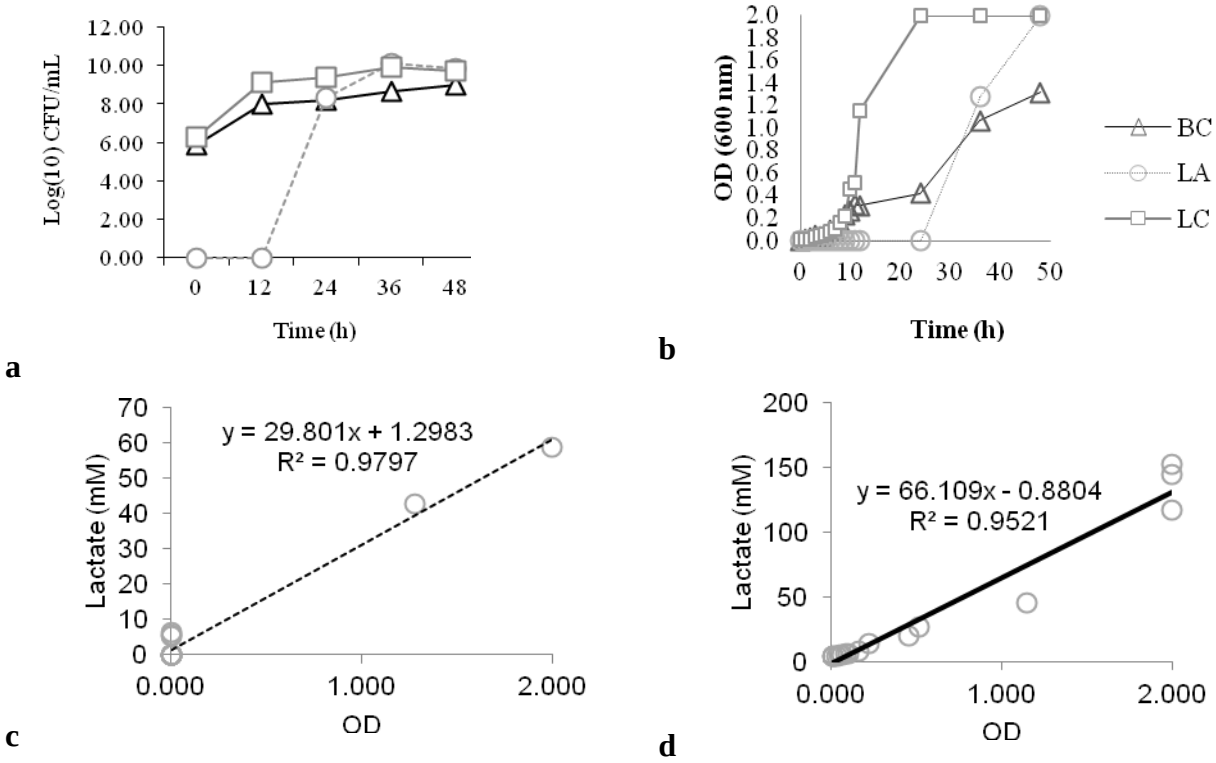

Figure 1. Bacterial growth and lactate production. Growth: Log10 (a) and Optical density (OD, b), Lactate production of *L. acidophilus* (LA; c), *L. casei* (LC; d).

On the other hand, *BC* showed 2 Lag stages, related (R^2= 0.5177) and not related (R^2= 0.1657) to lactate production (**Fig. 2**). LA and LC present the typical growth kinetics of any lactic acid bacteria in which the growth is directly proportional to lactate production, however BC did not present such growth, since it has

two stages of growth. One of the factors may influencing this behavior may be due to the amount of glucose in the medium

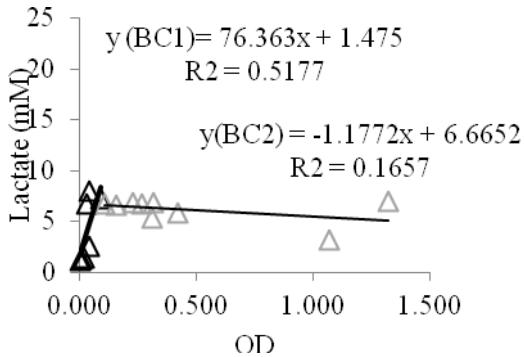

Figure 2. Growth (OD) and lactate production of *B. coagulans* (BC)

LA and LC present a typical growth kinetic and at the same time they consume glucose produce lactic acid, but BC uses another pathways using another kind of substrate, another studies as CO_2, acetic acid or ethanol production are needed to confirm this, But what is certain is that the type of the strain influences in their behavior. Das y Sen (15) reported that total depletion of glucose in a MRS broth (20 g/L) is reached approximately at the 14th hour when harvesting BC (RK-02) and in just 8 hours in a medium at a 4 g/L. The BC strain used in this study stopped using glucose at the 5th hour, and turn on using another carbon source until 48h. A plausible explanation to this phenomenon is a heterolactic fermentation. While homolactic bacteria uses the Embden-Meyerhof route to perform glicolisis and convert one mol of glucose into two mol of lactic acid, heterolactic ones uses monosaccharides via 6-p-gluconate to produce a mol of CO_2, one mol of ethanol and/or acetic acid and one mol of lactic acid (16-19).

After ME, irregular microspheres prepared with LA, LC and BC and coating materials (Alg or LMP) were obtained, with diameters between 400 and 500 μm (**Fig. 3**).

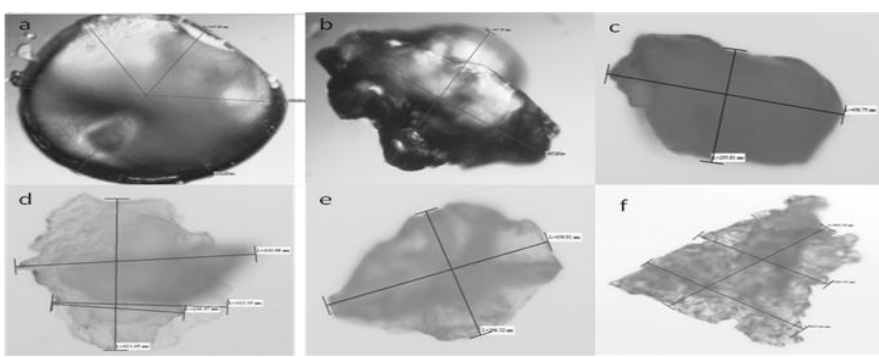

Figure 3. Microshperes: a) Alg-LC; b) Pect -LC; c) Alg-BC; d) Pect-BC; e) Alg-LA; f) Pect-LA.

It is well known that microspheres diameter depend on the microencapsulation method and the coating material used (20-22). By the extrusion technique, beads diameters varies from 2 to 4 mm (23,24), another factor to consider is the nozzle size and the concentration of the coating material by the fact that beads are found to increase as the concentration of sodium alginate increases (25). However, there are a few studies about microencapsulation of probiotic with LMP, however one study found microcapsules with an average size of 0.89 and 1.08 mm when they are mixed with alginate (0.5 % w/v) and LMP (1 and 2%)

respectively (26). These findings indicate that the obtained microcapsules had a smaller size and when the concentration of the coating material increases the size increases as well.

Lastly, in **Fig. 4** shows the viability of LA, and BC before and after microencapsulation (25°C), and the viability of these two bacteria when exposed at higher temperatures (48, 60, 98°C).

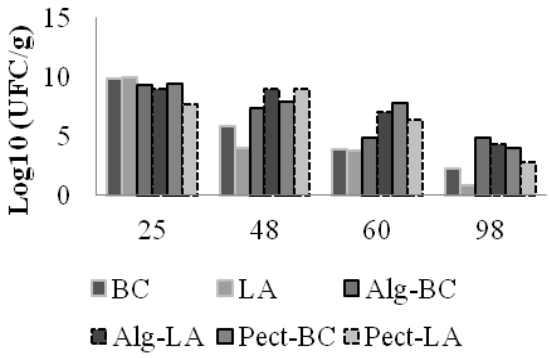

Fig 4. Viability and thermotolerance of three probiotic bacteria.

For free cells their thermo resistance felt down to 10^4 and 10^2 (BC) and 10^1 (LA) cells at 60 and 98 °C, respectively, being *BC*-ALg the most thermo tolerant combination. This results were confirmed by DSC for Alg alone (data not shown) as the transition temperature of alginate is higher that the LMP; another factor possibly influencing the thermoresistance is the type of strain, since BC is a spore former bacteria and for this reason it could be more resistant to temperature (7). Mosilhey (9) evaluated the thermoresistance of LA observing an important decrease at 55/30min, and 60 °C was chosen as the lethal temperature for free cells. The author also microencapsulated LA in different capsule materials (gum arabic, gelatine, soy protein+gum Arabic, whey protein+ gum Arabic and soy milk and gum arabic) and exposed them to different temperatures. At 60 °C/30 min, the controls started at 10^9 and after the temperature treatment decreased at 10^5-10^3. When these microcapsules where submitted at 65 °C/30 min all cells died excepting those coated with whey protein+ gum Arabic (10^2). In our study the viability was higher, remaining at 4.8 Log for BC Alg at 98 °C/30 min. However, further studies are needed on the thermoresistance of probiotic bacteria as there are very few.

In conclusion, besides the expected functional effect, the critical selection of the strain and the coating material is crucial when thermo tolerance is required.

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Antimicrobial Effect Of Water Produced During The Extraction Of Oregano Oil (*Poliomintha longiflora* Gray) By Steam Distillation.

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Abstract

One of the most used methods for extraction of essential oils from aromatic plants, including oregano (*Poliominthalongiflora* Gray), is steam distillation.This process generates approximately 1 liter of water from steam condensation and about1 to 2 ml of essential oil out of 50 g of dry leaves. Sinceno use is given to this water, the present study evaluated its antibacterial effect on aerobic mesophilic bacteria growth. Four different treatments were generated corresponding to the dilution of the water from steam condensation and buffer phosphate solution, adjusting to a volume of 9 ml in a test tube (100%, 50%, 25%, and 0%) and having 5 repetitions for each treatment. One ml of a 24 h-incubated culture of mesophilic aerobic bacteria sampling from a work table surface were added to each test tube (20). After 10 minutes of the application of bacteria, a 1-ml inoculum was put in a petri dish, covered with nutrientagar and put into incubation for 48 h at 35 °C. Bacterial colonies were counted and gathered data was analyzed with SPSS software using a completely randomized design. Statistical difference among treatments was found (P = 0.01). From the mean comparison analysis, the following information was obtained (DMS):Petri dishes from the treatment with undiluted water (100%) showed only 1UFC/ml (C, P = 0.01). Treatment 2 (50% water from steam distillation) presented 12,787 UFC/ml (B, P = 0.01). Treatment 3 (25% water from steam distillation) showed 27,302 UFC/ml (A, P = 0.01). Treatment 4 (100% phosphate buffer) presented 29,145 UFC/ml (A, P = 0.01). It can be concluded that water from steam condensation generated during extraction of oregano essential oil has a drastic antimicrobial effect.
(Keywords: *Poliomintha longiflora*, oregano, mesophilic bacteria, antibacterial)

Introduction

It has long been known that oregano have antimicrobial properties which are attributed to the presence of essential oils thymol and carvacrol. These compounds could be used in the formulation of food preservatives (1, 2, 3), antibiotics or growth promoters in the livestock industry (4, 5). Maguregui et al (2003) evaluated the antimicrobial activity of extracts of different polarity (alcohol, acetone and hexane) from Mexican oregano (Lippia berlandierii) bagasse (waste material from the extraction of essential oil) and stover against food pathogens (*Listeria monocytogenes, Bacillus cereus, Staphyloccocus aureus, Escherichia coli* O157:H7 y *Salmonella typhimurium*). The microorganism on which oregano bagasse had the greatest effect was *Bacillus cereus* and the least affected was *Escherichia coli*. Oregano stover extracts showed no effect on any of the microorganisms.

Portillo and co. (**6**) evaluated the activity of the Mexican oregano powder against fungi and yeast (24 fungi strains and 5 yeast strains) that contaminate food. In thirteen strains, a strong inhibitory effect on microorganism growth was observed. It has been proved that the oregano oil decreases the microbial count of mesophilic bacteria when applied to different types of meat (chicken, beef and pork), and also gives a better physical appearance since the active components in the oregano act as antioxidants (**7**).

The antimicrobial activity of essential oil of oregano depends on the chemical composition of the essential oil, which is related to the species of oregano, geographical conditions, harvest seasons and extraction method (**8, 9**) The process of extraction of essential oils can consist on hydrodistillation or steam stripping. During this process, water is obtained duringsteam and oil condensation; nonetheless, no use is

given to this water and it ends upbeingtreated as a waste product from the process. The obtained water can be, in fact, a relatively large volume since about one liter of water is obtained during the process of oil extraction from 50 g of dehydrated oregano leaves. For this reason, the present experiment has the objective to evaluate the bactericidal effect of the water obtained during the steam stripping of the essential oil of oregano (*Poliomintha longiflora* Gray).

Methods and materials

This study was conducted in the Laboratory of Microbial Biotechnology at the Facultad de Agronomia of the UANL. The essential oil extraction was performed with water steam stripping using 50 g of dry leaves; 1.7 ml of oil and about one liter of water from vapor condensation were obtained after the process.

Treatments.

In test tubes, volumes of 9 ml were prepared with the corresponding proportions ofwater generated by distillation and phosphate buffer solution, four treatments were obtained: T1 = 100% water of distillation T2 = 50% distilled water and 50 % buffer solution, T3 = 25% distilled water and 75% buffer solution and T4 = 100% buffer solution. Five tubes were preparedfor each dilution.

Preparation of Inoculum

For the analysis, a 24-hour culture of mesophilic bacteria was used. Bacteria was obtained from the surface of a work table (10) using a swab on a sampling area of 25 cm^2. The swab was later deposited into a tube with 10 ml of buffer and placed into agitation in a vortex. After this, 1 ml of the suspension was poured into a flask with 200 ml of sterile nutrient broth and put into incubation at 35 ° C for 24 hours.

Applications of Treatments

The five tubes from each treatmentwere inoculated with 1 ml of the previously obtained bacterial culture and set to rest for 10 minutes. Then,1 ml of the contents of each tubewas transferred and placed into a petri dish, covered with nutrient agar and put into incubation.After 48 hours, the countingthe bacterial colonies (CFU / ml) wasperformed.

Statistical Analysis

With the values obtained from the counting, an analysis of variance was performed following a completely randomizeddesign, in order to make a comparison of means (DMS). Software used for the analysis was SPSS version 10.

Results and discussion.

Analysis of variance (ANOVA) showed a significant difference (P <0.01). Table 1 presents the analysis of the comparison of means (DMS) where it can be observed that Treatment 1 reduced growth to 1 CFU / ml (A) (P <0.01) showing the antimicrobial properties of the water from oregano essential oilsteamdistillation.Treatment 2 reduced more than half the amount of microorganisms in Treatment 4 (control).

Table 1.-Comparison of means for the variable colony-forming unit per milliliter during the application of water produced from oregano essential oil steam distillation(*Poliomintha longiflora* Gray)

Treatment	Mean (CFU/ml)	
1	1	C*
2	12,787	B
3	27,302	A
4	29,145	A

T1.- 100% water from steam distillation, T2.- 50% water from steam distillation,50% phosphate buffer, T3.- 25% water from steam distillation, 75% phosphate buffer, T4.-100% phosphate buffer. Different letters mean a significant difference (P< 0.1)

The Figure 1 shows the images of the plate cultures after 48 hours of incubation observing the bactericidal effect of water obtained by steam distillation.

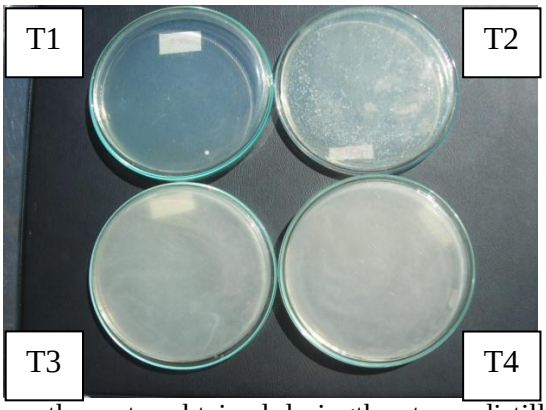

Figure 1.-Bactericide effect from the water obtained duringthe steam distillation of the oregano essential oil (*Poliomintha longiflora* Gray).T1.- 100% water from steam distillation, T2.- 50% water from steam distillation, 50% phosphate buffer, T3.- 25% water from steam distillation, 75% phosphate buffer, T4.- 100% phosphate buffer.

Aranda and co. (11) have demonstrated antimicrobial activity of different concentrations of essential oil of oregano (*Poliomintha longiflora* Gray) showing an inhibitory effect against *E. coli* 0150: H7-95, *E. coli* 0150: H7-932 at a concentration of 0.05% essential oil.

Rivera and co. (12) found that the essential oils from *Poliomintha longiflora* and *Lippia graveolens* displayed a moderate *in vitro* antibacterial activity, with minimum inhibitory concentration values ranging from 128 to 512 μg/mL against following bacteria *Bacillus subtilis* [ATCC6633], *Staphylococcus aureus* [ATCC25923], *Escherichia coli* [ATCC10536], *Salmonella typhi* [ATCC9992], and *Pseudomonas aeruginosa* [ATCC27853]

Conclusions

The obtained results demonstrated the antibacterial power of water obtained during steam distillation of oregano essential oil (*Poliomintha longiflora* Gray). This opensthe possibility of application of this product in sanitization of surfaces and utensils for mesophilic bacteria control.

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Observation Of Microbial Growth In A Food Processing Type Mole

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Introduction

Microbiology is a basic tool in the food industry to determine the quality and safety of food that we shipped. External factors, environmental or manufacturing responsible for the content and type of harmless microorganisms and pathogens in food. Within the study microbiological medium is vital to decide whether the conditions necessary for the growth of a microorganism.

Coliforms are Gram negative non-spore forming, from the environment or the intestinal contents. Its determination is important because they are indicators of poor hygiene (1) and therefore a bad manufacturing practice. Required for proliferation atmosphere with pH 6.9 ± 0.2 and temperature 36 to 38 ° C and water (2).

Water is a determinant factor in the preservation and food safety, because the amount of microorganisms that may contain a food is directly related to the amount of water therefrom, but not all can be used by microorganisms. A water available or free from microorganisms, and this can cause damage is known by the name of "water activity" (3)

These effects were studied in a product from a company stuffer city Matehuala, SLP. This company distributes its food-based mole type lean pork and no preservatives to convenience stores which presents the biggest problem with the rules adopted by each facility for sale, who do not apply the manufacturer's suggestion, "keep refrigerated by 4 ° C. "data to preserve the product life of 15 days. The company focused on the consumer and the bad habit that everything tastes better reheated mole or not reaching collect unsold, decides to investigate environmental conditions the lifetime of the product, focusing the analysis to determine the percent of moisture content in relation to total coliform plate (NOM-116-SSA1-1994 and NOM-113-SSA1-1994), this analysis when the Most Probable Number (MPN) is exceeded, ie more than 3 (NOM-145-SSA1-1995).

Experimental development

Preparationof culture media and solutions, NOM-110-SSA1-1994. All reagents used were of analytical grade, prepared in accordance with Mexican Official Norm (NOM) or producer BdDioxon. Solutions and growth media were sterilized in autoclave at a pressure AESA power between 15 and 16 lb/in2 for 15 min and in the case of the material using a drying oven for analog control Riossa temperature for 2 180 ± 2 ° C.

Sampling in the company. The company provided 15 samples of 150 g each, packaged in plastic containers with the same seal material placed manually. The analysis was prepared by opening a product every other day or so, using the required amount and discarding the excess.

Determination of moisture, NOM-116-SSA1-1994. Capsule was prepared with nickel gauze and glass rod inside, was placed in a drying oven for 2 Riossa 100 ± 2 ° C for drying. Once the time is removed and allowed to cool in a desiccator for 2 hours to determine its weight without the sample. The sample was prepared by blending the total content of the container for one minute. Was added to 5 g and the capsule

caps with the sample weight. Was introduced to the furnace for 4 hours at 100 ± 2 ° C. At the end was left cooled in the desiccator for 15 min and weigh again. The study was performed in triplicate.
Sample preparation, NOM-110-SSA1-1994. The liquefied sample solution was diluted using 0.2 M phosphate buffer or 0.1% peptone water, both at pH 7. The first analysis was performed with phosphate buffer and peptone water remaining. Which previously sterilized and distributed by placing 90 ml in each dilution bottle, 4 bottles to get thinner. The first dilution "primary call" was prepared with 10 g of food homogenate was stirred thoroughly and allowed to sit, the second dilution with 10 ml of the primary dilution transferred to another vial of diluent, this process is repeated as many dilutions required.
Total coliforms, NOM-113-SSA1-1994. Planting began pouring 2 mL of each dilution on a plate, plus 15 mL of violet red bile agar (RVBA), the mixture was homogenized in a circular motion and allowed to gel, attaching 4 mL and solidified the second spill, were reversed plates and incubated at 35 ° C for 24 h. Samples were run in duplicate and with a control plate. Elapsed time by Sol Bat colony counter type Quebec, was read the colonies formed, these are characterized by the size of 0.5 to 2.0 mm and a dark red to pink. Single plaques were selected from 15 to 150 colonies. The results are the average numbers of colonies determined for duplicate multiplied by the reciprocal of the dilution corresponding units are CFU / g CFU / mL.

Results and discussion

Moisture plays a role in microbial growth and the percentage present in the feed favors studied coliforms, although the percentage decreased as the days passed, Table 1. The amount of water is also suitable for the growth of molds and yeasts, microorganisms proliferated to a greater amount not permitting optimal development of coliforms, the probable cause the pH of the food acid, and less than 6 (unpublished data).
While it is clear the presence of coliforms (Table 1), the amount of yeasts and molds present (unpublished data) competitiveness by means of nutrition favored seconds. And as expected, in studies at room temperature decreased lifespan, about 11 days compared to approximately 15 days suggested for cooling.

Table 1. Total coliforms in food mole type according to processing time. (Account placa/10 g)

D	Average of two plates of colonies on each dilution quantified					UFC/g
	1:10	1:100	1:1000	1:10000	1:100000	
3	0	0	0	0	0	No development
5	0	**22**	**71**	-	-	37000
7	**52**	**73**	**78**	9	-	29000
9	**40**	10	10	5	4	400
11	**16**	**20**	3	2	3	22000
13	3	0	1	0	1	No development
Limit total coliforms ≤ 3NMP/go absence						

Conclusion

The coliform content alone is not a unique feature to evaluate procedures for personal hygiene and housekeeping practices. Sometimes this resides in a process step, packaging or storage. Thus, the processed food mole type presents a problem for the distribution stage, selected stores for sale refrigerator no exclusive use multiple products or have one for refreshments, where food can not maintain the optimal conditions for estimated last time by the company, approximately 15 days in comparison to the 3 study. You need to start a study microbiological quality, analyze and confront cooling product data for which the company has a wide range of possibilities to be applied during the process and increase hygiene measures,

adding conservative contemplate another way to package, keep staff updated regarding good manufacturing practices and hygiene and proper condition of facilities.

It is essential to inform conveni0encia stores where the product is distributed, the importance of this problem, it is committed to the health of consumers and mark them that reheating practice to improve the taste of a mole is not the best choice for your wellbeing .

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Antimicrobial Activity of the Essential Oil of Mexican Plant: *Piper auritum* Kunth (Yerba Santa)

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Introduction

Advances in biological and chemical analytical methods have raised questions regarding the safety of chemical food additives (4).Many research groups are examining the chemical nature and activity of natural antimicrobials in fruits, vegetables, grains, herbs and other foods (3). In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many uses, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (9).One of the plants with proven antimicrobial effects is the genus *Piper;* which has been demonstrated to inhibit the growth of foodborne pathogenic bacteria (17).

The genus *Piper*, with more than 1000 species, is widely distributed in the tropical and subtropical regions of the world and is used in a variety of ways (11), (15). The aromatic volatile oil of *P.angustifolium Lam* has been reported to exhibit bacteriostatic and fungistatic activity against *Trichophyton mentagrophytes, Pseudomonas aeuroginosa, Candida albicans* and *Aspergillus fumigatus* (18).*Piper canium L*. exhibited antibacterial activity against Gram positive bacteria *Bacillus cereus, Sthaphylococcus aureus and Streptococcus pneumonia* (17).México has several *Piper* species, including *P.auritum Kunth* locally known as Yerba Santa among the most important. Fresh or air dried, the plant is routinely consumed as a seasoning in foods such as curing meat and fish (5).The antimicrobial activity of *Piper auritum* Kunth has been attributed mainly to the presence of volatile compounds found in its essential oil, specially Safrole, which have been demonstrated to be responsible for the antimicrobial effect of *Piper auritum* as individual compound (16)The aim of this work was to carry out the microbiological study of the essential oil of Mexican *Piper auritum* Kunth (yerba santa).

Keywords: Natural antimicrobials, *Piper auritum*, essential oil

Materials and Methods

Plant Materials

Samples of *Piper auritum* Kunth were collected from Acapulco, Mexico in May 2011.

Extraction of Essential Oils

The fresh leaves and stems were subjected to hydrodistillation. The oils were dried over anhydrous magnesium sulfate and stored at 4–6 °C.

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS chromatograms were recorded using a Perkin Elmer Auto System XL. Helium was used as carrier gas at flow rate of 1 mL/min. Injector temperature was 250 °C. Oven temperature was programmed from 45 °C (5 min hold) to 260 °C at 6 °C/min and finally held isothermally for 15 min. For GC-MS detection, an electron ionization system, with ionization energy of 70 eV was used. A scan rate of 0.5 s (cycle time: 0.2 s) was applied, covering a mass range from 50–400 amu.(7)

Identification of Constituents

The constituents of the oils were identified by comparison of their mass spectra with reference spectra in the computer library and also by comparing their retention indices, with those of authentic compounds or data in the literature.

Antimicrobial Activity

Microbial Strains

The test microorganisms, *Staphylococcus aureus* and *Escherichia coli* were donated by the Microbiology and Immunology laboratory of UVM Chapultepec campus. The strains were grown on soy trypticase agar (Bioxon,México)

Disc Diffusion Assay

Antimicrobial activity of the essential oils of *P. auritum* was carried out by the disc diffusion method reported by the National Committee for Clinical Laboratory Standards (NCCLS) (14), based on Kirby-Bauer method. The discs (6 mm diameter) impregnated with different concentrations(5 - 250µg/mL) of the essential oil, were placed on the inoculated agar, and were incubated for 24 h at 37 °C, ceftriaxone(30µg) and gentamicin (120 µg) both from BD Company, wereused as the positive controls. Clear inhibition zones around thediscs indicated the positive antimicrobial activity. All tests and analysis were carried out in triplicates.

Results and discussion

The essential oils, pale yellow color were obtained by hydrodistillation of the leaves and stems of *P. auritum.* The chemical compositions of the leaves and stems oils were analyzed by GC-MS.The most abundant component in the oil was safrole (19.54 min). Figure 1.Similar to our results, safrole has been reported to be the major constituents of *Piper auritum* H.B.K. (Panama: leaves) (8), *Piper callosum* (Brazil: leaves oil), *Piper hispidinervium* C. DC. (Brazil: leaves oil) (12), *Piper betle* Linn. Flowers oil (Taiwan: flowers oil) (10), *Piper mikanianum* Kunth (Brazil: leaves oil)(1) and *Piperxylosteoides* C.DC. (Brazil: aerial parts) (6).*Piper caninum blume* (Malaysia: leaves and stems) (19), *Piper auritum Kunth* (Cuba: leaves)(16)and *Piper auritum Kunth* (Colombia)(7)

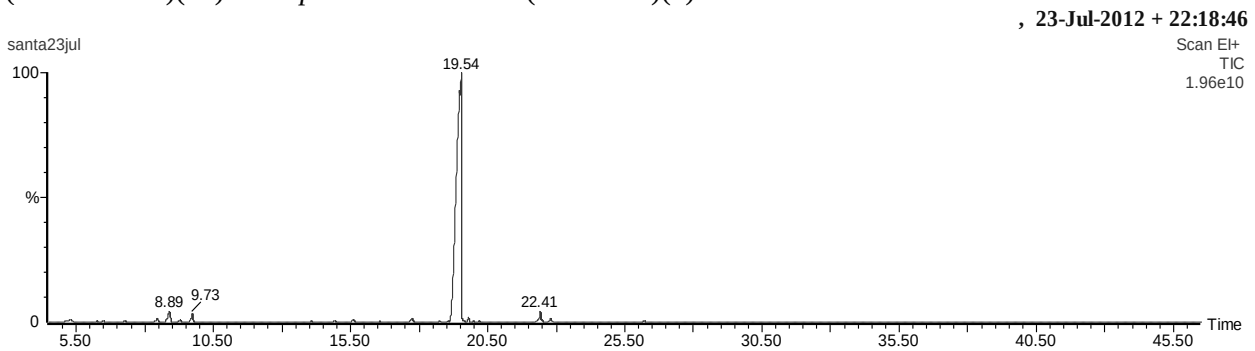

Figure 1. GC-MS Chromatogram of the essential oil of mexican*P. auritum*

Antimicrobial Activity

The antimicrobial activity of the essential oil against Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*E. coli*) was assessed by disc diffusion method. Figure 2 and 3, shows the antimicrobial activity of the essential oil of *P. auritum* against *E.coli* and *S. aureus.*

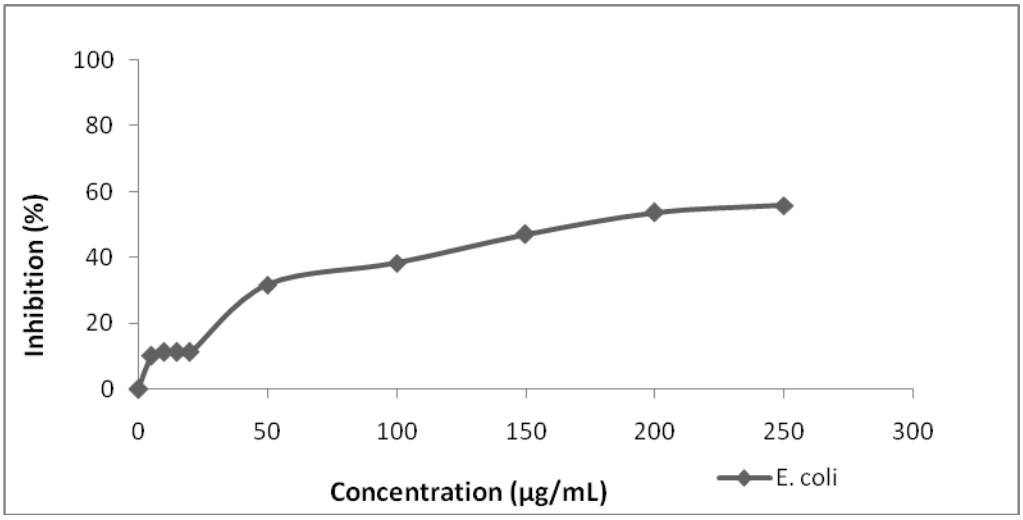

Figure 2. *Inhibition of bacterium growth (E.coli) caused by P. auritum essential oil.*

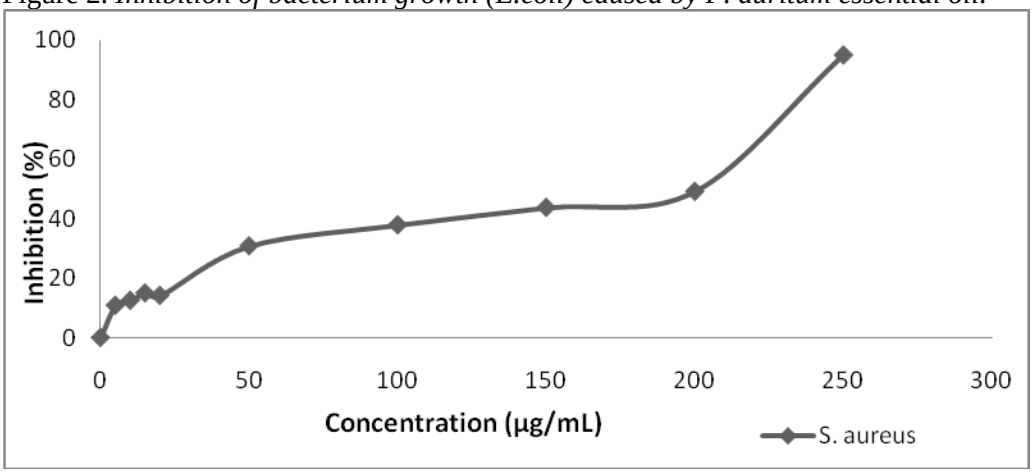

Figure 3.*Inhibition ofbacterium growth (S. aureus) caused by P. auritum essential oil*

The essential oil showed strong antimicrobial activities against both bacterial strains. In general, it showed better antimicrobial activity against the Gram-positive bacteria than the Gram-negative bacteria. Gram-positive bacteria (*S. aureus)*, were the most susceptible to this oil, with inhibition zone of 100% with concentrations250 µg/mL.Gram-negative bacteria were most resistant to this oil with inhibition zones above 50% with values of 250µg/mL. These results are consistent to those of previous reports in the literature, indicating that Gram-negative bacteria are more resistant to essential oils than Gram-positive bacteria due to its outer membrane (2). Some studies also concluded that the essential oils have greater antimicrobial activity due to their additive, synergistic or antagonistic effects (13).

Conclusions

The result demonstrated that safrole, was the main component from the leaves and stems oil of mexican *P.auritum Kunth*. The essential oil of *P. auritum* showed strong antimicrobial activity; therefore it might well be used as an antimicrobial agent, as well as in food preservatives or agricultural practices. This is the first microbiological study with *P. auritum* Kunth in Mexico.

Acknowledgments

The authors would like to thank Ph.D. Laila Muñoz Castellanos, Ph.D. Guadalupe Virginia Nevárez Moorillón and Ph.D María de Lourdes Ballinas Casarrubias, Facultad de Ciencias Químicas, Universidad

Autónoma de Chihuahua, Chihuahua, México by GC-MS technical support and RBT S.A. de C.V., by financial supports.

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Prebiotic Effect Of Vegetable Sources On *Lactobacillus casei* (B1922) Survival Described By The Weibull Model

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Abstract

A topic of great concern in food microbiology is the positive effect of prebiotics on the growth and/or viability of some probiotic bacteria. Several Lactobacilli bacteria are part of the healthy human gut and are particularly considered to have beneficial physiological effects, such as enhancing the immune system. In this work it has been studied the survival of *Lactobacillus casei* (B1922) in the presence or not of some vegetable sources in aim to demonstrate their prebiotic effect using the Weibull model. Different model systems were prepared: MRS broth (M1), MRS without dextrose (M2), M2 + inulin, M2 + dried vegetables (orange peel, grapefruit peel, green asparagus and globe artichoke). These model systems were inoculated with $2x10^{12}$ cfu/mL of *L. casei* and microbial count was determined using MRS agar, plates were incubated anaerobically at 35-37 °C for 48 h. The data of the survival *L. casei* in the different model systems were fitted with the Weilbull model obtaining the kinetic parameters b and n, for each survival curve. Results showed an adequate description of the survival kinetics of *L. casei* using the Weibull model (R^2>0.90). After 444 h of incubation, *L. casei* population decreased 8 log cycles with M1 ($4x10^4$ cfu/mL) in comparison with the model systems added with the vegetables sources. Among the model systems with dried vegetables, the one with grapefruit peel demonstrated the highest survival of *L. casei* with 5 log cycles ($1.4 x10^7$) after 444 h of incubation. However all vegetable sources enhanced the survival of *L. casei* significantly ($p<0.05$). In conclusion, the Weibull model was useful to describe and compare the survival of *L. casei* and demonstrate the prebiotic effect of the evaluated vegetable sources; we suggest the use of these in food product development for novel functional food.
Keywords: Weibull, *Lactobacillus*, prebiotics, vegetables.

Introduction

The prebiotics were first defined as a non-digestible food ingredient which beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improving health[1].

Some of the substances involved as prebiotics are oligofructose, inulin, lactulose, galacto-oligosaccharides (GOS), the transgalactic-oligosaccharides (TOS), fructo-oligosaccharides (FOS) and dietary fiber [2,3]. These substances are present in vegetables such as artichoke, tubers, quince, celery, beans, blackberries, broccoli, olives, oranges, Brussels sprouts, apricots, pears, cauliflower, avocado, banana, fennel, plum, nuts, leeks, raspberries, carrots and apples, among others[4]. The prebiotic effect of these substances is assessed in terms of its ability to stimulate the proliferation of "healthy" or desirable bacteria (bifidobacteria, lactobacilli) [2]. There are studies which show that fruits and vegetables can be used as prebiotics due to the high content of soluble fiber, inulin and fructans giving an advantage to certain agricultural products such as orange and grapefruit bagasse, which are discarded without a further use of its components[5,6].

Moreover, predictive microbiology is based upon the premise that the responses of populations of microorganisms to environmental factors are reproducible, and that, by characterizing environments in terms of identifiable and dominant factors, models of such responses may predict the behavior of those microorganisms in other similar environments[7].

A dose - response model is the Weibull distribution function which takes biological variation into account and is used to describe the spectrum of resistances of the microbial population under different conditions. Besides, this model presents the main advantage of remaining very simple and being sufficiently robust to describe both downward concave survival curves ($n > 1$) and upward concave curves ($n < 1$), as well as the traditional case where the survival curve is linear (n=1) [8, 9]. The aim of this work was to evaluated the prebiotic effect of some dried vegetable sources in the survival of *Lactobacillus casei* (B1922) and compare these kinetics by means of the Weibull model.

Methods and materials

Sample preparation. Vegetable sources of orange peel (*Citrus sinensis*), grapefruit peel (*Citrus paradisi*) both with albedo present, globe artichoke (*Cynara scolymus*) and green asparagus (*Asparagus officinalis*) were obtained in a local supermarket in Puebla, Mexico. The orange and grapefruit peel, artichoke and asparagus were cut into slices of approximately one centimeter thick and dried in a hot air dryer (Food Dehydrator, Model 3500, Excalibur, U.S.A) at 35 °C for 48 h. Dried samples were sifted passed through a sieve No. 35 (500 μm) (Cole - Parmer, U.S.A), and then packed in glass containers.

Bacterial strain and growth conditions. *Lactobacillus casei* (B1922) was obtained from the collection of the Food Microbiology Laboratory of the Universidad de las Americas Puebla. It was grown in MRS agar (Man, Rogosa and Sharpe) (Becton Dickinson, Mexico) anaerobically at 35-37°C during 48 h and then kept refrigerated (5°C). It was reactivated in MRS broth for 48 h at 35-37 °C.

Preparation of culture media. Different model systems were formulated: MRS broth (M1), MRS without dextrose (M2), M2 + inulin (Fructagave SP750 Monterrey, Mexico), M2 + dried vegetables (orange peel, grapefruit peel, green asparagus and globe artichoke). In the latter systems, 2 g of dried vegetable sources per 100 mL broth was used. In all cases, the vegetable sources and MRS broths were sterilized at 121°C for 20 min.

Survival curves. The model systems were inoculated with approximately $2x10^{12}$ CFU/mL of *L. casei* (B1922). After the inoculation, the broths were incubated at 35-37 °C for 444 h in an incubator (Imperial III Incubator, Lab Line Instruments, U.S.A), and samples were taken every 24 h for viable count determination. This was determined by spiral plating (Spiral Biotech, Inc. Autoplate 4000, Model 20814, Advanced Instruments Company, U.S.A) using MRS agar, plates were incubated anaerobically at 35-37 °C for 48 h. Finally, microbial count was performed using a scan and software (Spiral Biotech, Inc. Q-count, Model 02062, Advanced Instruments Company, U.S.A).

Modeling of survival curves. Survival data were fitted to the cumulative form of Weibull frequency distribution of resistances. This model assumes lethal events as probabilities and survival curves as the cumulative form for a distribution of lethal events.

$$\log \frac{N}{No} = -bt^{n} \qquad \text{(Eq. 1)}$$

where *No* is the initial number of viable cells (CFU/mL), *N* is the number of cell survivors (CFU/mL) after a time *t* (h), *b* and *n* are the scale and shape factors. Kaleidagraph (version 3.08c, Synergy Software) was used to determine *b* and *n* parameters. These values then were used to calculate the frequency distribution of resistances using the following equation:

$$d\phi/dt_c = bnt_c^{n-1} \exp(-bt_c^{n}) \qquad \text{(Eq. 2)}$$

where t_c is a measure of the organism´s resistance or sensitivity and $d\phi/dt_c$ is the Weibull distribution

corresponding to t_c, the distribution mode, tc_{mode}; mean, tc_{mean}; variance, σ_κ^2; and coefficient of skewness, $\upsilon 1$:

$$tc_{mode} = [(n-1)/nb]^{1/n} \quad \text{(Eq. 3)}$$

$$tc_{mean} = \{\Gamma[(n+1)/n]\}/b^{1/n} \quad \text{(Eq. 4)}$$

$$\sigma_\kappa^2 = \{\Gamma[(n+2)/n] - (\Gamma[(n+1)/n])^2\}/b^{2/n} \quad \text{(Eq. 5)}$$

$$\upsilon_1 = \frac{[\Gamma(n+3/n)/b^{3/n}]}{[\Gamma(n+2/n)/b^{2/n}]^{3/2}} \quad \text{(Eq. 6)}$$

where Γ is the gamma function. The tc_{mode} represents the treatment time at which the majority of the microbial population dies. The tc_{mean} corresponds to the inactivation time on average with its variance σ_κ^2. The $\upsilon 1$ represents the skew of the distribution and it is related to the most resistant members of the microbial population[10].

Results and discussion

The survival of *L.casei*(B1922) in the presence of different vegetables sources (orange peel, graprefruit peel, asparagus, artichoke and inulin) indicate that the microorganism was able to use them for growth largely after 200 h. The addition of citric peels in the culture medium (M2+citrus peel and M2+grapefruit) maintained the highest microbial population (Figure 1), followed in decrease order by the M2+asparagus > M2+artichoke and > M2+inulin, considering inulin as a prebiotic component[12]. Results showed an adequate description of the survival kinetics of *L. casei* (B1922) using the Weibull model R^2>0.90 (Table 1). After 444 h of incubation, *L. casei* (B1922) population decreased 8 log cycles with M1 ($4x10^4$ cfu/mL) in comparison with the model systems added with the vegetables sources. Among the model systems with dried vegetables, the one with grapefruit peel demonstrated the highest survival of *L. casei* (B1922) with 5 log cycles ($1.4\ x10^7$) after 444 h of incubation.
In Table 1, the *n* parameter of the Weibull equation, indicates the concave behavior of the survival curves, being all treatments concave upward ($n < 1$). The upward concavity indicates that the remaining cells have the ability to adapt to the medium formulation [11]. In this case, the microorganism was able to use the different dried vegetables sources to increase de its population.
Comparing the tc_{mean} values, the broths added with inulin, grapefruit and artichoke presented higher values, around 15 to 20 h, indicating higher survival with respect to the M2 broth (MRS without dextrose). None of the frequency distributions of the population had mode since the kinetics represents the frequency distribution of the survival microbial population of *L. casei* (B1922) with a high initial viable cells count (Figure 2). The distribution frequencies of *L. casei* (B1922) in the presence of M2+grapefruit peel and M2+inulin, show a similar behavior of the bacteria survival, demonstrating that this vegetable source acts as prebiotic.

The vegetable sources evaluated helped to maintain the microbial population after 18 days, around $1x10^7$ cfu/mL. Higuera et al.[13] evaluated the prebiotic effect in *Lactobacillus delbrueckii ssp. bulgaricus* and found that the bacterial growth were up to $2.79\ x\ 10^9$ cfu/mL and $2.14\ x\ 10^9$ cfu/mL using a culture medium containing chemical grade inulin, and agave juice. Altieri et al.[12] reported that some prebiotics induced biomass production in the presence of 5g of FOS and lactulose throughout 72 h of incubation; in this study, *L.casei* (B1922) had a biomass production, with only 2 g of these vegetable sources and 444 h of incubation.

Table 1. Weibull distribution parameters for *L. casei* (B1922) in different formulated broths added or not with inulin or dried vegetable sources.

Formulated broths	b	n	R^2	Mean (h)	Mode (h)	Variance (min^2)	Coefficient of skewness (-)
M1	0.57	0.44	0.96	9.29	-	632	0.01
M2	1.22	0.33	0.95	3.59	-	269	0.15
M2 + Inulin	1.97	0.16	0.91	20.40	-	648793	2.57
M2 + Orange peel	1.39	0.21	0.96	14.68	-	35218	0.07
M2 + Grapefruit peel	1.88	0.16	0.99	20.01	-	452050	1.34
M2 + Asparagus	1.53	0.23	0.98	7.39	-	6729	0.28
M2 + Artichoke	1.63	0.19	0.99	15.51	-	91156	0.29

Note: M1 (MRS broth), M2 (MRS without dextrose), b and n are the weibull factors (Eq. 1) used to calculate the frequency distribution parameters (Eq. 3 to 5), R^2 is the regression coefficient

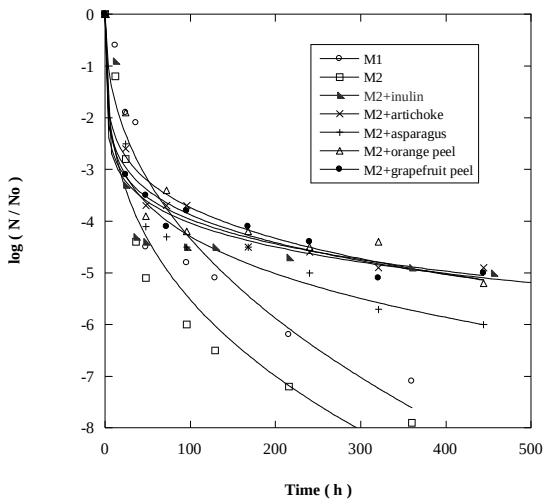

Figure 1. Survival curves of *Lactobacillus casei* (B1922) in MRS broth (M1), MRS broth without dextrose (M2) and in M2 added with inulin (M2+inulin), orange peel (M2+orange peel), grapefruit peel (M2+grapefruit peel), asparagus (M2+asparagus), artichoke (M2+artichoke), incubated at 37 °C. Each point represents experimental data average of 3 replicates and lines the fitted equation of Weibull.

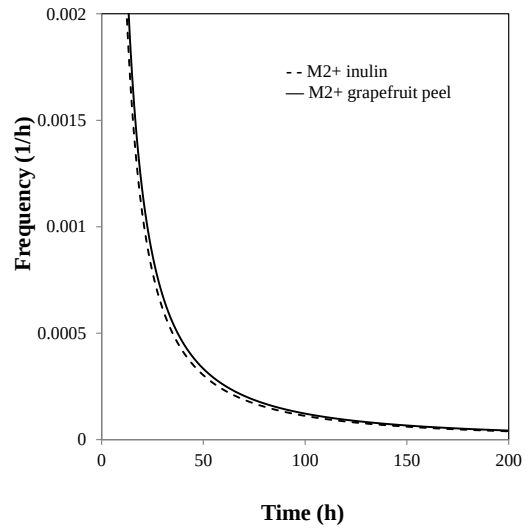

Figure 2. Survival distribution frequency of *Lactobacillus casei* (B1922) with Weibull model at 37°C in M2 broth added with inulin (M2+inulin) or grapefruit peel (M2+grapefruit peel).

Conclusion

In conclusion, the Weibull model was useful to describe and compare the survival of *L. casei* and demonstrate the prebiotic effect of the evaluated vegetable sources. The citrus peels behavior as prebiotics highlights a potential use of these in food product development for novel functional food, which are generally discarded as an agricultural residue.

Acknowledgements

Author Hernández-Carranza acknowledges the financial support for her PhD studies to the CONACyT and Universidad de las Américas Puebla.

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Use Of ICMSF Sampling Programs For The Detection Of *Listeria* spp In Pork Obtained From A TIF Slaughterhouse

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Abstract.

Listeria monocytogenes, considered an emerging and ubiquitous pathogen, is an increasing risk to public health due to its occurrence as Foodborne Disease (FBD). Given the aforementioned characteristics is essential to carry out permanent surveillance activities to detect and control it. So these were the objective of the study that was performed in a processed meats packaging plant located in Mexico City; the meat used came from pigs reared in Querétaro state, slaughtered in a Federal Inspection Type (TIF) slaughterhouse located in Guanajuato state and then carcasses were transported under refrigeration at 3-4 °C to their final destination. Sampling design was made according to recommendations of the International Commission on Microbiological Specifications in Foods (ICMSF). 60 samples of 5.5 cm2 X 1.5 cm from neck region muscles, considered the most likely contaminated zone, were collected from a total of 3 lots, (of 250 pork carcasses). Ten carcasses without any decontamination treatment were randomly selected for each batch. Moiety of collected samples (control group) were used to detect *Listeria* presence and the other were divided into two groups (test groups): 15 that would be treated to test the inhibitory effect of lactic acid with calcium sulfate and other 15 with peracetic acid on *L. monocytogenes*, if detected. As at that time was not known whether *Listeria* spp would be detected, treatments with acids were applied to test groups and then kept refrigerated at 4 ° C so that if *Listeria* was detected it could be possible to assess their potential inhibitory effect as a control measure in this case. Immunoprecipitant assay for detecting *L. monocytogenes* and other *Listeria* species in food, AOAC approved method 997.13, (LISTERIA VIP GOLD®) was used to process samples of control group, by triplicate. All samples tested negative. Therefore it was not possible to continue with the study nor was any statistical evaluation required. These results indicate that these meats were reliable in terms of *Listeria* risk contamination and could be used to produce safe foods.

Keywords: [Listeria, TIF, pork meat, rapid test, organic acids].

Introduction

Listeria monocytogenes, a Gram positive, non-spore-forming, catalase positive, aerobic, microaerophilic and facultative anaerobic bacteria, that sizes 0.5 mm in diameter and 1 to 2 microns in length, is considered an emerging and ubiquitous pathogen, is an increased risk for public health due to its occurrence as foodborne disease (FBD) (10). FBD's of bacterial origin are the most frequently reported worldwide. The list of pathogens involved has increased significantly including: *Campylobacterjejuni*, *E. coli* O157: H7, *Listeria monocytogenes*, *Vibrio vulnificus*, *Vibrio cholerae* and *Salmonella*, and others. *Listeria monocytogenes* is an organism that was not considered as an important pathogen until relatively recently due to its transmission through food.

These microorganisms are frequently associated with contamination by food handlers and the raw materials on site. Some of these pass naturally into the environment where food is processed, many are inactivated by heat treatment and others can be controlled by proper handling and storage (1.8). Main factors to meet safety in fresh processed meats are to limit its contamination and retard or inhibit the growth of spoilage or pathogenic microorganisms through their decontamination, as well as through the application of good manufacturing practices (GMP's) and cleaning and disinfection procedures in process areas (SSOP's) (8).

Decontamination must be understood as a microbicide treatment applied to fresh meat in order to achieve a significant reduction in the number of pathogenic and saprophytic (total microbiological load) present on the surface of carcasses, offal edible products, cuts, etc., that contaminate them during the slaughtering or cutting operations. It should be clear from the beginning that the possible use of these treatments must not lead to assume that hygiene practices in slaughterhouses may be less demanding but should remain core (9).

Given theseaforementioned characteristics, is essential to conduct ongoing monitoring activities to detect and combat it on statistical bases of design and selection of reliable samples. These were the objectives of this study conducted in meat by products packing plant located in Mexico City. The meat used came from pigs raised in the state of Querétaro, slaughtered in a Federal Inspection Type (TIF) establishment located in the state of Guanajuato and the carcasses were transported refrigerated at 3-4 ° C to their final destination.

Methods and materials

The materials used were: sterile bags mortar with pestle, beakers, pipettes, flasks, test tubes, distilled water, half peptone water powder, Demi Fraser powder medium, pork, Lactic acid (Sulac-02) and 80% peracetic acid (Titan-15) 15%.

In this study, a qualitative rapid test which indicates only the absence or presence of the microorganism was used; inmunoprecipitant visual assay, an AOAC approved method 997.13.was used to detect *Listeria monocytogenes* and *Listeria* species present in food

The sampling design was performed according to recommendations of the International Commission on Microbiological Specifications for Foods (ICMSF) (7).

Forthe sampling program, we sought classificationof *Listeriamonocytogenes*in Table"Biological agents that constitute food health risks", recommendedby ICMSF(Table 1), according toit,*Listeriamonocytogenes*is locatedin groupIIwithinmicroorganismsconsidered to be of moderated riskandimportantdissemination(7).

TABLE 1. ICMSF classification for biological agents that constitute food health risks

GROUP I(Severe risk)	GROUP II (Moderated risk and important diffusion)	GROUP III (Moderated risk and limited diffusion)
C. botulinum	***L. monocytogenes***	*Bacillus cereus*
S. dysenteriae	*Salmonella* spp	*Campylobacter jejuni*
S. typhi	*Shigella* spp	*Clostridium perfringens*
S. paratyphi	*E. coli* enteropatogena	*Staphylococcus aureus*
Brucela spp	*Streptococus pyogenes*	*Vibrio cholera* no 01
Vibrio cholerae 01	*C. parvum*	*Vibrio parahemolyticus*
Vibrio vulnifecus		*Yersinia enterocolitica*

Modified ofICMSF, 1988

Once the group that contains Listeria was identified, the Table "Sampling programs recommended for combinations of different degrees of risk for various health and handling conditions" (Table 2), from the same Commission (ICMSF) was used; since Listeria monocytogenes corresponds to the degree of risk category: "moderated direct danger and extensive dissemination" and taking into account the conditions in which this food is expected to be handled and consumed after sampling.

In this case, since this pork meat was going to be used for the production of cooked meat products, the corresponding column is the one named "conditions that reduce the danger", which places crossing cell that contains category 10, from where it was determined out that the sampling program must be one of two classes with a value of n = 5 and c = 0, where n is the number of samples to be taken for each batch tested and c is the number of positive samples that is allowed, which for this microorganism is unacceptable to have a greater number or otherwise, it would be a rejected batch.

60 meat samples of 5.5 cm2 x 1.5 cm obtained from the muscles of the neck region, considered the most likely area of contamination were tested, from a total of 3 batches (of 250 carcasses each). Ten carcasses were with no decontamination treatment were selected at random for each batch. Moiety of samples (control group) were used to detect the presence of Listeria and the other was divided into two groups (test): 15 which would be treated to test the inhibitory effect of lactic acid with calcium sulfate, and 15 for peracetic acid on L. monocytogenes, if detected.

TABLE 2. RECOMMENDEDSAMPLINGPROGRAMS FOR DIFFERENT DEGREES OF HEALTH RISKS AND VARIOUS CONDITIONS OF HANDLING COMBINATIONS

Degree of risk	Conditions expected for food to be handled and consumed after sampling		
	Conditions that low risks	Conditions no affecting risks	Conditions increasing risks
No direct health risks, shelve life, alteration	Shelve life increase CATEGORY 1 3 classes n = 5, c = 3	Without modification CATEGORY 2 3 classes n = 5, c = 2	Decrease in shelve life CATEGORY 3 3 classes n = 5, c = 1
Low health risk, indirect (indicators)	Decreased risk CATEGORY 4 3 classes n = 5, c = 3	Without modification CATEGORY 5 3 clases n = 5, c = 2	Increased risk CATEGORY 6 3 clases n = 5, c = 1
Moderated direct risk, limited spread	CATEGORY 7 3 classes n = 5, c = 2	CATEGORY 8 3 classes n = 5, c = 1	CATEGORY 9 3 classes n = 10, c = 1
Moderated direct risk, wide spread	CATEGORY 10 2 classes n = 5, c = 0	CATEGORY 11 2 classes n = 10, c = 0	CATEGORY 12 2 classes n = 20, c = 0
Severe direct risk	CATEGORY 13 2 classes n = 15, c = 0	CATEGORY 14 2 classes n = 30, c = 0	CATEGORY 15 2 classes n = 60, c = 0

ICMSF, 1988

Several factors were takeninto account for the collectionof the samples, such as the fact that in theslaughterhousecarcasseswereroutinelydisinfected, thus,forpurposes of this study they were asked tonot to applydisinfectant andtoidentified 12carcasses; 10 of which were sampledand the other twowere usedto separatethedisinfectedon the trail to avoid contamination.

Thecarcassescameto the plant every Wednesdayafternoon, where they were keptrefrigerated at 4°Cand dischargedon Thursdaymorning when the ten without disinfectant were recognized to take thesamplesinthe neck region.Sampling wasdoneover amonthsincethe secondweek it was not possible to samplebecausedisinfectantwas appliedto allcarcassesmaking not possible to have similar conditions.

Controlsampleswere obtained(ones without acid), andthen transportedtothe plant laboratory for further processing.

After that, Paraceticacid (15%, Diken -Titan-15®) was applied. A five liter solution of 1.33 mlofParaceticacidin1 literofwater (0.019%acid concentration) was preparedand then applied to 5 sampled carcasses perbatch. A five liters capacity spray pump was used to spread carcasses.

A Lacticacidwithcalcium sulfate (Chemital -Sulac-02 to80%®)solution was prepared using 30mLofLacticacidin1 literofwater, having a 2.4%dilutionofthe acid. This solution was spread over the other 5 carcasses of each sampled batch with other spray pump.

Sampling was performed three hours after acids application inthesame region of theneck;these sampleswere storedrefrigerated at4°C ± 1°C, awaiting the resultsof the control samples(without acid). This

3hour waiting time prior to samplingwas necessarymeanwhilesamples without acidwere been processed, as theminimum waitingtime recommendedby the manufactureris 15 minutesfor theParacetic acidandeffecton contactwithLacticacid. Sampleswith acid were kept incoolingbecause theVIPtest resultswould be obtaineduntil the third dayafter they were processed, so ifat that moment a positivesampleto *Listeria* be detected, conditionswould havechanged due tothenatureofthemeat. So this measure would allow reducingthese possible variations. Thevisual immunoprecipitant essay(Listeria VIPGOLD®) was used toprocess samplesfrom the control groupin triplicate.

Results and discussion

If only a red line was observed in the VIP kit, the absence of *Listeria* was considered, as well as the proper functioning of the test, so it is a control line; if two red parallel lines were observed would be indicative of the presence of *Listeria*. It is noteworthy that the test detects between 10^3 and 10^5 CFU / mL.

All samples were negative. Therefore, it was not possible to continue the study nor further statistical evaluation was required.

Although *Listeria monocytogenes* is a responsible for numerous infectious outbreaks and cases caused by eating contaminated meat and meat products pathogen, in this study was not possible to detect its presence by meansof the approved by the AOAC Rapid immunoprecipitant visual test. On this basis it can be said that this raw material complies with the *Codex Alimentarius* recommendations (2) respect to this bacteria. No indication of compliance with any NOM can be considered because no oneof them establishes such standards.

The presence of pathogens in meat depends on the species from which meat comes from (beef, pork, poultry, etc..), Some microorganisms of importance are *E. coli*, *Salmonella*, *L. monocytogenes*, among others, their presence and number in meat is variable because they can be affected by factors such as animal feed and the diseases affecting the mentioned species, among others. In recent reports Farzan *etal.* (2009) (5), in a study that sought the incidence of *Salmonella*, *Campylobacter*, *Yersinia enterolitica*, *E. coli* O157 and *Listeria monocytogenes*, 31 pig farms located in Waterloo region, Ontario (3 of end of cycle and 28 of complete cycle) were sampled, using 359 stool specimens for *Listeria monocytogenes*culture by approved AOAC method MFHPB; from which this bacterium was detected in 4 (3.3%) of 122 samples taken from 11 farms. Similarly Esteban *etal*. (2009) reported the absence of *Listeria monocytogenes* in 17 pigs, meanwhile in 17 sheep (14.2%) of 120, 38 beef cattle (30.6%) of 124 and 38 dairy cattle (46.3%) of 82 were positive, with samples obtained from the feces (4). Esteban *etal.* (2009) mentioned that although the number of pigs was limited and the presence of *Listeria* was negative, this bacterium can often be found in porcine origin products, making it more likely that serious contamination by this agent occurs particularly in slaughterhouses and processing plants (4)

Por lo que lo más factible seria que la contaminación por este agente ocurra sobre todo en mataderos y plantas de proceso [(4).]

As already mentioned, *Listeria* contamination can occur in slaughterhouses. Fernando H. (2006) (9), conducted a study in the U.S. where he worked with pig carcasses without treatment with organic acids (Lactic or Peracetic); using swabbing direct form carcasses technique to detect *Listeria*, without specifying the sampled area, reporting 1.5 % of positive from a total of 708 carcasses and 5% incidence of *Listeria monocytogenes* in meat cuts of the same carcasses, which would be consistent with results reported by Esteban (2009). By other side, Conter M *et al.* (2006) (3) did a study where they evaluated the microbiological conditions of pig carcasses and equipment used in the slaughterhouse. 70 halfcarcasses were analyzed immediately after evisceration, taking out pieces ofmeat from about 5 mm thick and 3 cm^2 fromloin, shoulders, jaw and the inside of the leg. Presence of *Listeria* was determined in 6 carcasses (8.6% of the 70 analyzed), while from equipment and facilities analyzed, 50% of the soil samples were positive and 33.3% of cut tables samples tested positive.

Conclusions

The reliability of results obtained in depends on the representativeness of the samples and the accuracy and precision of analysis; this is why it is important to work with an adequate sampling plan. Also it is

necessary to have a microbiological criterion for determining whether a food is of good or bad quality, assessing food safety, monitoring of good manufacturing practices, shelf life and utility of a food or ingredient for a certain purpose. So in this study we used the sampling method recommended by ICMSF.
However, it is necessary to take into account that this does not mean that it is possible to discard the possibility of *Listeria monocytogenes*presence in meat as there may be several causes of pollution, and therefore would have to rule out possible individual and combined factors to confirm their absence.
Finally it should be mentioned that statistics of the incidence of Listeria monocytogenes in Mexico is practically nil and the existing is not easily accessible, at present there is a major concern to be addressed, it is perhaps for this reason that, in our country sufficient information is not available. However, it should be given greater importance and implement preventive measures and appropriate management, have a program of early detection of *Listeria monocytogenes* in slaughter houses, meat processing plants and meat by products control, in order to take the steps necessary to prevent their proliferation due to the severity of the disease.

Aknowledgements
PAPIIT IT202312, DGAPA-UNAM;

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Evaluation Of The Fungicidal Properties Of Vegetable Extracts FromXopiltet (*Trichiliahavanensis*) In Agricultural Pests (*Aspergillusflavus* and *Fusarium*spp)

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Abstract

At present the indiscriminate use of pesticides used to control different pests that afflict humanity called second-generation insecticides, have caused environmental damage and health problems to humans. The Organization for Food and Agriculture Organization (FAO), has proposed several techniques for reducing problems by synthetic insecticides, thus proposing effective techniques for pest control, they consist on to study natural products to use as pest repellents and not affect ecological balance. In this research was studied methanol extract and seed flour of *Trichiliahavanensis*(Xopiltetl), for evaluate of the antifungal activity of *Aspergillusflavus* and *Fusarium*spp. The seed was pulverized in a mill to obtain flour and the plant extract was obtained by Soxhlet using methanol and concentrate in a rotavapor. We evaluated the fruit methanol extracts and ground seed, both of 10% and 30%, by using a chemical positive control (Mancozeb®) and a negative control with potato dextrose agar, the plates were examined every 24 h for five days and mycelial growth was measured. The evaluation of the antifungal activity of the methanol extract at 10 and 30% of *T. havanensis* showed an inhibition against *Fusarium*spp of 88.8 and 94.4% respectively and the ground seed treatment showed a mycelial inhibition of 18.5 and 61.1% at concentrations of 10 and 30% respectively. Moreover the methanol extract at 10 and 30% tested in *Aspergillusflavus* showed an inhibitory rate of 3.3 and 100% respectively. At concentrations of 10 and 30% ground seeds showed inhibition rates of 8.4 and 64.4%. So it showed that the methanol extract of *Trichiliahavanensis* have fungistatic activity for *Fusarium*spp to the slow their growth and good antifungal activity at concentrations of 30% versus *Aspergillusflavus* because it completely inhibited. So this plant can provide an alternative for its practical use in the field as an antifungal natural, compatible and non-aggressive to the environment.
Keywords: *Trichiliahavanensis, Fusarium*spp, *Aspergillusflavus*, vegetable extracts.

Introduction

The use of plant extracts for the control of pests and diseases in the context of sustainable agriculture is a promising alternative because of its high effectiveness, low cost and not polluting the environment.

Plants have been able to protect the plants themselves before the man played an active role in protecting[1]. Is known that they synthesize diverse array of secondary metabolites, also called natural products, involved in interactions plant insect. Also various vegetable products have antialimentary activity for insect. Natural compounds can serve as a basis for developing new pesticides more selective and less polluting[2].

Mexico is a large country with special food needs, which is why the task is to produce large quantities of these grains are an important part in the diet of Mexicans. Agricultural products are invaded by insects and fungi that degrade its nutritional value in various ways.In particular grains and seeds are infected by different fungi in the field, including *Fusarium* spp., *Alternaria* sp. and *Helminthosporium* spp. In other cases also stored grain is attacked by fungi such as *Aspergillus* and *Penicillium* spp., being the principal presented phytopathogens[3]. During its development in grains, fungi cause different damage such as loss of ability of germination, discoloration, production of mycotoxins, among others [4].

It is therefore important to seek alternatives to combat these pathogens that attack to grains and crops of great economic interest in our country, the main plants that attack *Fusarium* and *Aspergillusflavus* spp. They are corn, soy, amaranth, sorghum, wheat among others[5].

The use of plant extracts for the control of pests and diseases in the framework of a sustainable agriculture is a promising alternative, because of its high effectiveness, low cost, and be not pollutants of the environment. One option is the use of the fruit of *Trichiliahavanensis* (Meliaceae) (Nahuatl: Xopilcuahuitl).

According to various authors, there are numerous reports of the Meliaceae family plants (also belongs the species *T. havanensis*Jacq), widely distributed throughout the tropical regions of several countries of North and Central and South Americans, where farmers use their fruits and leaves to fight various plagues [6]. The fruits of this plant have been isolated numerous limonoids[6], having seen that the azadirona[7, 8], O-acetiltrichelenona and 1.7 + 3 (4: 1) mixture, 7-di-O-acetilhavanesina[9] have antialimentaria activity against insects. The objective of this study was to evaluate the antifungal effect of extracts methanol of the fruit of *Trichilia havanensis*, plant pathogens such as *Fusarium* spp. and *Aspergillusflavus*.

Materials and methods

Preparation of extract

The plant material consisted of dried seed of *Trichilia havanensis* which was collected the ripe fruit in the locality of Vicente Suarez Xochitlán, Zacapoaxtla Puebla. The seed was cleaned and disinfected with sodium hypochlorite to 20%, they are dried and pulverized. From 15 g of the sample it underwent Soxhlet extraction for 6 hours with methanol as a solvent for the subsequent concentration of the extract on the Rotary evaporator [10].

Antifungal activity assay

The strains were used for the test: *Aspergillusflavus*, *Fusarium* spp. of which was a suspension of spores to 1 x 106 spores/ml [11]. The test was carried out poisoning 10 ml of medium Agar potato dextrose (PDA) with 1 ml of the extract. The poisoned environment was distributed in Petri dishes and was left to solidify, later they were inoculated with 10 µL of the suspension of spores. Featured the following treatments: a) methanol extract *T. havanensis*, b) positive Control chemical Mancozeb ®, c) negative Control PDA, d) control treatment of plates which consisted of the same method of poisoning by changing the extract by chemical (Mancozeb ®) and another only with the potato dextrose Agar. The plates were incubated 26±2 °C for five days. The plates were examined every 24 hours and measured the Mycelial growth with a vernier, which graphically expressed according to the radial growth expressed in centimeters and the incubation time in hours [12]. The percentage inhibition of growth was calculated taking into account the inhibition is the inverse of the growth. All treatments were carried out in triplicate.

Results and discussion

Radial growth of fungi

The results shown in figure 1 presents of radial growth of *Fusarium* spp., effect revealed that *Fusarium* spp. presented a 92% inhibition. Similarly be noted that treatment with the antifungal chemical (Mancozeb ®) had a mycelial 100% inhibition compared to the witness who had no inhibition with a growth of 6 cm representing 100%.

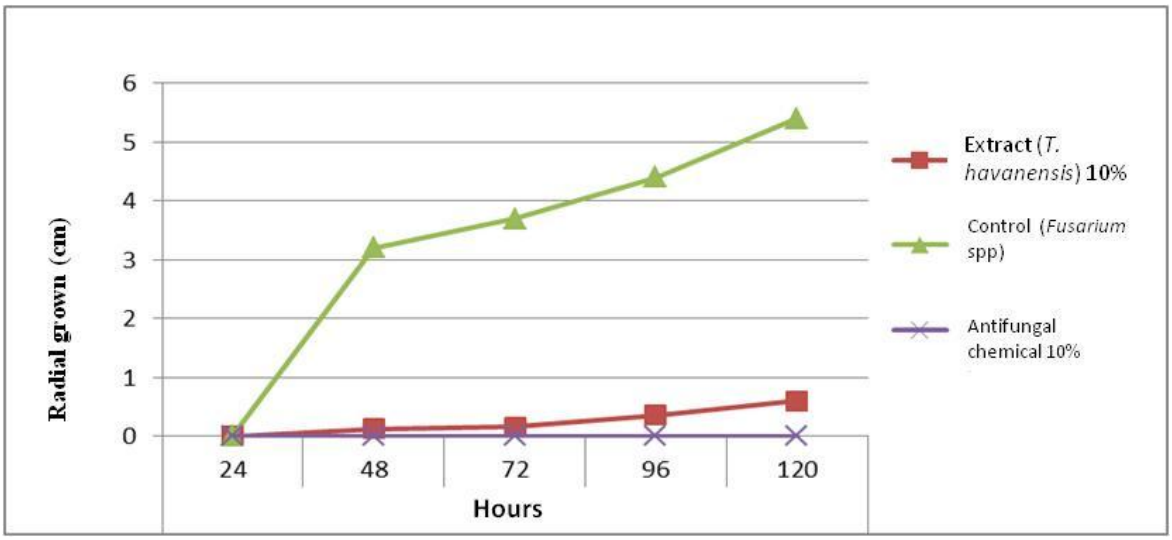

Figure 1. Effect of methanol extract of *Trichiliahavanensis* to 10% on the radial growth of *Fusarium* spp.

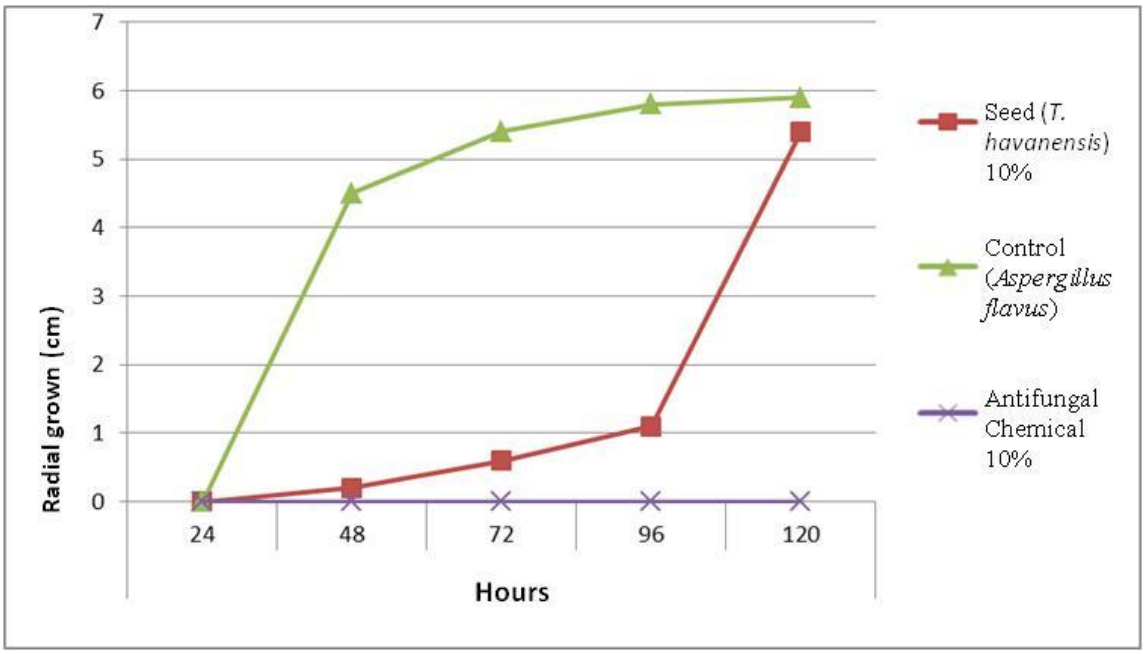

Figure 2. Effect of ground seed of *Trichilia havanensis* 10% on radial growth of *Aspergillusflavus.*

On the other hand the mycelial growth of *Aspergillus flavus* as shown in figure 2 to the second and third day noticed an inhibitory effect of 40% compared to witness the same days.OlguinLópez[8] observed activity of aqueous extracts from *Trichilia havanensis* on larvae of *Spodopteralittoralis*, finding antialimentary activity as shown in the results the ingestion of diet with *T. havanensis* (1 and 5%), induced a significant reduction in the weight of the larvae and a significant delay in its development. We observe that *T. havanensis* not only is effective to control larvae, but also, to control the growth of fungi.
Also have been observed in other three species within the genus *Trichilia* (*Trichiliaroka*, *T. connaroides*and *T. hirta*) effects Larvicides in *Lepidopterannoctuids* (*Spodopterafrugiperda* and *Peridromasaucia*), rehearsing with extracts of seeds and other plant parts [13].

Conclusions

The methanol extract of *Trichiliahavanensis* controlled the mycelial growth in *Fusarium* spp. with a 92% inhibition evaluated in five days. On the other hand to *Aspergillusflavus* in the 2nd and 3rd day only inhibited by 40%.

The methanol extract has antifungal activity with both study fungi. It is recommended to follow the line of research to be tested with more plant pathogens, as well as phytochemicals to identify the extraction and identification of the active compounds analysis, define the type of biological activity and mode of action.

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Microbiological Evaluation Of A Kind Food Made Mole From Pork

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Introduction

In food microbiology the presence of fungi and yeast is used as an indicator of poor hygienic practices during production and storage, as well as the result of inadequate raw material used for the manufacture of products. Although these organisms are part of the normal flora of the food itself, due to their low competitiveness and slow growth appear more in food where bacteria growth is less favorable, more tenaciously modifying nutritional value organoleptic characteristics (color, smell and taste) and of course the safety of the food. This last factor can cause poisoning, allergies and fungal infections.To avoid these risks in food and specifically on human consumption have been established permissible limits consumption of microorganisms as well as techniques to quantify, in our country is through decrees stipulated in the Mexican Official Standards (NOM). NOM-122-SSA1-1994 specifications for sanitary meat products was established as permissible limit of molds and yeasts a value less than 10 colony forming units per gram (CFU / g) and the NOM-111-SSA1-1994 determining the number of CFU using a reliable procedure for ensuring consumer safety, in principle the technique is based on providing a nutrient culture medium and the optimal conditions for the proliferation of plaque fungal. Media factors determining this aspect are temperature, nutrient content, light, food conservatives and pH. In fungi or yeasts, proliferation takes place in a pH range from 4 to 6 therefore involves a change or decrease growth. And as each food has a specific pH, their environment is sensitive to certain microorganisms, eg pork pH of 5.3 to 6.9, the minimum is ideal for the growth of fungi and yeasts, however when not processed meat thus maintaining the pH depend on the ingredients.In this project the microbiological quality was assessed by determination of fungi and yeasts of a finished food "type mole with pork and no preservatives" which was kept at room temperature without considering the label, in order to reproduce the conditions of a convenience store, where they consume a greater quantity of the product, so this analysis is the basis and strength of the company to implement prevention and control measures necessary to prevent the spread of food fungi produced.

Methods and materials

All analyzes were performed using the techniques and procedures indicated. The reactives used are of analytical grade. The company produces 40 kg per day of this food type mole with pork, packaged in a plastic container in presentation of 500 g with a hallmark of new product. To study the company provided 15 random samples packaged in a similar container, with a net content of 150 g of food which is kept at room temperature ignoring the label (refrigerate at 0 to 3 ° C) to simulate the process in a convenience store. Over a period of two consecutive weeks opened a container for microbiological tests then discarding theremainingproduct.

Preparation of growth media and solutions, NOM 111 SSA1 1994. A solution of 0.1% peptone, pH 7 which was adjusted with 1.0 N NaOH, this solution is known as a diluent. The growth media Potato Dextrose Agar (PDA) was prepared following the manufacturer's indication DBdioxon and adjusted to pH 3.5. The solutions and the sterilized culture medium, and the equipment required for testing.

Sample preparation, NOM 110 SSA1 1994. All content of the package was liquefied in 1-2 minutes and used for all studies immediately (pH, fungi and yeasts, and coliforms humidity, data not shown for the last analysis) to avoid any contamination.

Determination of pH, 130 NOM SSA1 1995. Was placed in a sterile beaker homogenized sample in sufficient amount to engulf the electrode and the pH value read obtained.

Preparation of samples diluted 110 NOM SSA1 1994. 10 g were taken from the homogenized sample and were added to 90 mL of diluent, the resulting mixture is called primary solution. With the above solution 5 diluted samples were prepared in the following proportion 1:10, 1:100, 1:1000, 1:10000, 1:100000, when transferred with a sterile pipette 10 ml of the primary solution to a vial 90 mL of diluent was shaken for 1 minute, repeating the procedure until 5 different concentration solutions.

Fungi and yeasts, NOM 111 SSA1 1994. In a plate count and placed under aseptic conditions 2 ml of the primary dilution plus 15 mL of potato dextrose agar (PDA), the mixture was stirred and circular movements until the hardening waited for inverting the plate agar. In an incubator at 25 ± 1 ° C led to the proliferation medium for 5 days. The process was performed in duplicate for each concentration and a control box (not shown). At 3 °, 4 ° and 5 ° of incubation the plates were removed from the incubator to quantify the colonies present in each using a colony counter type Sol Bat Quebec. According to standard is selected plates 10 and 150 colonies within colonies submit extensive colonies would be taken in the next few days. The results are the average number of colonies determined for duplicate multiplied by the reciprocal of the dilution corresponding units are CFU / g CFU / mL.

Results and discussion

Figure 1 shows the behavior of the pH of the food with respect to time, being clear food acidity and thus an accurate prognosis of the proliferation of mold and yeast at room temperature condition for this event.

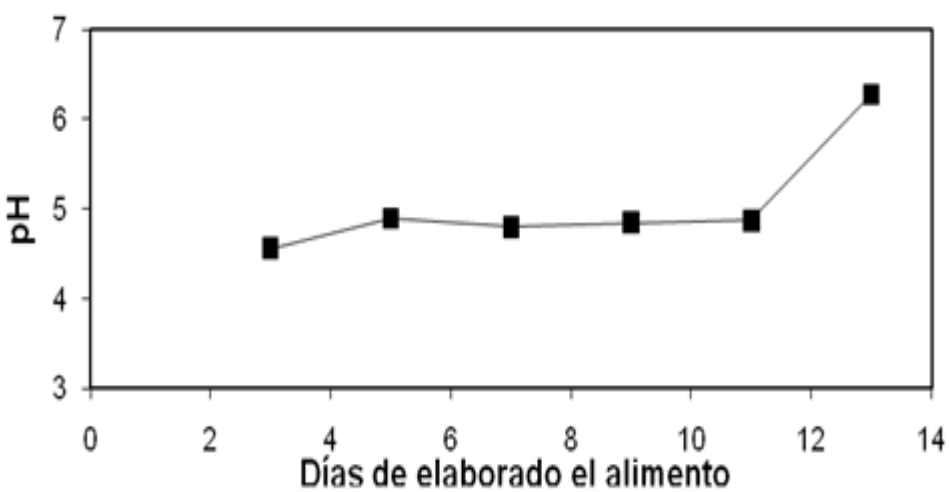

Figure 1. Changing the pH of the food from the first day of production

Figure 2 outlines the behavior of fungi and yeasts as the days of making the product. The results are in boxes between 10 and 150 colonies selected as the standard indicates counted on the 5th day and if you do not select meets the 4th or 3rd day. Only three cases in which the colonies grew strongly from 4th day so it was necessary to consider counting the colonies on the 3rd day. From the third day the CFU / g of molds and yeasts present in this product is beyond the permissible limit of <10 CFU / g upward growth of fungi

and yeast is the result of an enabling environment, the samples were kept at room temperature and pH results indicate a gradual and constant at the end. Visually, the food presented on the third day and fungus growth fell to fifth day, however this behavior is not observed in the product kept under refrigeration,which did not serve the convenience stores.

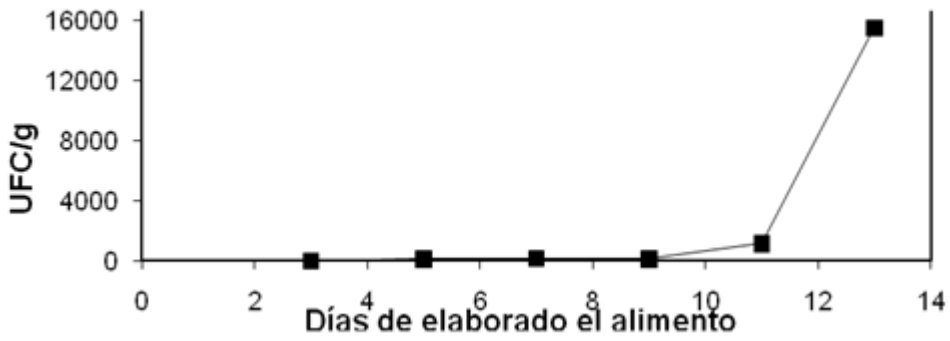

Figure 2. Colony growth of fungi and yeasts

The presence of fungi and yeasts and pH variation in food is a sign of poor manufacturing practices, however there is no guarantee that when there have been no cooling the product analysis which shows no growth before 15 days indicated as label for expiration. Therefore, it is essential to review the refrigerated shelf life compare results, and the company will take the necessary decisions to modify its production process, adding preservatives, other packaging implement, train staff and make changes in its facilities. It is essential to inform staff of the convenience store nearby as the consumption of this food may involve a serious risk to healthand the practice of more exquisite superheated to a "mole" is not recommended.

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Physicochemical And Phytochemical Characterization Of Pitahaya Fruit (*Hylocereus ocamponis*)

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Virtually unknown in the mid-1990s, the pitahayas (Hylocereus ocamponis) are already part of the world market and can be seen on the shelves dedicated to exotic fruits. However, some species of this fruit are still poorly known, so a physico-chemical and phytochemical characterization of the pitahaya would be very important. The aim of this work was to quantifying the content of phytochemicals present in pitahaya fruit, evaluate the antioxidant capacity of hydrophilic and lipophilic extracts obtained from the fruit, evaluated by two assays 2,2 '-diphenyl-1 -picrylhydrazyl (DPPH) and the ferric reducing antioxidant power (FRAP). In addition to establishing the source of the antioxidant capacity, we assessed the total phenolic, total carotenoids and the total betalain contents, and also a physicochemical characterization (pH, acidity, moisture, total soluble solids and color).

The total phenolic content was 139.81 ± 7.38 mg/100 g gallic acid equivalents, the total carotenoids pitahaya content was 2.02 µg (expressed as β-carotene), total Betalains was 1.36 ± 0.03 mg/100 gfw of betaxanthins and 4.29 ± 0.09 mg/100 gfw of betacyanins.

The antioxidant capacity measured by the DPPH assay in the hydrophilic extract was 5437.98 ± 103.41 µmol trolox equivalent/100 gfw, while in the lipophilic extract it was 186.91 ± 8.28 µmol trolox equivalent/100 gfw. The antioxidant capacity obtained by the FRAP assay in the hydrophilic extract was 4807.54 ± 300.02 µmol trolox equivalent/100 gfw, whereas that in the lipophilic extract was 185.40 ± 6.47 µmol trolox equivalent/100 gfw. In general, the lipophilic extract showed lower values than the hydrophilic extract, regardless of DPPH or FRAP assay employed. These results suggest that pitahaya fruit is a promising source of phytochemiclas.

Key words: Pitahaya, Antioxidant Capacity, Phytochemicals, Hydrophilic and Lipophilic Extracts.

Introduction

There is a growing interest in the use of natural phytochemical compounds for food and fruits because natural products are associated with quality and health promotion whereas synthetic compounds are critically assessed by consumers (1).

The attractive fruits of rare species, such as the pitahaya fruit (Hylocereus spp.), are highly prized in the world markets. Although the fruit is the most demanded product, the entire plant can be used as food and medicine. For these reasons, several countries, including Mexico, have been interested in cultivating this species (2).

Hylocereus biodiversity is poorly known, including types cultivated in home plantations and newly initiated. Some efforst have been invested in Nicaragua to selected materials that are most cultivated in that country (3). In Mexico, research is emerging (4).

Elsewhere, pitahaya is considered to be a new, promising fruit species; it is cultivated on different scales in Australia, Israel and Reunion Island. This success can be explained in part by the fruit qualities and characteristics (attractive colors and shape) and also by the commercial policies of some producing and exporting countries (e.g., Vietnam, Colombia and Israel) (5). The aim of this study is physicochemical and phytochemical characterization of pitahaya fruit (Hylocerus spp.), which is grown in several parts of the state of Guanajuato, through the physicochemical characterization and phytochemical study of aspects

related to the production fruit of genotypes from home gardens (mainly the state of Guanajuato) to initiate the selection of the most promising and future cultivation in the region.

Materials and methods

Chemicals and Solvents. Reagents were obtained from Sigma-Aldrich (St. Louis, MO), unless stated otherwise, and standard purity was 97% for β-carotene, 2.2-diphenyl-1-picrylhydrazyl (DPPH). High-performance liquid chromatography (HPLC)-grade methanol, acetone, *n*-hexane, and ethanol were purchased from J.T. Baker (Baker Mallinckrodt, Mexico). HPLC-grade water was prepared by a Milli-Qplus purification system (Millipore Corp., Bedford, MA).

Materials

Materials. Pitahayas red with pink pulp (Hylocereus ocamponis) was selected randomly from several home orchards in Celaya and Comonfort, Guanajuato Mexico; 25 to 30 days after flowering, considered by the growers as the best time of crop maturity, bearing a single fruit as a result for each and every one of the tests to be conducted for the evaluation of phytochemical compounds.

Physicochemical Characterization.

Upon arrival to the laboratory, fruit were washed and physically and chemically characterized by measuring weight, internal and external color, pH, firmness and total soluble solids content (TSS, °Brix), and representative samples were freeze-dried until reaching a constant weight, for moisture content analysis. °Brix was measured in the juice obtained from a representative portion of each fruit, using a temperature adjusted hand refractometer (ATAGO, Co. Ltd., Osaka, Japan). pH was measured in the juice with a potenciometer (Orion research digital pH/millivoltmeter 611). Firmness measurement was carried out using a penetrometer (Fruit Pressure Tester FT 011 0-11lbs., Italy). Color was measured with a Minolta spectrophotometer (Minolta, Co. Ltd., Osaka, Japan), which was calibrated with the white pattern during each sampling time. External color was longitudinally determined on three points of each flat side of the fruit (six points for each fruit). For flesh (internal) color, a big slice from a flat side of each fruit was obtained and color was determined longitudinally on three equidistant points. L^*, a^*, b^*, C^*, and $h°$ values were recorded. All different samples were then frozen with liquid nitrogen and kept at -80 °C and freeze-dried within about 1-4 weeks. Freeze-dried samples were kept in the dark at -80 °C during the whole period of analysis, which lasted about 8 weeks.

Analysis of Antioxidant Compounds. Total phenolic compounds (TPC) were extracted as reported by (6), with some modifications. A total of 1 g of freeze-dried powder samples was homogenized in 20 mL of 80% acetone using an Ultra Turrax model T25 basic homogenizer (IKA Works, Willmington, NC) at room temperature. The homogenate was sonicated for 5 min in a Bransonic 2510 sonicator (Bransonic Ultrasonic Co., Danbury, CT) and then centrifuged at 19000 *g* for 15 min at 2 °C in a Hermle Z-323-K centrifuge (Hermle Labortechnik in Germany). The supernatant was collected, and the residue was subjected to an additional extraction process under the same previously mentioned conditions. Both supernatants were mixed and aliquots were taken for analysis. The extraction process was performed in triplicate. For TPC quantification, aliquots were diluted 1:10 with HPLC-grade water, and 30 μL of diluted sample per hole was placed in 96-hole plates, and 150 μL of Folin-Ciocalteu reagent (dilution 1:10) and 120 μL of 7.5% Na_2CO_3 were added. The plates were incubated for 2 h in the dark, and absorbance at 630 nm was measured using a Dynex MRX microplate reader (Dynex Technol. Chantilly, VA). Results were expressed as milligrams of gallic acid equivalents (GAE)/100 g of fresh weight (fw).

The total carotenoids (TC) extraction was performed using the 970.64-AOAC method (6), with some modifications (7). Samples of 0.5 g of freeze-dried powder were mixed with 10 mL of hexane/acetone/toluene/ethanol (10:7:7:6, v/v/v/v) solution and 1 mL of 40% KOH in methanol, stirred at 56 °C for 20 min, and cooled with faucet water, and 10 mL of 10% Na_2SO_4 was added, stirred, and incubated in the dark until phase separation (approximately 10 min). The top phase was separated and used for analysis. TC quantification was measured on top-phase aliquots in a Beckman DU-65

spectrophotometer at 450 nm. A calibration curve was established using β-carotene in hexane as the standard and hexane as the blank.
Betalains extraction was done by weighing 5 g of fresh sample and added 20 ml water, homogenized for one minute using an Ultra Turrax tissue homogenizer model T25 IKA Works (Laborterchnik inc., Wilmington, USA) (8). Samples are maintained for 10 minutes in the dark, then centrifuged at 12000 g, and 15 °C for 15 minutes in a Hermle Z323K centrifuge (Wehingen, Germani).
Betalains were quantified using a Spectrometer (UV / VIS Beckman, USA) equipped with UVWinLab V 2.85.04 software, and the absorption spectrum was read in triplicate to 483 and 535 nm for betacyanins and betaxanthines, respectively. Betalains content was calculated based on the following equation:

BC (mg / g) = [(A (FD) (MW) vd / εLWD)]

Where A is the absorbance value, DF is the dilution factor, vd is the volume of fresh pulp solution (mL), WD is the weight of the fresh pulp, MW is the molecular weight and molar coefficient ε, for betacyanins MW = 550 g / mol, ε = 60000 L / (mol cm) in water. For the quantification of the average betaxanthins a molar extinction coefficient ε = 48.000 L / (mol cm) in water, MW = 308 g / mol were used (8).

Extracts Preparation and antioxidant capacity (AOC) Analysis. Lipophilic (LPE) and hydrophilic extracts (HPE) were obtained as reported by (6), with some modifications. Samples of 1 g of freeze-dried powder were homogenized in 10 mL of hexane/dichloromethane (1:1, v/v) using an Ultra Turrax model T25 Basic homogenizer (IKA Works, Willmington, NC). The homogenate was sonicated for 5 min in a Bransonic 2510 sonicator (Bransonic Ultrasonic Co., Danbury, CT) and then centrifuged at 15000 *g* for 10 min at 4 □C in a Hermle Z-323-K centrifuge (Hermle Labortechnik in Germany). The supernatant was collected, and the residue was again subjected to the same extraction process. Both supernatants were mixed and dried at 40 □C and low pressure. The dried extract was resuspended in 10 mL of HPLC-grade acetone, and designated as LPE. The residue, after the second extraction process, was dried with nitrogen, and the dried residue was homogenized in 20 mL of acetone/water/acetic acid (70:29.5:0.5, v/v/v), then sonicated, and centrifuged as previously described for LPE. The supernatant was collected, and the extraction process was repeated with the pellet. Both supernatants were mixed and designated as HPE. LPE and HPE were used for AOC analysis using the DPPH and FRAP assays.
DPPH assay was performed as reported by (6), with some modifications, using a microplate reader. Aliquots of 280 μL of 100 μM DPPH/methanol solution per well were placed in the same row of a 96-well plates, and then, 20 μL of extracts, diluted to different concentrations, was added to each well to complete 300 μL. Aliquots of 300 μL of methanol were placed in the first row as a blank. The plates were incubated for 30 min in the dark, and measurements were taken at 490 nm in a MRX microplate reader (Dynex Technology, Chantilly, VA). Results (AOC) were expressed as Trolox equivalents (TE) in μmol/100 g fw (9).
For FRAP assay (10), aliquots of 280 μL of FRAP reagent were placed in 96-well plates, and 20 μL of extracts, diluted to different concentrations, was added in each well. The plates were incubated for 30 min in the dark and read using a filter of 630 nm in a MRX microplate reader. Calibration curves were prepared using Trolox as a standard, and results were expressed as TE in µmol/100 g fw. FRAP reagent was prepared with 50 mL of 300 mM acetate buffer (pH 3.6), 5 mL of 10 mM 2,4,6-tripyridyl-2-triazine (TPTZ) in 40 mM of HCl, and 5 mL of 20 mM $FeCl_3$.

Results and discussion

Physicochemical Characterization. Table 1 presents the average values (mean ± standard deviation) of some physicochemical characteristics of pitahaya fruit (pH, firmness, ° Brix, moisture, color). Fruit moisture was 83.17%, pH was 3.87, °Brix was 16.73 °Brix, firmness was 0.0017 N, these data are typical for similar fruits from cacti, (11), found similar data in a study conducted for quality control of the pitahaya fruit to the use of this as a natural coloring with antioxidant, while the color present in the fruit

peel values, L * = 3.05 to 35.67, C = 32.37 to 33.54 and Hue = 20.51 to 86.21 and pulp: L * = 19.09 to 31.68, C = 43.69 to 48.03 and Hue = 339.14 to 342.59, indicating that values of the cactus fruit color was brillant pink-red, this is probably because the mucilag content in the pulp adds brilliant color to pink-red pitahaya fruit, very similar data are reported by (6) in a study they did with various fruits especially in prickly pear fruit.

Table 1. Physicochemical characteristics of the pitahaya fruit.

Physicochemical parameters				Color parameters		
Firmness (mN)	**°Brix**	**pH**	**Moisture (%)**	**L***	**C***	**h°**
1.70 ± 0.01	16.73 ± 0.14	3.87 ± 0.01	83.17 ± 0.22	29.41 ± 3.37	43.7 ± 4.76	342.59 ± 0.62

Antioxidant Compounds.

TPC, are presented in Table 2, Pithaya fruit had 139.81 ± 7.38 mg EAG / 100 gfw. The concentration of total phenolics compounds in pitahaya (*Hylocereus ocamponis*) had lower concentrations than those reported by (12) in berry cactus *Myrtillocactus* fruit with 9.12 g/Kg.

Total Carotenoids (TC), are presented in Table 2, in which, one can see that the pitahaya has an approximate value of 2.02 μg β-carotene equivalents / 100 gfw. The concentration of total carotenoids in pitahaya (*Hylocereus ocamponis*), was higher to results reported by (13) in dragon fruit (*Hylocereus polyrhizus*).

Total Betalains (TB), are presented in Table 2, indicating that pitahaya fruit had an approximate value of 5.65 mg / 100 gpf this. The concentrations of total betalaínas in pitahaya was higher 2.30 mg/100 gfw in pitahaya (*Hylocereus polyrhizus*) reported by (14) but lower than Camuesa 8.15 mg/100 gfw reported by (8).

Table 2. Phytochemicals presents in pitahaya fruit.

Total Phenolic Content (g GAE/100gfw)	Total Carotenoids (μg β-carotene/100gfw)	Total Betalains (mg TB/100gfw)	
		Betaxanthins	**Betacyanins**
139.81 ± 7.38	2.02 ± 0.14	1.36 ± 0.05	4.29 ± 0.09

Antioxidant capacity (AOC).

The hydrophilic extracts presented higher AOC compared to the lipofílic extracs, and in some cases, the AOC was even undetectable in lipofilic extracs. The hydrofilic extracs contributed to a 96.6% of AOC, while the lipofilic extracs contributed to a 3.4% of AOC when we used the DPPH assay (Table 3). The FRAP results were a little different in relation to the percentage of contribution to the AOC, where hydrofilic extracs contributed 96.2% and, lipofilic extracs a 3.8% of the total AOC (Table 3), these are values found in the pitahaya fruit. In other studies that have evaluated both hydrophilic and lipophilic extracts, in which results have been pretty close (15), which were found in this study to extract hydrophilic and the lipophilic extract. They used the method to measure DPPH and FRAP, AOC of the hydrofilic extracs and lipofilic extracs of pitahaya fruit similar results but with *Hylocereus undatus*.

Table 3. Antioxidant capacity of lipophilic and hydrophilic extracts of the pitahaya fruit by DPPH * and FRAP assays.

DPPH* (μmol trolox equivalent/100gfw)		FRAP (μmol trolox equivalent/100gfw)	
HPE	**LPE**	**HPE**	**LPE**
5498.98 ± 103.41	186.91 ± 8.28	4807.54 ± 300.02	185.40 ± 6.47

Conclusions

The high content of nutraceutical compounds containing the fruit of the pitahaya, among which are carotenoids, betalaínas and phenolic compounds which makes this fruit have high nutritional value in the national and international markets there to take advantage of all its compounds and be part of therapies against to chronic degenerative diseases in both rural and urban populations of all Mexico and other countries, and so in this way to promote cultivation in Mexico.

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Biological Activity Of Gallic Acid On Isolated Human Leucocytes

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Introduction

Gallic acid (GA), also known as 3, 4, 5-trihydroxybenzoic acid (Figure 1) is a phenolic monomeric compound which attached to sugars molecules and is included into the group of hydrolysable tannins. GA mostly results from the hydrolysis of tannic acid by bioconversion with the tannase enzyme, which results in hydrolyzable tannins and gallic acid esters.[1] Also, this phenolic compound is obtained from a) plants: gobernadora and damiana, b) fruits: grape, pomegranate, walnuts, banana, strawberry, lemon, apple and mango peel c) vegetables: chard and spinach; d) drinks: coffee and green tea.[2]

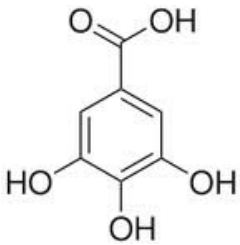


Figure 1. Chemical structure of gallic acid

Currently GA is known to be applied in different areas such as: 1) pharmaceutical manufacturing of broad-spectrum antibiotics such trimethoprim; 2) industrial processing of tanning animal hides,[3] photodeveloper and production of inks;[4] 3) food such as oils and fats antioxidant and food and beverage additive.[5] Furthermore, its been found to have various biological effects such as anti-inflammatory, antibiotic, anticancer and cardiovascular protection.[6,7]

Some authors reported that GA is a potent antioxidant, involved in absorbing and neutralizing free radicals produced by cells.[8] Paradoxically, it also promotes cellular oxidative stress, causing a cytotoxic effect in some cell types[9] In addition, some authors mentioned that the generation of reactive oxygen species (ROS) in response to GA, induces apoptosis in cultured cells *in vitro*.[10]

Therefore, in the present study we investigated the effects of GA on human leukocytes *in vitro*, and determined its median lethal dose (LD_{50}). Furthermore, we verified the protein expression profile of human leukocytes exposed to GA. The results indicate that high concentrations of GA, more than 2,000 µg/mL, generate toxicity in leukocytes, the LD_{50} of DIA GA was 2.323 ± 5.5 and LD_{50} SIGMA GA was 2.360 ± 4.3. However, the protein analysis suggests that GA could activate some proteins that promote apoptosis.

Methods and materials

We evaluated two types of gallic acid, one of them donated by the Department of Food Research of UA de C (DIA)[11] and the other was purchased from Sigma-Aldrich (G7384).

Leukocytes isolation

Leukocytes were obtained by venipuncture of healthy subjects, after signing informed consent. The leukocytes were isolated by centrifugation at 2500 rpm for 5 min at 4 ° C. The leukocyte pellet, was recovered and re-suspended in 1 mL of phosphate buffer (PBS), pH 7.4. Finally, the leukocyte concentration was adjusted to 500,000 cells/mL by counting the cells in a Neubauer chamber.[12,13]

Cell viability assay

After adjusting cell density, increasing concentrations of GA were tested for cytotoxicity (10, 100, 1,000, 2,000, 4,000, 6,000, 8,000 and 10,000 µg/mL).[14] Briefly, cells were incubated at 37 ° C for 1 h with constant agitation. After incubation, cell viability was determined by staining with tripan blue.[15]

Protein analysis of treated leukocytes

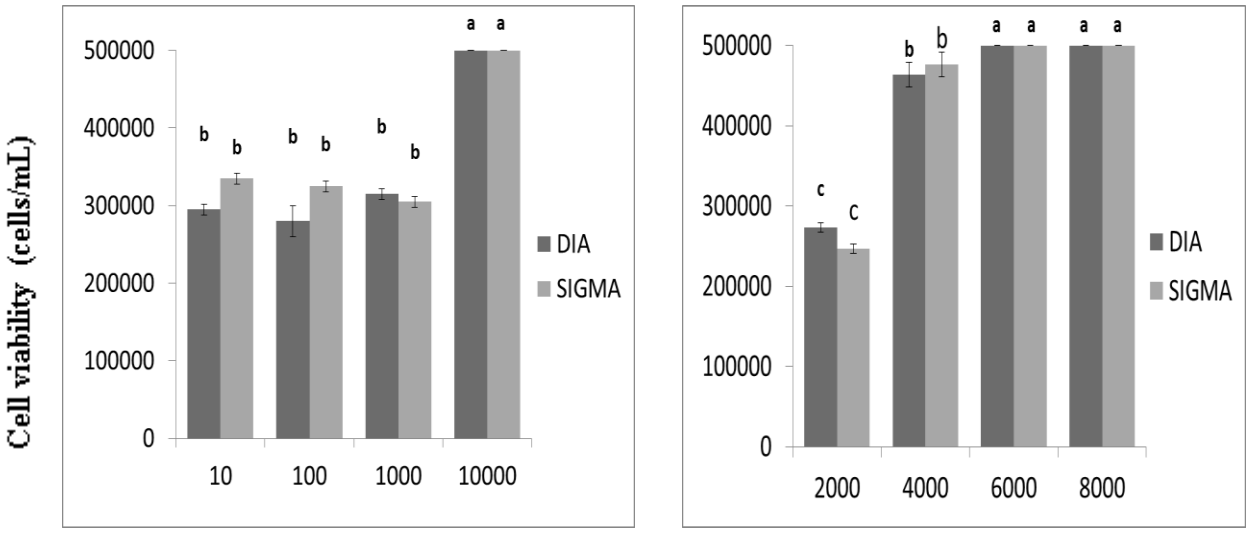
Treated cells were recovered for semi-qualitative analysis of protein profile. Cells were centrifuged and pellet was recovered, washed twice with PBS, and submitted to cell lysis treatment, adding 250 µL of cell lysis solution then cell lysates were stirred for 10 min and centrifuged at 2500 rpm for 5 min at 4 ° C.[16] Subsequently, protein of crude extract was quantified.[17] Once the proteins were quantified, concentration was adjusted to 20µg and resolved by electrophoresis in polyacrylamide gels under denaturalizing conditions with sodium dodecyl sulphate (SDS-PAGE) 12%. Finally, proteins were stained using Coomassie blue for analysis.[18]

Results and discussion

***In vitro* Cytotoxic effect of GA**

It was observed that increasing concentrations of GA result in decreasing cell viability. We also observed a 100% cell death upon treatment with a 10,000 µg/mL of GA. Figure 2A, shows that the treatment with 10, 100 and 1,000 µg/mL did not show significant differences compared with 10,000 µg/mL and positive control. In Figure 2B, it was observed that treatment with 4,000, 6,000 and 8,000 µg/mL have similar cytotoxic effect as the highest concentration tested. Finally, applying the Tukey mean equation, we obtained the median lethal dose (LD_{50}) for both cases, DIA GA equal to 2.323 ± 5.5 and the SIGMA GA was 2.360 ± 4.3.

a) b)



GA concentration (µg/mL)

Figure 2.Cell viability with different concentrations of gallic acid. Black: DIA GA; gray: SIGMA GA.

Protein analysis by SDS-PAGE

Protein profile assays. Figure 3 shows protein profile obtained from the supernatants after leukocytes treatment with GA. In lanes 6 and 8, protein bands are observed in the range of 30, 26 and 21.5 kDa which are not present in the other lanes corresponding to lower concentration or untreated cells. These mentioned proteins could be correlated with Bcl-2 (26 kDa), Bcl-XL (30 kDa) and BAX (21 kDa), proteins involved in cell apoptotic pathways.

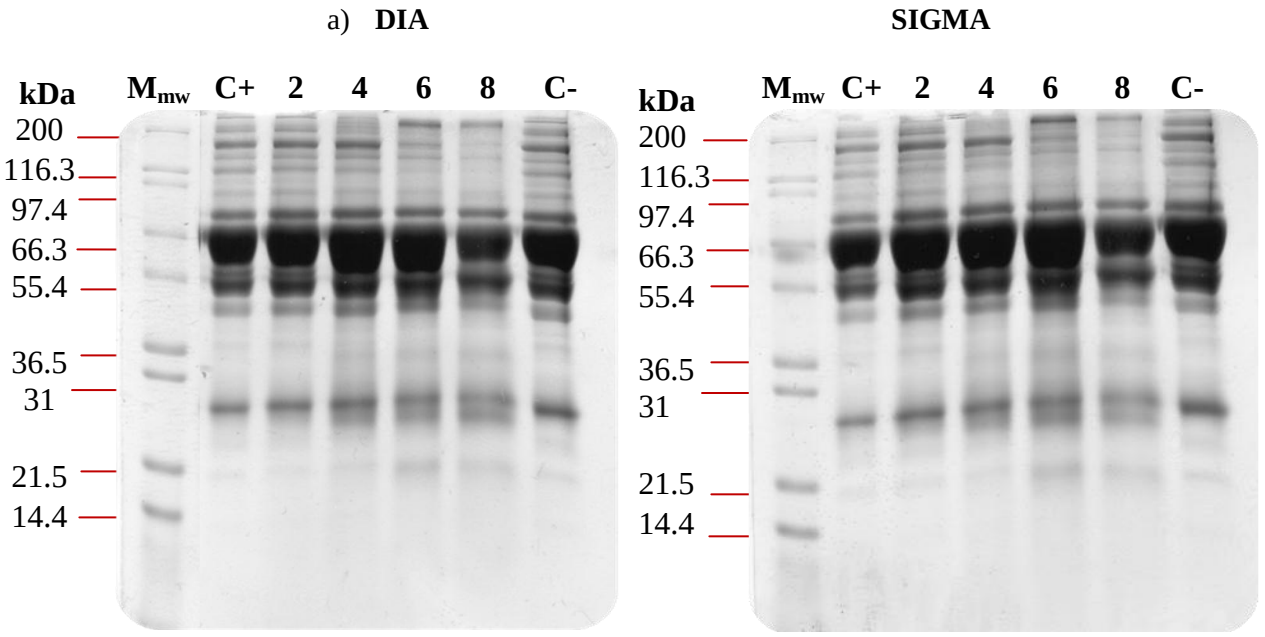


Figure 3. Polyacrylamide gels. Proteins obtained from supernatant after GA treatment. The first lane shows molecular weight marker, Mark12 (M_{mw}), following lanes correspond to different concentration 2,000 (2), 4,000 (4), 6,000 (6) and 8,000 (8) µg/mL of either GA, negative (C-) and positive (C +) control.

Our studies allowed us to determine that the effect of GA at high concentrations (above 2,000 µg/mL) results in a significant cytotoxic effect in human leukocytes, where lower concentrations did not had any effect on cell survival. The National Cancer Institute, considers that the values of a natural extract, purified or crude, should not exceed 30 µg/mL to be considered for anti-cancer therapy.[19] Furthermore, the protein study allowed us to suspect the presence of Bcl-2 (26 kDa), Bcl-XL (30 kDa) and BAX (21 kDa), which could be activated by the GA to promote apoptosis.[20] Although studies by our working group are not conclusive, this will help us to design experiments on cancer cells and verify GA treatment as a potential anti-cancer therapy because stimulate apoptosis.[21,22,23]

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Antioxidant Properties And The Potential Hypoglycemic Effect Of Lambrisco (*RhusvirensLindh*) Leaf Extracts Obtained Under Different Solid-Liquid Extraction Conditions

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Abstract

Diabetes mellitus is an endocrine disorder of worldwide awareness since at least 220 million of people suffer from it. Previously leaf extracts of Lambrisco (*RhusvirensLindh*), Huereque (*Ibervilleasonorae*) and Nispero (*Eriobotrya japonica*), commonly use for the treatment of diabetes in Northern Mexico, were evaluated and it was observed that Lambrisco had the most significant α-glucosidase inhibitory activity (AGH). Numerous studies indicate that the phenolic compounds present in plants possess inhibitory effects on the α-amylase enzyme thus acting as AGH bio-compounds. In the present study through the application of a 2^k factorial design during the solid-liquid extraction of Lambrisco leafs we evaluated the effect of solvent polarity, plant size and extraction time on the amount of phenolic compounds present in the liquid fractions. The total phenolic content in Lambrisco extracts was determined by the Folin-Ciocalteu method. A one-way ANOVA fora general linear modelwithTukey'stest multiple comparisons were used to analyze the data. The results of this study showed that the total phenolic content in the samples were in a range of 0.652 - 48.91 de grGAE/gr of Lambrisco. Thus the extraction parameters had a significant effect ($P \leq 0.05$) on the phenolic compounds obtained. The sample with the highest content of phenolic compounds (48.91 grGAE/gr of Lambrisco) was the one obtained under the following conditions, particle size of 2 mm, extraction time 90 min and polar solvent (ethanol). Among the samples extracted with ethanol the extraction time and particle size were the most significant factors inducing the extraction of phenolic components. In the same sense ethanol extractions had significant greater α-amylase inhibition activity (in vitro) with levels above 30% when compared to the blank samples. The present study reveals that the phenolic profiles of Lambrisco differed among extraction conditions. The phenolic compounds present in Lambrisco leaf extracts are positively correlated with the α-amylase inhibition activity. Thus Lambrisco leaf extracts emerge as potential α-amylase inhibitors and could be used in the treatment of diabetes or in the formulation of functional foods.

Introduction

TheWorld Health Organization defines diabetes as a chronic disease that occurs when the pancreas does not produce enough insulin or when the body does not effectively use it. The effect of uncontrolled diabetesis hyperglycemia (highblood sugar), which eventually damages the nervous system and blood vessels(5).

Conventional drugs used in the treatment of this illness include insulin, sulfonyl ureas, biguanides, alpha-glucosidase of human intestine and tiazolidneonas, which are widely accepted(4). Recent investigations have searched for plants with inhibitory effects on α-amylase that lower postprandial glucose levels, (6). Probably due the high costs in the treatment of diabetes with conventional drugs. The Mexican population in rural and urban areas, useas an alternative treatment of diabetes plants or they combine these with drugs for type 2 diabetes mellitus (1).

One approach for the treatmentof diabetes is to decrease the post-prandial hyperglycemia. This is done by the reduction of the glucose absorption through the inhibition of the carbohydrates hydrolysing enzymes of the digestive tract such as α-glucosidase and α-amylase. Inhibitors of these enzyme systemscause a delay of the carbohydrates digestion time.Thus,producing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise. Many plant origin materials have been investigated regarding their ability to suppress the glucose production rate in the gut or glucose absorption from the intestine (2).

The importance of polyphenols in the prevention and cure of diseases has been reported in previousstudies.Phenoliccompounds are known to interact with proteins and can inhibit enzymatic activity including α-glucosidase and α-amylase (3) Currently it is not possible to replace the conventional drug treatment of diabetes by herbal products, which is known as phytotherapy. However, the prevention and control of diabetes could be aid through plant-derived products (4).
Therefore research regarding the identification and characterization of the plant components that hold α-glucosidase and α-amylase inhibitory activity is of significant awareness. As, these chemical components could be used in the treatment of diabetes. Moreover the components identified could be incorporated in the formulation of food developing novel functional food products for the treatment of diabetes. Inthisstudywe selectedLambrisco(*RhusvirensLindh*) a plant of thefamily*Anacardiaceae*in order to carry out solvent extractions at different conditions and evaluate the relation in the content of polyphenolswith the inhibitory capacityoftheα-amylase enzyme (*in vitro*) of the extracted solvent fractions.

Materials and methods

Plant material

Lambrisco leaves (*RhusvirensLindh*) were obtained from an herbal store located in the city of Chihuahua Mexico and stored at room temperature. The whole plant was ground in an industrial blender and sieved through a mesh 10 and 18 (particle size of 1 mm and 2 mm) and stored at 5 ºC until the solvent extractions assays were performed.

Preparation of lambrisco solvent extracts

A 2^k factorial design (shown in Table 1)was applied to evaluate the effect of extraction conditions such as type of solvent, particle size and extraction on the content of polyphenols and α-glucosidaseandα-amylase activity. The dried plant material (10 g) of grind leaf fractions (particle size 1 mm and 2 mm) were extracted with 150 ml of ethanol (80 ±2° C) and hexane (70 ± 2 ° C) with a solvent / plant ratio of 15:1 during two different times (45 and 90 min) in a Soxhlet apparatus. The extracts were evaporated to dryness with a rotary evaporator under reduced pressure at 40 ° C and subsequently the flasks containing the extractions were transferred to a desiccator cabinet. The extracts were kept at room temperature before testing.

Table 1. 2^k factorial design applied during the solvent extraction of lambrisco leafs under different conditions.

Extraction	Treatment	Time (min)	Particle (mm)	Solvent
1	1	45	2	Hexane
9		45	2	Hexane
2	2	45	2	Ethanol
10		45	2	Ethanol
3	3	90	2	Hexane
11		90	2	Hexane
4	4	90	2	Ethanol

12		90	2	Ethanol
5	5	45	1	Ethanol
13		45	1	Ethanol
6	6	45	1	Hexane
14		45	1	Hexane
7	7	90	1	Ethanol
15		90	1	Ethanol
8	8	90	1	Hexane
16		90	1	Hexane

Total phenols

The determination of total polyphenols was performed by the modified method of Folin-Ciocalteu (Quettier-Deleu et al., 2000). Calibration curves were made with gallic acid at concentrations between 100 to 40 mg / mL, which had interval linearity.In a test tube there were placed 3 mL of distilled water, 0.2 ml of Folin-Ciocalteu reagent and 0.04 mL of the extract. After 10 min 0.6 mL of sodium carbonate solution 20% were added and incubated at 40 ºC /15 min, the extracts were diluted in appropriate proportions prior to absorbance readings obtained in the calibration curves (dilutions between 3 mg of extract in 1 ml of methanol) at an absorbance of 755 nm. Results were express as gGAE / g Lambrisco (dry leafs fractions).

Enzymatic inhibition activity

The enzymatic activity inhibition of lambrisco leaf extracts was determined throughout the evaluation of starch converted to glucose by a starch assay kit (Kit-1KT STA20, SIGMA Aldrich**)**. The hydrolysis of starch into glucose is catalysed by α-amylase and amyloglucosidase, then the glucose is oxidized to gluconic acid and hydrogen peroxide by glucose oxidase. The hydrogen peroxide reacts with o-dianisidine to form a coloured product, oxidizing o-dianisidine reacts with sulfuric acid to form a more stable pink colour. The pink colour intensity was measured at 540nm.

Statistical Analysis

Data was analysedby a one-way analysis of variance (ANOVA) method using a general linear model. ATukey test was used to analyse any significant mean difference among the results from the factorial design 2^k. Thus, the interaction of the extraction factors (time, particle size and solvent) was evaluated in order to analyse their effect in the concentration of total polyphenols and inhibition of the enzyme α-amylase activity exhibit by the Lambrisco (*RhusvirensLindh*) extracts.

Results and discussion

Accordingtothe 2^k factorial design 16 extracts (8 treatments) were obtained from *Rhusvirens Lindh*, polyphenol concentrations and inhibitory activity of the α-amylase were of significant different values.

Levels of polyphenols in Lambrisco leafs extracts

The interactions of the three extraction factors evaluated during the polyphenols assay are shown in Figure1. It is observed that thetype of solventhad the most significant effect in the amount of polyphenols extracted. Particle size and time did not show any significant effect during the polyphenols extractions and their particular interaction was as well not significant.Thetotal phenolics content in the Lambrisco leaf extracts are shown in Figure 2. The concentrations varied from0.652to48.91ofmgGAE/Lambriscog(dry) and treatment 4 (90minextraction, 2mm particle size and ethanol assolvent) exhibited the highest value. In previous studies were the amounts of total polyphenols indifferent types of plants, used in the treatment of diabetes, was done it was observed that the final concentrations obtained were above 140 (mgCatequina/gdry) in the plant

OcimumbasilicumL. This had polyphenols amounts of 146 ±5.26(mgCatequina/gdry) (3). Whereas, our values are higher than those reported in Gojiberries with 2.4mg/g (mg gallic acid per 1 gram of Gojijuice) (4). Thus, the amounts of polyphenols detected in lambrisco leafs are comparable to those reported in other plants used in the treatment of diabetes.

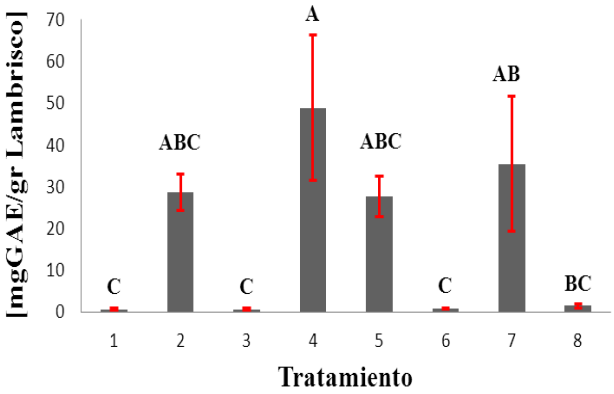
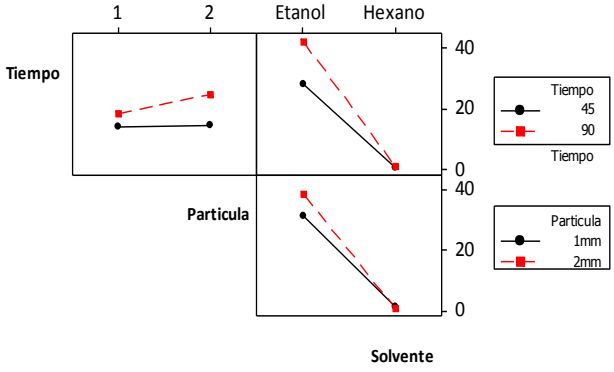


Figure 1.Interactionsfactors: time (min), particlesize(mm)andsolvent type (polar:ethanol and non-polar:hexane), ofthe 8treatments studiedfor determinationoftotalpolyphenolsbytheFolin-Ciocalteu method



Inhibition of α–amylase activity

The interactionsofthe three factorsevaluated during the solvent extraction of Lambrisco leaf extracts and their effect in the inhibitionof α–amylase activity (in vitro) are shown in Figure 3. It was observed that the type of solvent was the factor with the highest effect among the extraction of compounds with α–amylase inhibition activity (in vitro). Therefore the solvent ethanol was the solvent with greater significance during the extraction rather than the factors particle size or extraction time. In the same context the interaction among the factors did not shown any significant effect among the extraction of compounds with α–amylase inhibition activity. The percentages of inhibition effect of α-amylase among the extracted Lambrisco fractions are shown in Figure 4 were it is observed thatthe percentages ranged from10 to 31%. The extraction performed with the solvent ethanol using a particle size of 2 mm and an extraction time of 90 min had significant grater α–amylase inhibition activity.These results are comparable to other studies were the percentages of α–amylase inhibitionreachedup to 60%forthe case of the plant extracts of *Trianthemadecandra*and55% for*OcimumbasilicumL (2,3).*

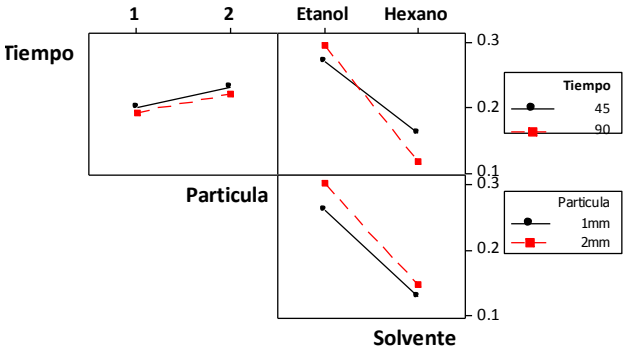


Figure 3. Interaction of the extraction factors: time (min), particle size(mm) and solvent evaluated for the inhibition of α-amylase in Lambrisco leaf extracts.

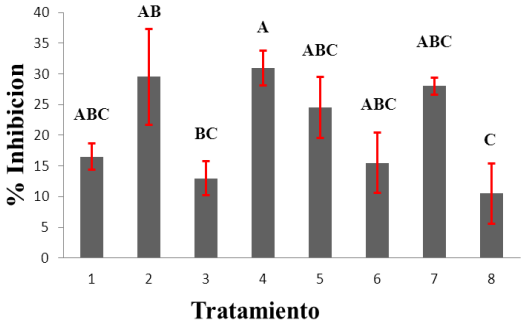


Figure 4. Percentage of inhibitory activity for the α-amylase assay on Lambrisco leaf extracts. Barrs with difrent letters showed significant diffrence acording to the Tukey mean analysis.

Conclusion

The data obtained in this study confirms that polyphenol compounds have a direct correlation with α–amylase inhibition activity. It emerges that Lambrisco leaf extracts could aid the conventional drug treatment of diabetes. Moreover, the therapeutic properties attributed to Lambriscoare possibly due to its content of polyphenols. It is suggested that additional testsshould be carried out in biological systems (in vivo) for more precise evaluation of the hypoglycemicinhibition activity related to the amount of polyphenols. In order to achieve Lambrisco extracts with high α–amylase inhibition activity that could be further used in the formulation of functional foods with diabetic treatment properties.

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Evaluation Of Antinutritional Factor Phytic Acid On Triticale AN66 From Coahuila State (Mexico), At Different Phenological State

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Introduction

Animal diets are composed mostly of plant foods such as cereals, which provide nutrients for good physiological and productive development. On a dietare important the macronutrient, responsible of the metabolic energy, and essential micronutrients in processes such as the physiological development of the skeletal system. Among macronutrients, one of the most important is phosphorus which is present in high quantities on the diet, however is not assimilated well in majorityof cases. Of totalconsumption of this mineral only 33% is absorbed, the 67% remaining is disposed of in the stool. This is mainly due to the presence of antinutritional factors in foods [1.2].

One antinutritional factor related withphosphorus metabolism is phytic acid, an organic acid which comprising six molecules of one molecule of phosphate and inositol. It is abundant in cereals and legumes [5,6].

Its function is not completely defined, but is believed to relate to following:

- Energy reserve and cation source.
- Reserve phosphorus regulates the concentration of this mineral in the seeds.
- Source of myoinositol, a precursor of the cell wall polysaccharides.
- Antioxidant which prevents lipid peroxidation and increased longevity of seeds [10].

Phytic acid, permits chelation and strong structure on essential minerals and proteins and inhibits digestive enzymes. This acid is insoluble and it cannot be assimilated by animals[9, 10].Deficiency of compounds in animals causes pathological conditions such as osteoporosis, loss of appetite, decreased fertility and production, among others. The excreted phytate, cause accumulation problems in rangelands, which promote eutrophication, killing fish and aquatic life, and the release of nitrous oxide, a potent greenhouse gas [7]. To counter these problems has proposed the use of phytases, which improves the bioavailability of nutrients which are attached to phytates [2].

Phytase enzymes are hydrolases type phosphate acid, breakingphosphomonoesterbonds act degrading phytate to inositol and inorganic phosphorus. Natural sources of phytases are grains in which it is reported that the presence of endogenous phytase is essential to produce the hydrolysis of phytic acid during germination processes, the intestine of ruminants and certain wildlife other organisms such as fungi and bacteria [7].

Microbial phytaseshavehigh potential to be employed in the agricultural industry as a feed additive and other feed. Several authors has developed various biotechnological processesfor the production of phytase, mainly from strains of *Aspergillus* spp., producing them mostly by fermentation on solid medium, using natural supports from cereals [1, 10].

This paper evaluates the phytic acid content in six different phenological stages of triticale AN66 from start tasseling to maturity total. The main objective on this investigation is obtain a complete study on phytic acid as important antinutritional factor, the evolution of its content in each phonological stage, and select the appropriate stage to use in the production of phytase.

Materials and Methods

Collection of samples

Samples of triticale were collected from experimental fields located south of the city of Saltillo, Coahuila, belonging to Universidad AutonomaAgraria Antonio Narro during the period from May to July of 2012. Triticale belong to the kind of late season type (AN66) and wheat as control, Coahuila late season type. Table 1,show the phenological stages used, the age in days after sowing (das), and the characteristics of this stage.

Table 1. Stages phenologies and sample characteristics

Daysaftersowing (das)	Stagefeature
94	Beginning of tasseling
108	Grainaqueous
118	Grainmilky
127	Softdoughgrain
144	Harddoughgrain
159	Drygrain

Moisture determination.

100 g were taken separately for each sample (stem and spigot) and placed in an oven at 70 ° C for 24 hours until a constant weight was determined and the amount of moisture loss based on the initial weight.

Pretreatment and preparation of biological material

Samples were separated on shank and stem respectively, and were separately washed with distilled water and disinfected with a 400 ppm solution of sodium hypochlorite during20 minutes to inactivate microorganisms and enzymes. They were then brought to the drying oven at a temperature of 70 °C, the sample was maintained for 24 hours or until constant weight. The samples were ground in an electric mill Thomas Wiley, with a particle mesh size of 0.5 mm, which were stored in a sealed pouch until use [12].

Samples chemical analysis

To carry out the valuation of phytic acid, the method used was Haug and Lantzchand validated by Soteloet *al*. [12],0.5 grams of sample was take and extracted with 20 ml of 0.2 N HClsolution, with vigorous magnetic stirring for 30 minutes. The extract was centrifuged at 12,000 rpm for 15 minutes. After, at 0.5 ml sample was added 1 ml of ferric solution: 0.2 g of ferric ammonium sulfate *12H_2Odissolved in 100 ml of 0.2 N HCl, to 1000 ml volumetric. The sample was placed in boiling for 30 minutes. After, 2 ml of a solution of 2,2-bipyridinewas added, 1 g2,2-bipyridine and1mlthioglycolic acid of this was graduated with 100 ml distilled water. The sample was mixed and the absorbance was read at 519 nm 30 seconds after adding the last reagent.Protein determination was performed by the methodKjeldahl[13].

Results and discussions

Figure 1 shows the marked decrease of moisture indicating as the grain is coming to its full maturity, which is common due to grain formation process and in the case of stem by own maturation process, this indicates the loss of some minerals and some important factors such as proteins which are less necessary for the cereal in advanced stage of maturity. We can also observe entity relationship moisture loss and decreased phytic acid (Fig 2).

In a figure 2, we can see the values of quantification of phytic acid in both samples of triticale in six phenological stages and wheat Coahuila, in phenological stage of 108 days. This shows that the triticale showed higher concentration of phytic acid in an early stage of development that was at 108 days, similar to wheat that was taking as reference. Values of phytic acid on triticale were compared against one of his ancestors, the Coahuila wheat at the same phenological stage for to observe the presence or the strengthening of its features, as mentioned by Shimadaet *al.* [11]. The analysis showed that the ancestor contains a smaller amount of phytic acid, both in the grain and in stem which differs to that reported by

Hernández *et al.* [6] in an analysis of antinutritional factors. A remarkable fact was that the present shank highest amount of phytic acid, since two-thirds of phosphorus is concentrated in the form of this compound as reported Cortés *et al*. [3], stored in the aleurone and endosperm mainly, which was tested in this study. This compound is required subsequentlyduring germination which agrees with some authors, such as Frontela*et al*. [4] mentioned it as a store of phosphorus or energy source for the plant.

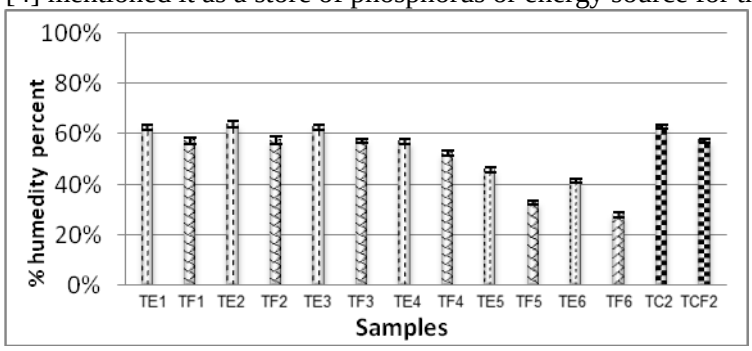

Fig 1. Percentage of moisture present in the sample at different phenological stages

TE: Tang triticale, TF: forage triticale, TEC: wheat spike Coahuila, TCF: Coahuila wheat forage. 1 = 94 das, 2 = 108 das, 3 = 118 das, 4 = 127 das, 5 = 144 das and 6 = 159 das.

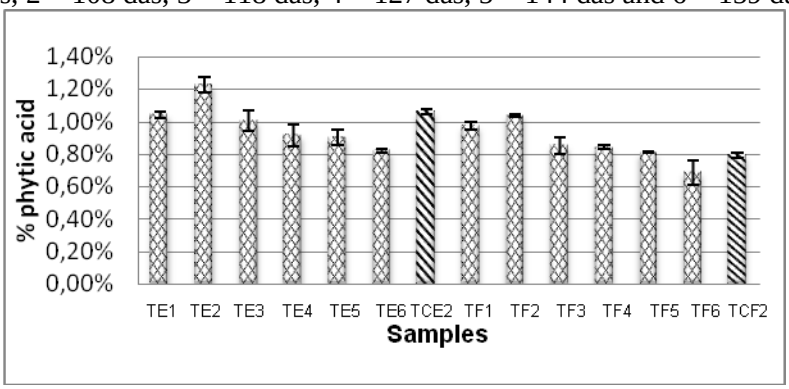

Fig 2. Amount of phytic acid present in samples of wheat based triticale Coahuila.
TE: Tang triticale, TF: forage triticale, TEC: wheat spike Coahuila, TCF: Coahuila wheat forage. 1 = 94 das, 2 = 108 das, 3 = 118 das, 4 = 127 das, 5 = 144 dasand 6 = 159 das.

As seen in graph 3 the study showed a decrease in protein maturation evident as triticale, which is common due to the germination process in which plant needs more nutrients. In its final stage triticale showed a protein concentration of 14.85%, similar to that reported by Sotelo*et al.*[11] for one ancestor of this hybrid, the wheat that amount comes from the characteristic high protein.

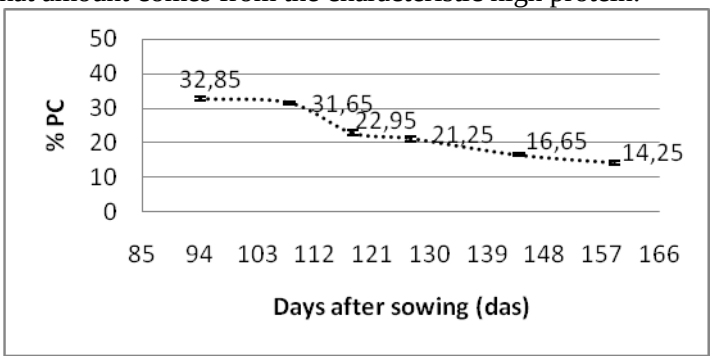

Fig 3. Protein content in phenological stages
Tang and forage together1 = 94 das, 2 = 108 das, 3 = 118 das, 4 = 127 das, 5 = 144 das and 6 = 159 das

Conclusion

In summary, is notedhigher presence of phytic acid when the plant is in its early stage of development so that when the harvest takes place this antinutritional factor is lower. This is because the phosphorus present as phytic acid is the main source of this mineral and is taken in the period of maturation of silver, the results observed in a gradual decrease of this compound conform the plant develops. Therefore the maximum production of phytic acid was shown to the 108 das which is when the spike is fully formed and grain filling begins. This shows that the triticale showed higher concentration of phytic acid in an early stage of development that was at 108 days, similar to wheat that was taking as reference. This research shows a significant difference in antinutritional factors present in the grain, particularly of phytic acid. This background provides a selection of cereal phytasesprduccion based on this cereal as well as nutritional information on this factor often affecting.

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Hybrids Development Of Quality Protein Maize For Hihglands Valleys Of Mexico

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Introduction

The demand of corn for human and animal consumption is increasing, for the humans this crop provides about 20 % of caloric intake and 15 % of protein consumed worldwide (4). This cereal, has reduced quantity and quality protein and it is a fundamental constitute of the mexican diet, and there are broad social sectors (rural or marginalized) with high protein requirements, such as children and pregnant or nursing women, whose sole provision of this molecule are particularly vegetables and tortillas, a situation that causes chronic malnutrition. It is therefore important to improve the nutritional composition of the maize grain whose endosperm accumulates 75% starch and 15% protein (2), and the zein is the predominant protein which is deficient in the essential amino acids lysine and tryptophan, and is not synthesized in mammals (6) so it should be part of the daily intake. There maize germplasm with high content of essential amino acids by the effects of the mutant gene opaco2 (3) and this enables the generation of varieties of grain quality protein through conventional breeding, the molecular marker assisted or by transgenesis (2), this type of maize are called modified or protein quality (QPM).The objectives of this study were 1) to estimate the heterotic response in crosses of six lines of quality protein maize in terms of their contents of lysine and tryptophan and quality of protein, and 2) Select the best hybrid combinations for the characters mentioned.

Methods and Materials

We used six inbred lines of maize with quality protein (M1 to M6) and their 15 single crosses included three witnesses, a quality protein maize CML 503B and two with different quality protein BA-2192-CML HEW 503-2 (middle) and H 70 (low).

Samples of 100 seeds (125 mg) of each treatment, are finely ground and treated with hexane in the Soxhlet apparatus for six h, 100 mg of this flour was used for the colorimetric determination of the essential amino acids lysine was taken for based on the reaction of 2 - chloro, 3, 5-dinitropiridine (390 nm) and the glyoxylic acid reaction for tryptophan (560 nm) (1). For total nitrogen (%) microkjeldahl method it was used and estimated amount of protein with the algorithm: Protein (%) = N_2 (%) (6.25). Protein quality index was calculated in relation to the tryptophan present in total protein: IC = [Tryptophan (%) x 10^2] [Protein (%)] $^{-1}$. We used a randomized block design with two replications of 100 seeds, the variables were analyzed with the statistical analytic system (SAS) (5) through the corresponding variance analysis, comparison of means was performed by the Tukey method ($\alpha = 0.05$).

Results and Discussion

It were detected significatives differences (α = 0.01) in the analysis of variance for lysine, tryptophan, protein quantity and quality protein, this indicated heterogeneity in the comparison of the characteristics evaluated (Tables 1 and 2).

Table 1. Tukey method for lysine and tryptophan in the maize genotypes.

Genotype	Tryptophane (%)		Genotype	Lysine (%)	
M2 x M4	0.12	a†	M2 x M6	0.49	a
CML 503B	0.12	a	M2	0.49	ab
M2	0.12	a	M2 x M4	0.48	abc
M3	0.12	ab	M3	0.47	abc
M2 x M6	0.12	ab	CML 503B	0.47	abcd
M3 x M6	0.11	ab	M3 x M6	0.44	abcde
M6	0.10	bc	M6	0.43	abcde
M2 x M5	0.09	cd	M5	0.41	abcde
M3 x M5	0.08	cde	M5 x M6	0.36	abcdef
M4	0.08	cde	M4 x M6	0.35	abcdef
M5 x M6	0.08	cde	M2 x M5	0.34	abcdef
M1 x M4	0.08	def	M3 x M5	0.34	abcdef
M4 x M5	0.07	def	M2 x M3	0.32	abcdefg
M3 x M4	0.07	def	M3 x M4	0.31	bcdefg
M1 x M3	0.07	def	M1 x M4	0.31	bcdefg
M2 x M3	0.07	def	M1 x M6	0.31	bcdefg
M1	0.07	def	M4	0.30	cdefg
M5	0.07	def	M1	0.29	defg
M4 x M6	0.07	def	M4 x M5	0.29	defg
M1 x M6	0.07	ef	M1 x M2	0.28	efg
M1 x M2	0.06	ef	M1 x M5	0.28	efg
M1 x M5	0.06	f	M1 x M3	0.26	efg
CML BA-2192-503 HEW-2	0.06	f	CML BA-2192-503 HEW-2	0.24	fg
H 70 (F1)	0.04	g	H 70 (F1)	0.18	g

† = Means with the same letter are significantly equivalents (α = 0.05).

Table 2. Tukey method for protein and quality protein in the maize genotypes.

Genotype	Protein (%)		Genotype	Quality protein	
M2 x M3	14.8	a†	CML 503B	1.07	a
M3 x M6	14.5	ab	M2 x M4	0.89	b
M2	14.5	ab	M3	0.85	bc
M2 x M6	14.2	abc	M2	0.85	bc
M2 x M5	14.1	abc	M6	0.83	bc
M3	13.9	abcd	M2 x M6	0.81	bc

M2 x M4	13.8	abcde	M3 x M6	0.75	cd
M1 x M2	13.8	abcde	M5 x M6	0.63	de
M1 x M3	13.7	bcdef	M3 x M5	0.62	de
M3 x M5	13.5	bcdefg	M5	0.62	de
M1 x M4	13.5	bcdefg	CML BA-2192-503 HEW-2	0.60	ef
M4 x M6	13.2	cdefgh	M2 x M5	0.60	ef
M1	13.2	cdefgh	M4	0.59	ef
M3 x M4	13.1	defgh	M4 x M5	0.58	ef
M4 x M5	12.9	defgh	M1	0.57	ef
M4	12.8	efgh	M3 x M4	0.57	ef
M5 x M6	12.6	fghi	M1 x M4	0.56	ef
M5	12.6	ghi	M1 x M6	0.53	efg
M1 x M6	12.6	ghi	M1 x M3	0.52	efg
M6	12.5	ghi	M4 x M6	0.51	efg
M1 x M5	12.3	hi	M1 x M5	0.51	efg
CML 503B	11.7	i	M2 x M3	0.47	fg
CML BA-2192-503 HEW-2	10.4	j	M1 x M2	0.47	fg
H 70 (F1)	10.1	j	H 70 (F1)	0.40	g

† = Means with the same letter are significantly equivalents (α = 0.05).

Table 3. Lineal correlation between essential amino acids, protein content, and protein quality.

	Tryptophan	Lysine		Protein		Quality protein	
Tryptophan	1.0	0.8	**	0.5	**	0.9	**
Lysine		1.0		0.5	**	0.7	**
Protein				1.0		0.2	ns
Quality protein						1.0	

**** = Significative (α = 0.01).**

The used controls were comported as the planned, the negatives had significantly (α = 0.05) lower content of essential amino acids (Table 2), as well as the quantity and quality of protein, although in this CML BA-2192 and HEW503-2 reached an intermediate value (Table 3). The positive control (CML 503B) showed the highest values (α = 0.05) in tryptophan, lysine and protein quality index (Tables 2 and 3). These results support the quantifications and served as referencefor to classify the genotypes analyzed for their protein quality, the classified as QPM reaching approximately twice tryptophan and lysine that the controls. Were obtained in the treatments significantly (α = 0.05) higher proportions of lysine, tryptophan and protein than controls, but protein quality was difficult to achieve in all the crosses, as anyone equaled the positive control in index quality and also the amount of protein not correlated with that (Table 3), while amino acids had a high and significantly correlation (r = 0.9) with the quality protein and for this reason they were a good amino acid balance indicator of the treatments. Inbred lines M2 and M3 had significantly (α = 0.05) the best content of tryptophan, lysine and protein with twice the content of essential amino acids from both negative controls (Tables 2 and 3), and for this reason they were sensu stricto the only lines with protein quality in the experiment.

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Evaluation Of Fungal Pigments Extracted from *Pycnoporussanguineus*against the Phytopathogenic Fungal (*Colletotrichumfragariae, C. gloeosporioides* and *Botrytis cinerea*)

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Abstract
The objective of this study was the raw extracts production of two isolated (H1 and H2) of *P. sanguineus* and to prove their effect on in vitro control of fruit and vegetables fungi.The best conditions for the orange pigment fungus production were determinated. For this, a randomized design in a factorial arrangement treatment was made, the factors and levels were: pH (8.0, 8.5 y 9.0), culture medium (potato dextrose broth and malt extract) and kind of light (daylight and full time white light). The orange pigment was extracted with ethyl acetate. The pigment was concentrated and tested in postharvest fungi (*Colletotrichumfragariae, C. gloeosporioides and Botrytis cinerea*).The best conditions for orange pigment production were pH 8.0, potato dextrose broth and full time white light. The fungicidal effect was observed against phytopathogens fungi with a reduction of 70% and 60% in *B. cinerea* growth with 10 and 20 mg/mL raw extract of *P. sanguineus* pigment, respectively. In *C. fragarie*, the inhibition was major of 50% and only 40% in *C. gloeosporioides*. Therefore, fungal extracts could be potentially active such as biofungicide in postharvest diseases.
Keywords: *Pycnoporussanguineus* extract, fungal pigments, antifungal activity, postharvest diseases.

Introduction
Wilson and Lawrence (1985) mentioned that in the case of post-harvest losses caused by microorganisms, had not received the attention of problem magnitude. It had been difficult to have a complete estimate of losses due to disease. However, conservative approaches reveal U.S. losses in fruits and vegetables 24% of the total harvest. These authors also report that in less developed countries are even greater losses due to lack of proper cooling and hygiene. Thus, they concluded that in the world, losses are approximately 50% of the harvest and much of it is caused by the decay caused by microorganisms. Some postharvest diseases which concern the researches are mentioned ahead.Anthracnose is caused by*Colletotrichum* species, such *as C. gloeosporioides*, *C. acutatum*, *C. fragariae*, and *C. demantium*. These species attack the fruit, primarily in the field, but the disease can develop after harvest. Lesions of anthracnose lesions develop as sunken, circular, tan or light brown in mature or nearly mature fruit. An example is *C. gloeosporioides* causing anthracnose of many fruits such as apple, avocado, mango and papaya (2).The infection can cause fruit to fall prematurely, but the biggest losses are presented in the maturation phase (3). Though there are no accurate statistics, it is estimated that losses in mango postharvest anthracnose fluctuate between 30 and 60% of the total production (4). Other disease is rots by gray mould (*Botrytis cinerea*) are of a light brown or brown hue, sometimes dark brown. It is the most serious postharvest disease commonly found in strawberries in modern refrigerated warehouses and transport (5). The disease is common in ripening strawberry and ripe infection occurs in unripe or green, particularly in wet conditions. The fungus can attack the material stored in the strawberry spread. This pathogen is able to affect 95% of the fruits after 48 h of harvest (6).For many years, a variety of chemical products and synthetic compounds have been used as antimicrobial agents to inhibit the action of phytopathogens. Research on plant-derived fungicide applications in agriculture, is being intensified as it is having a huge potential to inspire and influence modern agrochemical research (7). In recent years it has been used antimicrobial chemicals, such as benzimidazols, aromatic hydrocarbons and inhibitors of the biosynthesis of sterols, are often used in controlling plant diseases in agriculture. However, there are a number of

problems against the effective use of these chemicals in areas where fungi have developed resistance. To overcome this problem, higher concentrations of these chemicals increase the risk of high-level toxic residues in products (8).Therefore, there has been increasing interest in the investigation of the possible use of essential oils and plant extracts which are less harmful for the control of pests and diseases in agriculture (9). Also another form is "biocontrol" refers to the use of a natural source of microorganisms which are antagonistic to postharvest pathogens that we wish to eliminate (10). One most applications of antagonists for controlling postharvest diseases are performed before harvest. Postharvest applications have not been fully exploited (11). Fungi especially *Basidiomycetes*produce a wide range of natural products spanning structural components with antitumor activity and immunologically active until antimicrobials, antifungals, antivirals, cytostatics, enzymes, growth regulators and fragrances (12).Example *Pycnoporussanguineus* is a fungus belonging to *Basidiomycetes*, used in popular medicine since ancient times, have great importance in the search for substances antiviral, antifungal and antibacterial secondary metabolites (13).The major compounds are produced by *P. sanguineus* having antimicrobial activity isPoliporin. The antimicrobial activity of *P. sanguineus* has been known since 1946, where an active compound was isolated (poliporin) against Gram-positive and Gram-negative bacteria which had no toxicity against animals (14) and Cinnabarine. It is an antibiotic substance produced by *P. sanguineus* is an orange pigment having a basic 3-phenoxazine structure, with a carbonyl group at C-1, an amino group at C-2 and a hydroxyl group at C-9 (15, 16). Smania*et al.*, (1995 & 1997) has shown that the activity in cinnabarina*B. cereus*, *E. faecalis*, *E. faecium*, *E. coli*, *K. pneumoniae*, *L. mesenteroides*, *L. plantarum*, *P. aeruginosa*, *S. typhi*, *S. aureus* and various *Streptococcus spp*. and their activity is higher in Gram (+) to Gram (-).

Materials and Methods

Sampling and collections were made from *P. sanguineus* in Villa Guerrero, Estado de Mexico (H1) and Paracuaro, Michoacán (H2). For isolation and purification of fungus was taken basidiocarp tissue portion (fruiting bodies) and were grown in culture medium potato dextrose agar (PDA).

Pigment production by *Pycnoporussanguineus*.For this, a randomized design in factorial arrangement treatments was made the factors and levels were evaluated:pH (8.0, 8.5 and 9.0), liquid culture medium (potato broth, malt extract) and lighting conditions (daylight, continuous white light). Units under continuous white light were placed inside a wooden box (25 ± 3 °C). The treatments were maintained at ambient temperature and each treatment was made in triplicate. The production of extracts was based on experimental design, to obtain crude extracts of the pigment *P. sanguineus*, the following: depositing PDA's disk (5 mm diameter) of mycelium of *P. sanguineus*(H1 and H2) for 20 days in glass bottles (350 mL) containing 150 mL of potato broth to which they adjust the pH according to the proposed experimental arrangement. The inoculated flasks were incubated at 25 ± 3 °C for 30 days in day white light (12 h) and continuous light (24 h). After the incubation period, the broth was decanted potatoes with mycelium orange and filtered with Whatman No.1 previously weighed to determined mass. An aliquot of each sample was taken and dried at 105 °C for 20 h. The remaining mycelium was macerated with 10 mL ethyl acetate to release the pigment. It was adjusted to a volume of 50 mL with the same solvent. The extract was allowed to stand for 2 days. Subsequently, the extracts were concentrated in a rotary evaporator Büchi 461 at 75 °C. The extracts were weighed and stored in glass bottles.

The response variables were: concentration, mycelial dry weight and yield (dry weight of extract /dry weight of biomass). The concentration was determinate and quantity of orange pigment, it became a standard curve with known concentrations of methyl red dye (0.0024, 0.0048, 0.0097, 0.019, 0.039, 0.078125, 0.15625 mg/mL of ethyl acetate) by comparison with previously selected other pigments using a thin plate chromatography. By means of a linear regression equation was determined from the straight line ($y = 18.813x-0.0153$, $r^2 = 0.9972$) which was calculated with the pigment concentration.

Tests decontrol of fungal pathogens in vitro.The tests were made with fungi *Colletotrichumfragariae*, *C. gloesporoides* and *Botrytis cinerea*, previously grown on PDA. For this, we used three concentrations of the extracts of H1 and H2 (2.0, 10.0 and 20 mg/mL) which were added to the culture medium (PDA) before solidifying. The pigment was dissolved in ethyl acetate. The positive control was a commercial

fungicide (Captan®) at a concentration of 200 mg/mL PDA medium also added before solidification. The negative control was boxes PDA with 1 mL of ethyl acetate. PDA pieces were seeded (5 mm diameter) with mycelia of each of the fungi in all treatments. There were three replicates per treatment. Thus, treatments were: T0 (absolute control) only PDA; T1 (negative control) acetate, T2 (positive control) capture to 200 mg/mL, T3 (H1 pigment, 2 mg/mL), T4 (H1 pigment, 10mg/mL) and T5 (H1 pigment, 20mg/mL). This was repeated for the case of H2. Growth inhibition halos were measured.

Results and discussion

Table 1 presents the comparison of treatments (Tukey, $p<0.05$) on the growth of phytopathogenic fungi in postharvest *Botrytis cinerea*, *Colletotrichumfragariae*, and *C. gloeosporioides*treated with extracts isolates of *P. sanguineus*H1 and H2.

Table 1. Comparison of average growth radius (mm) of fungal pathogens in fungal extracts PDA *Pycnoporussanguineus* (H1 and H2) and commercial fungicide (Captan®)

Treatment	*Botrytis cinerea*		*Colletotrichumfragarie*		*C.gloeosporioides*	
	H1	H2	H1	H2	H1	H2
T0	33.183[a]	33.183[a]	25.000[a]	25.000[a]	25.000[a]	25.000[a]
T1	25.000[b]	25.000[b]	22.833[b]	23.333[b]	22.833[a]	23.333[a]
T3	25.000[b]	25.000[b]	17.500[c]	22.000[b]	21.833[b]	25.000[a]
T4	18.000[c]	10.500[c]	14.333[e]	12.166[c]	18.000[c]	18.666[b]
T5	16.500[c]	13.166[c]	15.000[d]	12.000[c]	20.833[b]	14.500[c]

[a,b,c,d,e]. Means in the same column with same letter have not significant difference (Tukey, $p<0.05$)

Botrytis cinerea noteworthy that grew and reached the total space of the Petri dish in 3 days (absolute control, T0) while *Colletotrichumfragariae* and *C. gloeosporioides* did in 7 and 9 days, respectively.From the above table it can be said that in general, had greater growth inhibition with extracts of *P. sanguineus*as the extract concentration was higher, noticing an increased effect with the extract of the isolation of H2 in the three fungi tested. In the case of*B. cinerea*was reduction nearly 70 and 60% for treatments with 10 and 20 mg/mL, respectively, compared to the negative controls (T0). In some work on biocontrol fungal extracts were used extracellular (EFE) of *Penicillium sp*., *Beauveria sp*., *Aspergillus sp*. and *Phyalophora sp.* in germinating *B. cinerea* and found an inhibition between 85% and 90% (19). Another study work with ethanol extracts (25%) of four plant species: plantain (Plantago major L), rue (Rutagraveolens L), prompt relief [Lippiaalba (Mill) NEBROS] marranero fern [Pteridiumaquilinum (Kaulf) Maxon] found that decreased the development of fungi *Botrytis cinerea* and *Colletotrichummusae* up to 68% of the growth (20). This study found that for *C. fragariae*, inhibition was greater than 50% and only 40% for *C. gloeosporioides.* At work with living organisms control (biocontrol) of Carrillo *et al.* (2005) tested the effectiveness of *Bacillus subtilis* and*Rhodotorullaminuta*against *C. gloesporoides* in mango; found that applications of these two microorganisms reduce anthracnose allowed up to 86.7% in the fruits.

Conclusions

Succeeded in reducing the growth of phytopathogenic fungi in vitro postharvest using crude extracts of orange pigment *P. sanguineus*. *B. cinerea* growth reduction was nearly 70 and 60% for treatments with 10 and 20 mg/mL, respectively. For *C. fragariae*, inhibition was greater than 50% and only 40% for *C. gloeosporioides.*

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Estimation Of The Physicochemical Properties Of The Valencia Orange Using Digital Images Analysis

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Abstract

The texture of an image is an important characteristic in the analysis of images due to that it represents a spatial variation in the brightness spectrum of the digital image. The correlation between properties was carried out during this work; such as color and texture with physicochemical properties estimated by analyzing the images of this kind of orange. To achieve this objective a lot of 25 oranges was selected and analyzed from which a digital image was taken to each orange . such images went through a process of segmentation and conversion of the model of the color RGB to CIEL L*a*b*, as well as it was gotten the measurement of Entropy and Energy to estimate textural characteristic which allowed to correlate them with physicochemical color properties, texture, PH, Bx, and hardness obtained through laboratory methods.

Sensorial characteristics of critics relate in a meaningful and direct way into the physicochemical properties, determining with this an evaluation alternative that allow us to shorten the times, and above all to avoid the use of destructive tests for quality criteria assessment.

Color and texture are major characteristics in critics and they can be factor of analysis through computer techniques and be applied in the food industry for the selection and evaluation of quality through the analysis of images.

Introduction

Image analysis through computer is a job whichis carried out in different industryfields ,for example in the health field there have been meaningful scientific advances going from molecular analysis to the detection of substances in organisms.

In the agricultural fields it is important to stand out recent works in the precision agriculture during the harvesting and post-harvesting stages in which state of the art technologies are used involving vision systems through computer for the analysis of physical properties , for example, vision system for the location of fruits in trees, where efficiency is a requirement achieved through vision sensors through computer [1], we find areas of opportunity in the packaging systems during the post-harvesting, in which physical properties are considered one of the most important variables in the agriculture products [2], at the end the quality of fruits and vegetables is defined by a wide range of characteristics like ripeness, size, shape, color and the presence of spots and damages that make fruit to be attractive or unattractive to consumers. [3]Artificial vision systems have become a powerful tool for automatic fruit inspection [6], through the estimation of external characteristics and internal parameters.

This work presents the results in the application of Images analysis through computer in the citric industry, in which meaningful advances have already been shown for the selection and evaluation of quality [3,6] Moreover, here it is shown another viable alternative to contribute even more to the evaluation and selection of oranges.

The methodology that was developed corresponds to a general model in the digital images analysis, the adjustments to the model in this project were retaken from works made in the image analysis of fruits [4,7,8] where it has been proved the efficiency on the implemented technique.

Method and Materials

Oranges used for the sampling were obtained form a local market in the Xalapa, Veracruz region.30 oranges were selected (Valencia kind) with different color schemes which went from the greenest to the

most orange in color. Also, it was taken into consideration that the oranges were of different sizes and had different levels of pitted texture in the oranges peel.

Image acquisition.

Imageswere takenwith a 10.0 megapixels digital camera(CCD), Power Shot model A 640 (Canon, USA 2006) which was set vertically on a bottom surface where sample was put 22 centimeter far. As a standard conditions for the image capture, images were taken on a black surface with the following camera settings; manual mode, without zoom, without flash, imageresolution at 3648 x 2736 pixels. Images were stored in a JPEG format. To get and visualize images directly, the camera was connected to the USB slot of a laptop Architecture Sony Vaio EBSerie with 64 bits, 4 gigabytes of memory RAM and Intel core processor.

Software

Pre-processing algorithms were implemented, segmentation, estimation on textural characteristics was carried out through the result of entropy and energy, such algorithms were implemented in the C programming language with the compiler gcc 4.1.2 and graphic libraries OpenGL Mesa 7.3, on an operative system Slackware Linux version 12.1

Image pre-processing.

Original color images taken by the camera CCD were made into monochromatic images (to a scale of gray colors). The image conversion to a scale of gray colors is essential to carry out the implementation of the textural analysis algorithms, so it is necessary to do this procedure first. For this, the image was analyzed completely, estimating in each pixel the intensity of gray that corresponds accordingly to the following illumination equation[5]:

$$I = 0.3Ir + 0.59Ig + 0.11Ib$$

Where Ir , Ig y Ib correspond to the values of the intensities of the red, green and blue components, respectively.

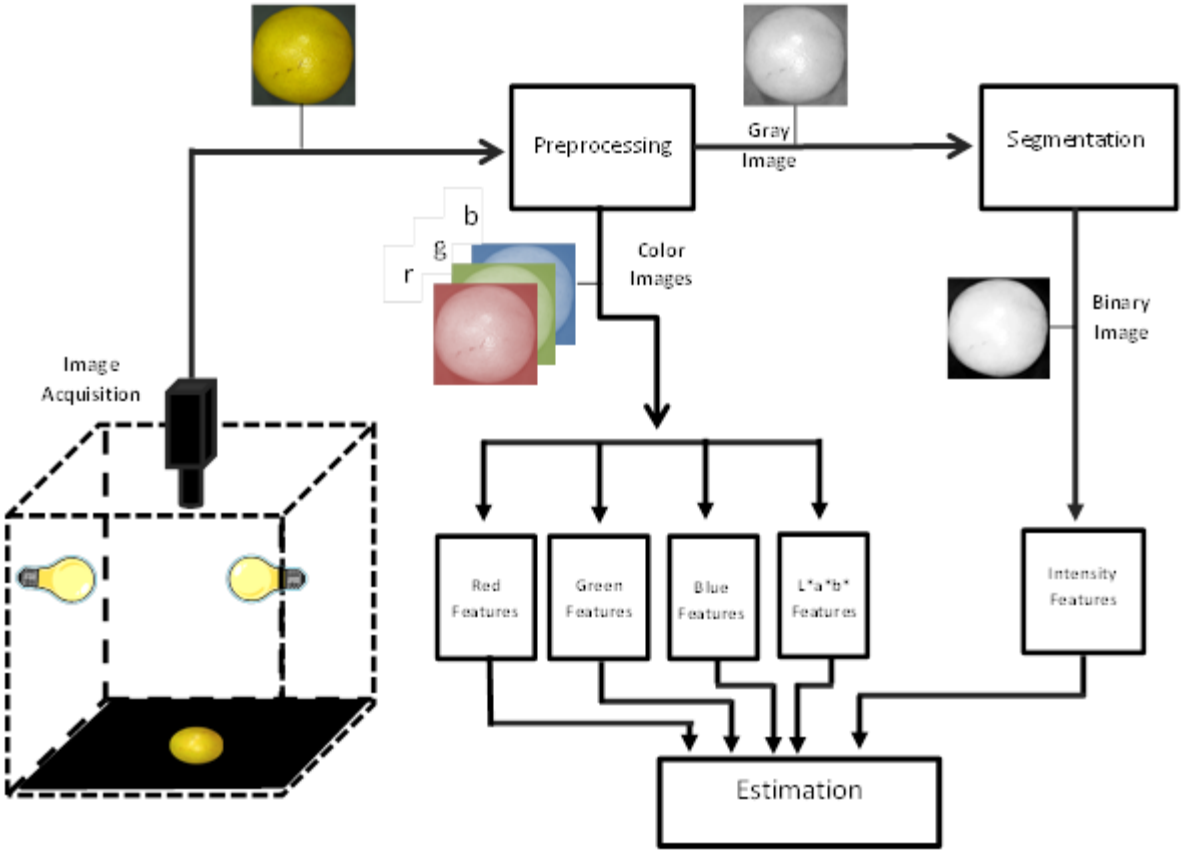


Figure 1.General scheme of theimage analysisof theOrange.

Estimation of Textural Characteristics.

Entropy and energy are calculated from the image histogram on scale of gray colors. The histogramshape of an image shows several useful clues to determine the shape of an image.

Results and Discussion.

A lot of 30 oranges wasanalyzed through diverse tests and statistics parameters. Results on entropy and energy provide us with valuable information on the texture of digital image due to that they are directly related one to another (figure2) Thus, the texture in a digital image with more entropy shows evidence of a texture with diverse imperfections (Figure 3.b) On the other side, in an image of a texture without spots entropy tends to decrease.

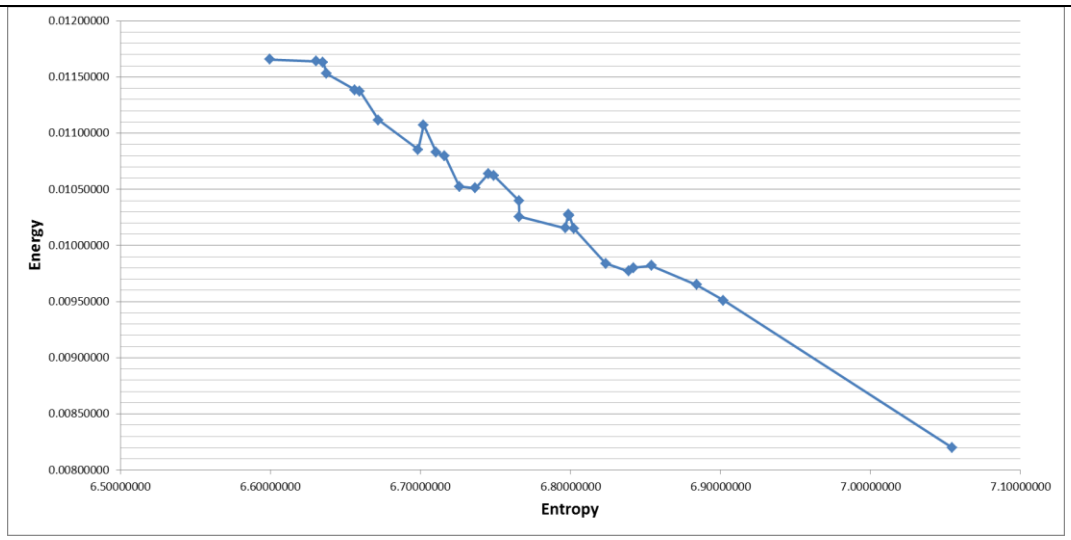
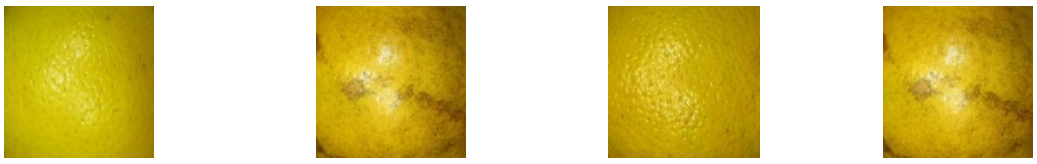


Figure 2. Representation of entropy and Energy calculus

Energy calculus provides such valuable information that texture of digital image with the most energy shows a texture free of spots (Figure 3.c) and a texture with any kind of imperfection shows less amount of energy. (Figure 3.d)



(a) Entropy = 6.599 (b) Entropy = 7.054 (c) Energy = 0.0116 (d)Energy = 0.0081

Figure 3.Maximum and minimum value of entropy and energy calculus in the lot of oranges

The following graphic shows that the orange epidermis presents two characteristic peaks, the first one is due to the outside and most external layer which presents a behavior shown in Figure 4. In which as the penetration distance increases, the force is still. As for the second peak, shows a lineal behavior (R=0.825) until reaching a maximum which is directly proportional to the penetration distance and hypodermis thickness. This is possible due to the distribution of the oil glands spot on the orange outside layer/ exocarp.

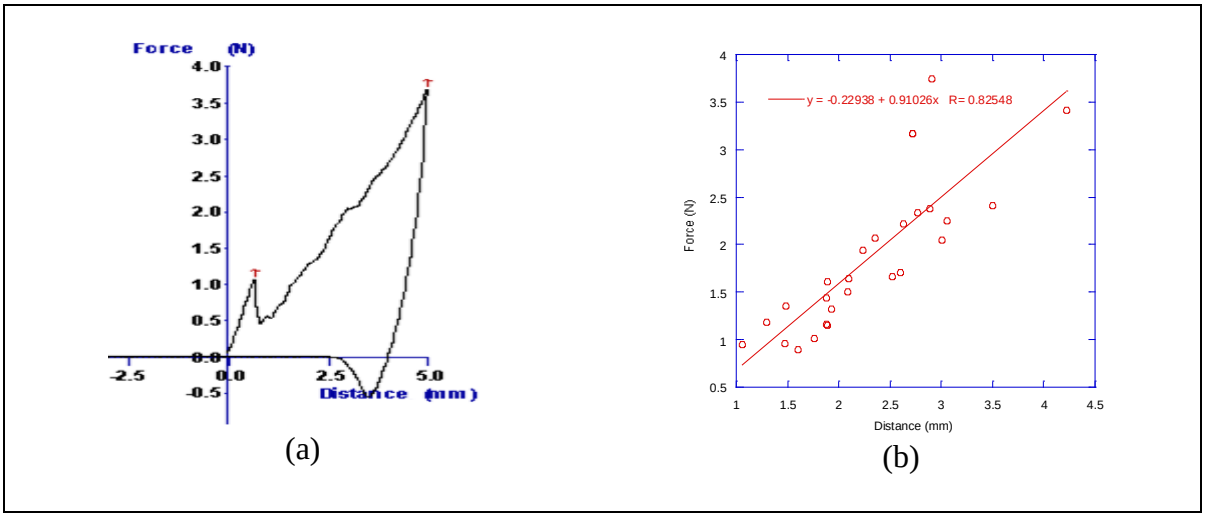


Figure 4.**(a)** Orange penetrometer test using a 2 mm needle **(b)**. Relation between the necessaryforce and de distance of the orange hypodermis (*Citrus sinensis*var. Valencia).

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Effect Of 1-Methylcyclopropene On Firmness And Translucency Of 'Maradol' Papaya Fresh-Cut

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Introduction

Papaya (Carica papaya L) is a tropical fruit with nutritional and digestive properties.Mexico has the second highest worldwide in production after Brazil with952, 194 ton (1),this fruit is very important in the Mexican economy. It is grown mainly in the state of Veracruz, Chiapas, Michoacán, Tabasco, Guerrero, Yucatan, Oaxaca y Nayarit (2). A feasible way to increase the value and marketingof this fruit is processing it as fresh-cut product. The worldwide consumption of these products has increased but their relatively short shelf life is a barrier for commercialization.The increase in consumption and demand for fresh-cut products has impulsed to the researchers in the design and implementation of methods that improve and extend the quality of these highly perishable products. In fresh-cut papaya, the main problem is the softening and increased translucency. The 1-methylcyclopropene (1-MCP) has been successfully applied before or after processing in fresh-cut fruits. The aim of this study was to evaluate the effect of 1-MCP on the firmness and the translucency of fresh-cut 'Maradol' papaya with additives.

Materials and methods

The mature papaya was washed in chlorinated water (200 ppm) and it was air-dried. All the fruits, before cutting,were sprayed with hydrogen peroxide (2%, v/v)to remove impurities. The papaya was cut out transverselyin slices and the slices measured 1x5x2.5 cm (thickness x length x width).In all the slices was applied, by immersion,calcium chloride (1%, v/v) for 2 min, after was appliedisoascorbic acid (0.1% v/v) and sorbic acid (0.05%, v/v) by aspersion.The treatments were as follows:

1. Control (T1),slices with additives stored in polystyrene containers at 5 ° C.
2. Slices with 1-MCP (T2):the application with1-MCP (1000 ppb) was in chambers 12 Lfor 12 h at 10 ºC, then slices were sprayed with additives and stored in polystyrene containers at 5 ° C.
3. Whole fruit with 1-MCP andfresh-cut (T3):the whole fruits were treated with 1-MCP (1000 ppb) for 12 h at 23 ºC, immediatelyit was fresh-cut, with additives and stored in polystyrene containers at 5 ° C.
4. Whole fruits with 1-MCP, fresh-cut andslices treated again with 1-MCP (T4). The application of 1-MCP and storage of slices were as mentioned above.

Textural firmness. Resistance to penetration was measured on both sides of the slides with a manual Shimpo penetrometer model FGV-100. Firmness was measured in three different points: one in the centre and one on each end of the slice, on both sides. Mean values were calculated and expressed in Newtons (N).

Pectinmethylesterase (PME) activity.Enzyme extracts were prepared at 4 °C and pectinmethylesterase activity (PME) was based on the colour change of a pH indicator during the PME catalysed reaction (3). This change was continuously monitored by a spectrophotometer at 620 nm. Substrate consisted of 2 ml of 10 g pectin/l (of distilled water), 0.3 ml of bromthymol blue and 0.25 ml of deionized water. Substrate was incubated at 37 °C for 15 min, its pH value was adjusted to 7.5 and 0.1 ml of enzyme extract was added, allowing the reaction to proceed for 60 min. One unit (U) of PME was defined as the production of 1 µmol H+/h at 37 °C. PME was reported as U/mg protein (4).

Polygalacturonase (PG) activity.Extraction was performed at 4 °C according to Lazan et al. (4). To test for activity, 0.2 ml of 4 g/kg solution of polygalacturonic acid were mixed with 0.4 ml of acetate buffer 0.05 M at pH 4, and incubated at 37 °C for 20 min. Then, 0.2 ml of enzyme extract were added and

allowed to react for 3 h. The reaction was stopped by adding 2 ml of sodium borate buffer (100 mM) and 0.6 ml of a solution of 1 g cyanoacetamide/l (of distilled water) heated in a water bath for 20 min, then cooled quickly with ice. Absorbance was measured on a GBC spectrophotometer at 276 nm. One unit (U) of polygalacturonase activity (PGa) was defined as the production of 1 µmol of D-galacturonic acid/h at 37 °C. PGa was reported as U/mg of protein (5).

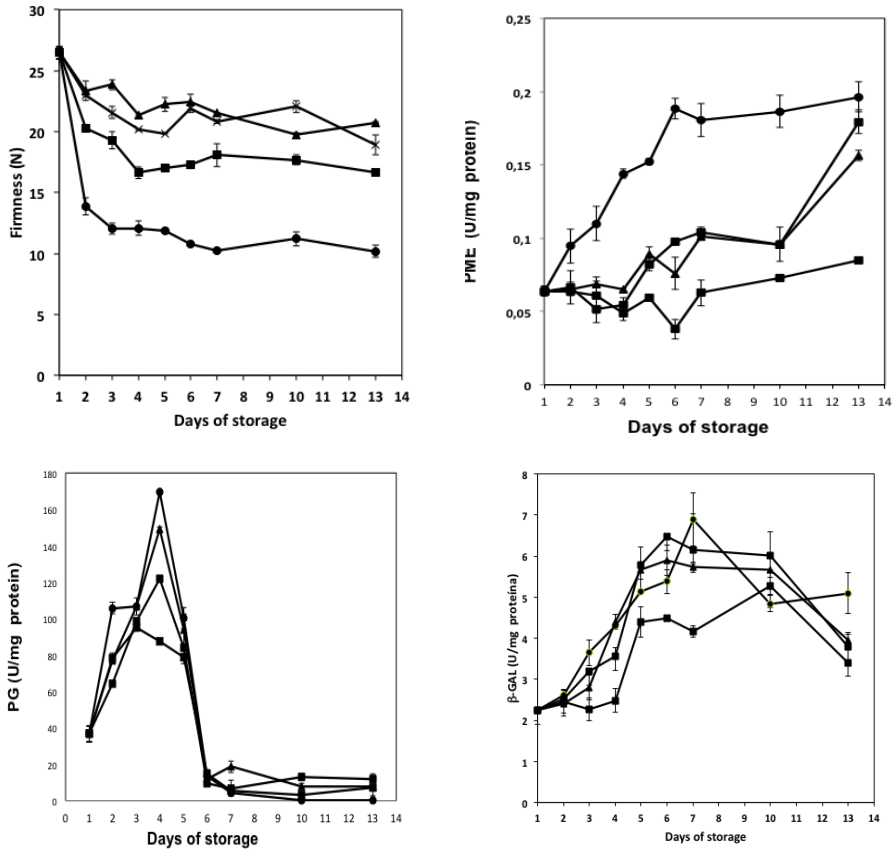
β-galactosidase (β-GALactivity. Extraction was performed at 4 °C according to Lazan et al. (3). The reaction mixture consisted of 0.52 mL sodium citrate buffer 0.1M (pH 4.1) and 0.4 mL of p-nitrophenyl-β-D-galactopyranose 13 mM as substrate. This mixture was incubated at 37 ° C for 10 min, then was added 0.08 mL of enzyme extract and allowed to react 15 min at the same temperature. To stop the reaction was added 2 mL of 0.2 M sodium carbonate P-nitrophenyl groups formed were determined by measuring the absorbance in a spectrophotometer at 415 nm mark Jenway. One unit (U) of b-galactosidase activity was defined as the production of 1µmol of p-nitrophenyl/h at 37 ºC.

Translucency.It was measured with RED TIDE USB 650 (Ocean Optics) spectrophotometer provided with optical fibers both sides of slides.

Statistical analysis. Data were analyzed by ANOVA using the statistical package SAS (The SAS System for Windows). Mean comparison was made by the least significant difference (LSD). All tests of significance were at the 0.05 significance level.

Results and discussion

The loss of firmness of control slices was observed (fig. 1). It has been reported that loss of firmness in fresh-cut fruits is attributed to the rapid changes for the damage caused to cells during cutting or peeling (6). In T2, the 1-MCP delayed loss of firmness. The combination of 1-MCP/$CaCl_2$ delayed the ripening process in fresh cut watermelon and this is attributed to the retention of firmness and lower hydrolytic enzyme activity in relation to control (7). T3 and T4



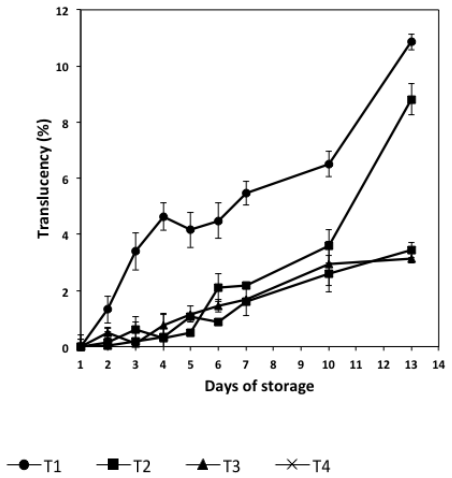


Figure 1.Firmness, pectimethylesterase (PME) activity, polygalacturonase (PG) activity and b-galactosidase activity of fresh cut papayacontrol (T1), fresh cut papaya with 1-MCP (T2), whole papaya with 1-MCP and then cut (T3) and whole papaya with 1-MCP and then cutwith 1-MCP (T4). Data points represent the mean of four measurements and their standard deviation.

treatments decreased by approximately 50% loss of firmness of papaya slices throughout storage. Results similar to those reported bySaftner et al. (8).

T1 presented the highest values of the PME, PG and β-GAL activity during storage; it coincided with the rapid loss of firmness (fig. 1). This is attributed to that activity of the enzymes could increase by cutting (8). The treatment with 1-MCP after cutting (T2) had significant effect to reduce enzyme activity, while the application of 1-MCP in the whole fruit and then these were cut without 1-MCP (T3) and 1-MCP (T4) decreased the activity of the enzymes. This is likely due to that application of 1-MCP in the whole fruit may remain for longer in the cells (9).

Translucency was increased regardless of treatment, but with significant differences between them ($p<0.05$) and none was greater than 15% of translucency, a percentage that is considered commercially unacceptable (10). In T1 (fig. 1c) was observed a higher percentage of translucency and at day 13 of storage there was 10.84%. Portelaand Cantwell (11) concluded that the severity of the cut affects the development of translucency. In T2 the development of this physiological disorder was slower (8.81%).

In treatments T3 and T4 also decreased significantly translucency, to the last day of analysis were 3.15 and 3.44%, respectively. This could be attributed to the application of 1-MCP could slow this physiological disorder.Ergun et al. (12) observed that on the fifth day of storage at 5 ° C, the minimally processed melon, not treated with 1-MCP had higher ethylene production that whole fruit treated.

Conclusion

We conclude that the 1-MCP had a significant effect ($p <0.05$) in firmness, with lower activity of enzymes β-galactosidase, polygalacturonase and pectinmethylesterase and translucency decreased significantly.

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Analysis Of Some Mechanisms Of Inhibition By Chitosan In Cell Germination Of *Colletotrichum* sp. Isolated From Banana Fruits (*Musa sapientum*)

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Introduction

In Nayarit, the agricultural sector represents an important part of its economy, but this production is affected by various postharvest diseases caused by pathogens, which reduce the quality of the fruits and generating economic losses. Some of the pathogens that cause a number of diseases is*Colletotrichum* sp. which has the ability to penetrate the fruit, established and developed until the fruit has the characteristics necessary. One of the main events involved in the infection process of this fruit is germination. At present, the methods for controlling this disease are the chemical methods, mainly fungicides, which pollute the environment and develop resistance to pathogens. Therefore it is necessary the development and understanding of new control methods such as chitosan, which shows a high inhibition of the germination process different pathogens without causing alterations both fruit quality and the environment. Such inhibition is favored by their different mechanisms of action, but these mechanisms are not fully tested. Because little information exists about the mechanisms of action of chitosan and reports generated on both structural and genetic changes that occur in the fungus through its application is necessary to study the mode of action of chitosan in the events present during the inhibition of the fungus. Therefore, the present study is to analyze one of the mechanisms of action of chitosan involved in the pathogenic processes of *Colletotrichum*sp banana.

Methods and materials

Banana fruits were collected in Nayarit, México in physiological ripeness. The fruits were washed with water and disinfected with a solution of 2% NaClO for 1 min. We performed a spore suspension with *Colletotrichum*sp strain, adjusted to a concentration of $1x10^6$ ml^{-1} using a Neubauer chamber. Chitosan solutions were prepared: 0.5, 1.0 and 1.5%, dissolving low molecular weight chitosan (LMWC) in distilled water (w/v) with acetic acid, stirred for 24 h and Tween 80 was added. Chitosan solutions adjusted to pH 5.5 with 1N NaOH (2). The treatments were inoculated with 50 µl of the spore suspension previously adjusted, and allowed to dry for 30 min in sterile area.For fluorescence microscopy of a disc was taken from each of the treatments (0, 0.5, 1 and 1.5% chitosan), placed on a slide and inoculated with 50µlspore suspension, then were added 20 µlof Calcofluor White. Was placed in a fluorescence microscope coupled to mark DM6000B Leica CTR 6000 BA300 source structure and determining fluorescence intensity of each of the treatment and its possible structural changes was observed. In the evaluation of pH of the medium to Falcon tubes, was added 10 ml of Potato Dextrose Broth plus chitosan at different concentrations (0, 0.5, 1.0 and 1.5% of LMWC), and inoculated with 100 µl of spore suspension. Were incubated at 25°C with constant agitation. We evaluated the pH of the medium in a short time (0, 2, 4 and 6 h) and prolonged time (0, 24, 48 and 72 h) with a potentiometer 300 brand pH Hanna Instrument. For enzymatic activity of PG, we proceeded to the Grant of crude extract (5) 10 g of frozen fruits were homogenized with 30 ml of buffer solution of sodium acetate / acetic acid 0.05 M, PVPP 1% w / v (pH 6.0). The mixture was centrifuged at 12,000 xg for 30 min and the supernatant was discarded. The solid residue was washed with 30 ml sodium acetate / 0.05M acetic acid (pH 6.0) and then centrifuged at 12,000 xg for 30 min. The supernatant was discarded and the wash step was repeated once more. The solid residue from the second wash was extracted with 20 ml of sodium acetate / 0.05M acetic acid (pH 6.0), 1 M NaCl with stirring at 4 ° C for 3 h. Was centrifuged at 12,000 g for 30 min and the supernatant was dialysed for 14 h against 20 volumes of solution of sodium acetate / acetic acid 0.05 M,

pH 6.0. The dialyzed extract was used to determine the overall polygalacturonaseactivity, this was done in test tubes by placing 2 ml of the solution 0.2% polygalacturonic acid plus 0.4 ml of 0.2 M acetate buffer at pH 4.4 and incubated for 10 min at 37 ° C. Then added 1 ml of the enzyme solution and reacted at incubation for 20 min at 37 ° C. Reaction of 100 ul were taken and added 3 ml of borate buffer 0.2 M sodium cyanoacetamide and 100 l of 1% to stop the reaction, then heated in a water bath for 10 min, cooled, and finally the absorbance was taken in a spectrophotometer at 276 nm. The quantification of the enzyme activity of the PG was performed using a calibration curve of galacturonic acid, with the readings obtained chart was performed absorbance (276 nm) vs. concentration (micron galacturonic acid) was obtained from the equation the line and the slope was used to determine enzyme activity, and subsequently expressed in U / ml.

Results and discussion

Figure 1 shows the effect of chitosan on *Colletotrichum*spin fluorescence microscopy. As can be seen the affected Chitosan and fluorescence intensity which is different in each of the treatments. For the control, the fluorescence intensity is higher than in all treatments where to Chitosan concentration increased, considerably decreased intensity. The shape and size of spore was changed in each of the treatments (Fig. 1a). Figure 1b shows that chitosan inhibited spore germination in all treatments and prevented the growth of the germ tube, showing complete inhibition at higher concentration treatments. For the greater was observed in both fluorescence intensity and in the spore germination tube, being higher in this, this may be because the tube be forming spore germination of the cell wall, intact.

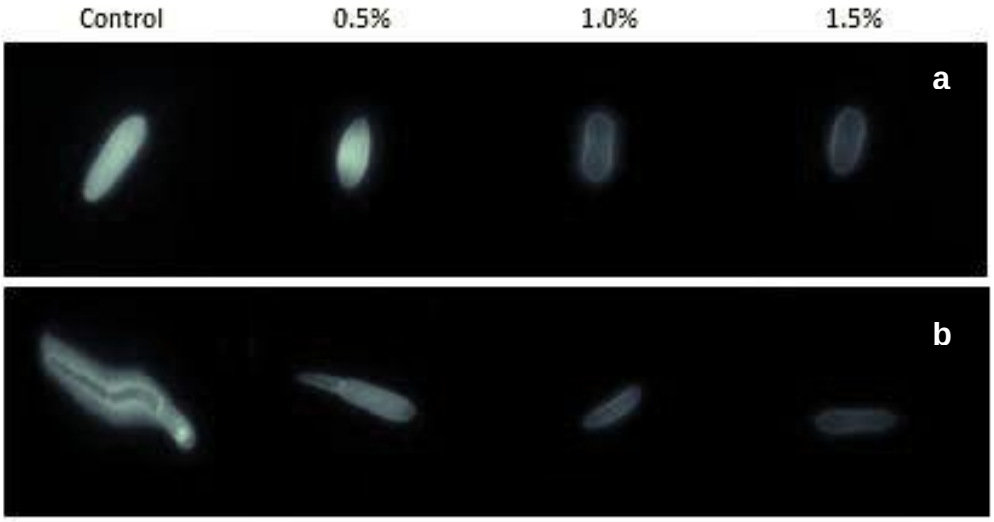

Figure 1.Effect of Chitosan in the fluorescence intensity of the spores of Colletotrichum sp. a) shows the fluorescence intensity as well as the structure of the spores, b) shows the effect on the fluorescence intensity and germination.

The fact that Chitosan alter the intensity and the structure of the spores is associated with the alteration of the permeability of the resulting membrane pores therein, which causes a lower affinity to Calcofluor to the wall.It was reported that chitosan binds to lipopolysaccharide cell plasma membrane changing its structure by removing the metabolic activity due to nutrient exit generating consequently cell death(4). Regarding the effect of chitosan and pH of the medium can be seen in Figure 2, the effect of chitosan on the pH of the medium at short and long times, showing that chitosan affecting the pH of the medium in all the concentrations applied over all measurement times. For short times, the greatest effect is shown at concentrations of 1.0 and 1.5% no statistically significant difference between the two, with values at the end of the 6 h of 5.53 and 5.59 respectively. As the above concentrations treatment with a 0.5%

concentration increased exponentially LMWC pH of the culture medium with a final value at 6 h of incubation of 5.4 although the increase was less as compared to concentrations of 1.0 and 1.5 % of LMWC showing statistically significant differences with these treatments. The control treatment (uninoculated) kept the same pH of 5.24 to over time. Chitosan treatment without inoculated with *Colletotrichum* sp. tended to decrease the pH of the culture medium with an initial pH of 5.24, at the end of the incubation the pH was 4.62. At different incubation times of this treatment the pH was lower compared to treatment with chitosan. Changing the pH of the medium was prolonged very similar to above, showing the greatest effect on the concentration of 1.5% LMWCwith values at the end of the 72 h of 6.21. The treatments with concentrations of 0.5 and 1.0% LMWC also able to increase the pH of the medium to a lesser extent, in comparison with the 1.5% concentration, existing statistically significant differences among the treatments. Control behavior was the same until the end of analysis.
In the case of the absence of treatment with chitosan showed a decrease of the medium pH values of 4 to 72h term trends showing alkalizing medium.

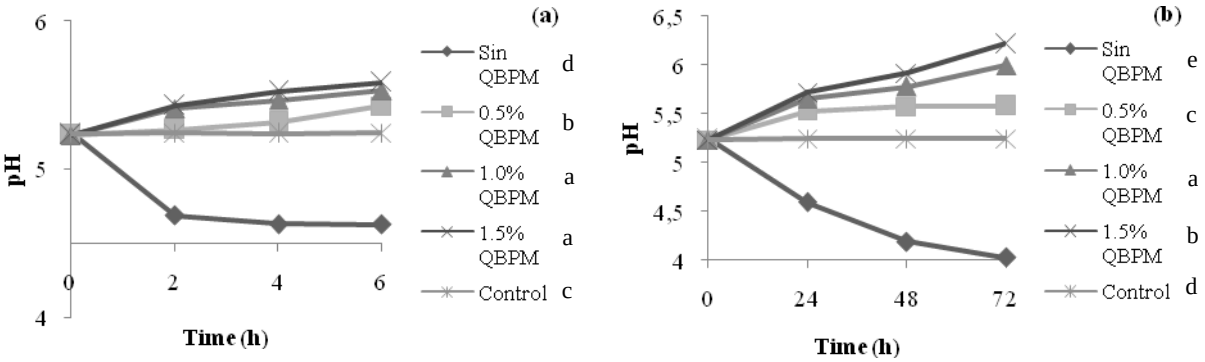

Figure 2.Effect of chitosan on the change in pH of the medium.a) in short time periods (0, 2, 4 and 6 h), b) periods of time (0, 24, 48 and 72 h).

This may be because the chitosan to be in contact with the fungus induces major changes in its integrity and alterations in their metabolism which is reflected very clearly in the pH of the culture medium. One possible explanation for this phenomenon could be due to the departure of basic molecules of low molecular weight peptides primarily, potassium, ammonium, etc.., As a defense mechanism against phytopathogenic stress of chitosan. Similarly, it has been observed that treatment ofNeurospora crass hyphae with antifungal defensins (cationic molecules) induces alkalinization of the incubation medium and inhibits fungal growth (6). Some authors, found a similar effect in evaluating the effect of chitosan on *Rhizopusstolonifer* plasma membrane (3).Theeffect of chitosanontheenzymatic activity ofPGcan be seenin Figure 3, chitosan modifiestheenzymatic activity ofPG, decreasing itsactivityinall treatmentsin the presence ofchitosan, being themost effectiveconcentrationsof1.0%and 1.5% LMWCshowingnostatistically significant differencebetween them.

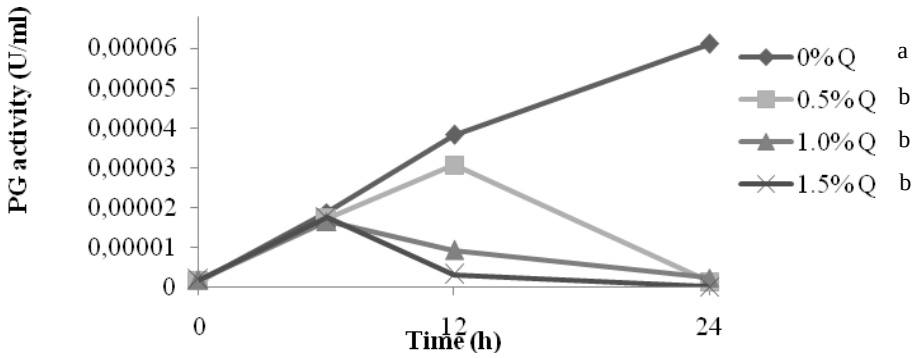

Figure 3.Effectof chitosan onthe enzymatic activity ofpolygalacturonase(PG)

This decrease in activity may be due to the PG that chitosan affects processes pathogenesis of *Colletotrichum* sp. necessary for reproduction. The PG in the fungus is responsible for breaking the cell wall of the spore to make way for the generation of the germ tube. This enzyme cleaves the chains cell wall peptic adding a water molecule and hydrolyzing the bond between two galacturonic acid molecules to achieve degrade the cell wall (1).This decrease inactivityof thePGmay be due that chitosanmodifiedprocessespathogenesisof*Colletotrichum*sp. affectingthe expression ofPGsynthesisby interfering withmRNAor thetranslationprocessof this enzymealtering the permeability ofthe cell wall of*Colletotrichum*sp. (3)

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Effect Of Chitosan In The Induction Of Some Enzymes And Gene Expression Related To Defense And Protection Against *Colletotrichum* sp In Mango (*Mangiferaindica* L.) Tommy Atkins

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Introduction

In Nayarit, the mango fruits are affected by various diseases that reduce the quality and quantity production, causing economic losses, highlighting its importance, anthracnose, a disease caused by *Colletotrichum gloeosporioides* (1). A new method for controlling pathogens, is to induce the defensive mechanisms of the fruit by resistance elicitors, been one of the most relevant chitosan (2). Induced resistance is a defense involving differential gene expression and metabolic changes that occur as a consequence of a specific recognition between the plant and the pathogen (13). The main redox enzymes that are involved in these signaling processes are peroxidase (POD) and Polifenoxidasa (PPO). Assumed the economic importance of the mango as an export product for Nayarit, the application of chitosan emerges as a potential treatment for the control of pathogens, however, the mechanism of action of chitosan has been little studied, so is necessary to study the events occurring at enzymatic and genetic level to have a more complete understanding of the total process. For the foregoing reasons, this project aims to evaluate the effect of chitosan on the induction of defense enzymes and gene expression as one of the mechanisms of action of chitosan in theinteraction *Colletotrichum*sp and mango fruits c.v Tommy Atkins.

Methods and materials

Mango fruits (Tommy Atkins) were collected in Atonalisco (Nayarit, México) in physiological ripeness. The fruits were washed with water and disinfected with a solution of 2% NaClO for 1 min. We performed a spore suspension with *Colletotrichum*sp strain isolated from mango, adjusted to a concentration of $1x10^6$ ml^{-1} using a Neubauer chamber. Chitosan solutions were prepared: 0.5, 1.0 and 1.5%, dissolving low molecular weight chitosan (LMWC) in distilled water (w/v) with acetic acid, stirred for 24 h and Tween 80 was added. Chitosan solutions adjusted to pH 5.5 with 1N NaOH (7). Those mangoes were inoculated with 20 µl of the spore suspension previously adjusted, and allowed to dry for 30 min in sterile area. Were subjected to an immersion treatment at different concentrations of chitosan for 1 min, allowed to dry for 1 h. Tissue sections were cut from all treatments using a sterile scalpel, wrapped in aluminum foil, frozen with liquid N_2 and stored at -80°C. For enzyme activity, we proceeded to obtain the crude extract (9). Were grounded 2 g of tissue of each treatment with liquid N_2 and homogenized with 10 ml of 100 mM Na_3PO_4 buffer (pH 6.4), and 0.2 g of polyvinylpolypyrrolidone (PVPP) at 4°C. Was centrifuged at maximum speed for 30 min at 4°C. The supernatant was taken and this was again centrifuged at the same conditions. Finally the pellet was discarded, and the supernatant was kept on ice as enzyme source. For the POD activity, the reaction mixture included 0.5 ml of the crude extract and 2 ml of buffer guaiacol. It was incubated for 5 min at 30°C. The reaction was stopped by adding 1 ml of H_2O_2 (24 mM), and enzyme activity was determined in a UV/Visible spectrophotometer, by measuring the absorbance at 460 nm (8). In the PPO activity of the reaction mixture consisted of 0.5 ml of crude extract, 3 ml of catechol as substrate and measured the absorbance at 420 nm (15). Enzyme activity was expressed as Umg^{-1}protein. For RNA extraction, were grounded 0.25 g of frozen mango tissue in a mortar with liquid N_2, were added 4 volumes of lysis buffer and vortexed for 1 min. Homogenate were added 0.25 volumes of absolute ethanol and 0.11 volumes of 5 M potassium acetate. Was vortexed for 1 min, followed by extraction with

one volume of chloroform:isoamyl alcohol (49:1) and centrifuged at 15,000 rpm for 10 min at 4°C. The aqueous phase was recovered and extracted with one volume of phenol:chloroform:isoamyl alcohol (25:24:1), and centrifuged under the same conditions. The aqueous phase was recovered and proceeded to another extraction with a volume of chloroform:isoamyl alcohol (49:1). The aqueous phase was removed,and the RNA was precipitated withLiCl (3 M final Concentration) at -20°Covernight. RNA was collected by centrifugation (15,000 rpmfor 60 min at 4°C). The liquid was discarded and the pellet was washed with 500 µl of 70% ethanol. The pellet was resuspended in sterilemilliQ water and stored at -20°C until use (20). For analysis of gene expression by RT-PCR, the RT reaction was performed using the SuperScript III kit. The reaction mixture consisted of 1 µl of dNTPs, 1 µl of oligo dT_{18}, and the rest of DEPC water (final volume 10 µl). And a second prepared mixture with 2 µl of 10X reaction buffer, 1 µl of RNaseOUT, 2 µl of 0.1 M DTT, 4 µl of 25 mM $MgCl_2$ and 1 µl of enzyme SuperScript III. The PCR products were evaluated by electrophoresis on agarose gel 1.2%, and was run in 1X TAE buffer at 80 volts for 90 min. The RNA gel was stained with GelRed. For enzyme activity, was used a randomized complete block design with a 2x4 factorial arrangement. The results were analyzed statistically by analysis of variance (ANOVA). The comparison of means was done with LSD test (P <0.05), using the SAS System 9.0.

Results and discussion

In Figure 1, we see the fruit inoculated with *Colletotrichum* sp. It can be seen that at concentrations above 1.0% chitosan, was controlled anthracnose disease. Chitosan can form a film on the surface of the fruit, to protect the mango from pathogen infection (6).

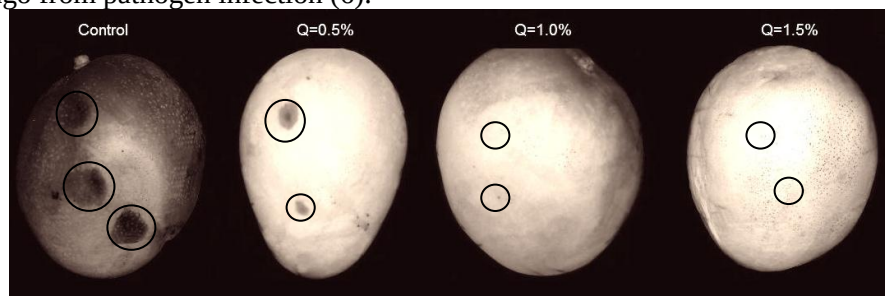

Figure 1. Effect of chitosan at different concentrations on mangoes fruit, 72 h after applying treatments, stored at 25°C.

The effect of chitosan on POD enzyme activity without pathogen inoculation, were statistically significant differences between treatments (P <0.001), seeing that in early stages of fruit ripening, increased POD activity (Figure 2A), with the highest enzyme activity at 24 h, the 1.0% concentration of chitosan exhibited the highest enzyme activity.

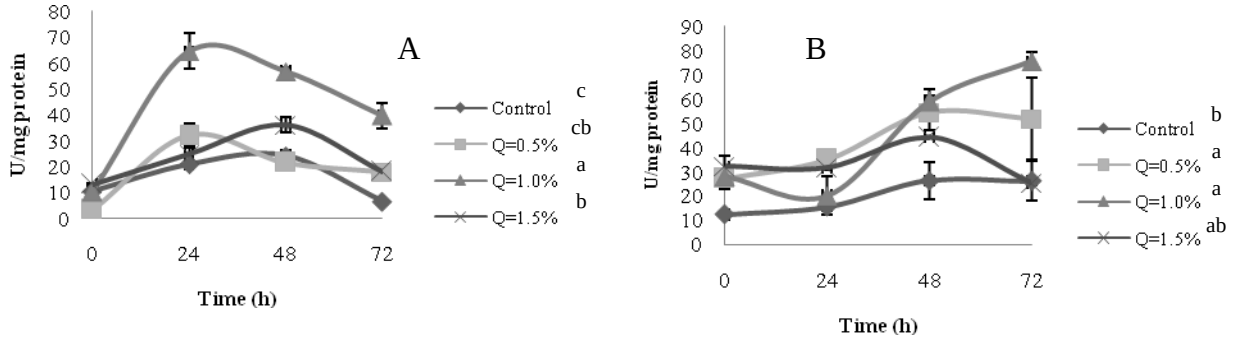

Figure 2.Effect of chitosan on the enzymatic activity of peroxidase (POD) in mango c.v Tommy Atkins without inoculation of *Colletotrichum*sp (A), and with inoculation of *Colletotrichum*sp (B).

In the treatments with *Colletotrichum*sp inoculation, it was observed POD increased over time (Figure 2B), having statistically significant difference between treatments (P <0.001). The concentration of 1.0% chitosan was more effective, achieving the most induction of the

enzymatic activity of POD, showing maximum activity at 72 h. This may be because when the pathogen is inoculated, chitosan can form physical barriers around the sites of penetration of the pathogen, preventing spread of disease (8). Other studies found that applying chitosan to fruits inoculated with *Botrytis cinerea* increased POD activity (3, 11). Regarding the effect of chitosan on the enzymatic activity of the PPO without inoculation of *Colletotrichum*sp, showed an increase in this enzyme over time in all treatments, observing a significant difference (P <0.001) yielding the best results the 1.0% concentration of chitosan (Figure 3C), showing the highest enzyme activity at 72 h.

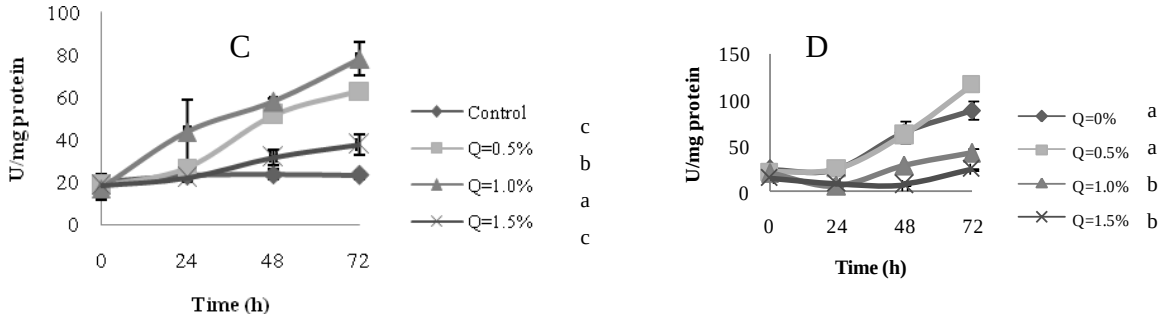

Figure.3. Effect of chitosan on the enzymatic activity of polyphenol oxidase (PPO) in mango cv Tommy Atkins without inoculation of *Colletotrichum*sp (C), and with inoculation of *Colletotrichum* sp. (D)

In the treatments with *Colletotrichum*sp inoculation, we observed an increase in enzymatic activity over time, being the 0.5% concentration that achieved further induce the PPO, showing the highest activity at 72 h (Figure 3B). There wasn´t significant differences from control (P <0.001). This may be due to the chitosan forming a film on the surface of fruit, reducing the respiration rate and delaying ripening, suggesting that perhaps at concentrations above 0.5%, the fruit entered a process of preventing the induction of stress the PPO as a defense mechanism. In the analysis of gene expression, RNA was extracted from the 8 samples, showing that the bands are well defined, suggesting that RNA is not degraded and has good integrity (Figure 4).

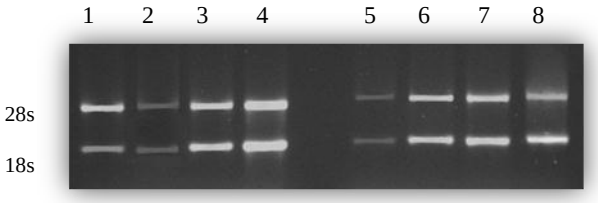

Figure 4. Gel electrophoresis of total RNA extraction from all samples. Lanes 1-4 treatments without inoculation; lanes 5-8 pathogen inoculation treatments. Lane 1, Q = 0%, T = 0 h, lane 2, Q = 1%, T = 0 h, lane 3, Q = 0%, T = 24 hr, lane 4 Q = 1%, T = 24 hours , lane 5 , Q = 0%, T = 0 h, lane 6, Q = 1%, T = 0 hrs, lane 7, Q = 0%, T = 24 hr, lane 8 Q = 1%, T = 24 h.

In the gene expression encoding the PPO, (Figure 5), it can be appreciated that in those without inoculation treatments, 1.0% chitosan was able to induce gene expression of the PPO at 0 and 24 hours, suggesting, this biopolymer which acts from the time of application, from an early stage can interact through a signal transduction mechanism. In those fruits that were inoculated with *Colletotrichum*sp, 1.0% chitosan showed higher expression in the PPO at 0 hours, in contrast, at 24 h, chitosan remained the same expression, and the fruits inoculated showed increased expression. This may be because the pathogen-host interaction, the fruit sends signals as a defense mechanism to induce gene expression of enzymes. This suggest that PPO gene expression induced by chitosan behaves constant throughout the processto attack by *Colletotrichum*sp, as an ignition system for a prolonged time which allows the fruit's defense pathogen.

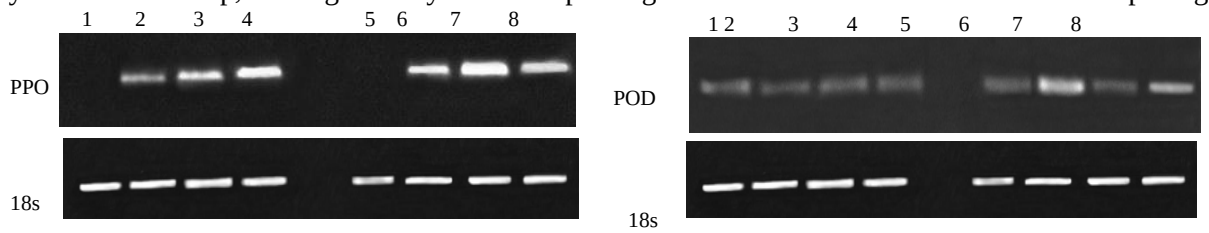

Figure 5. Gel electrophoresis of the amplification of the gene encoding the PPO. Lanes 1-4 treatments without inoculation; lanes 5-8 pathogen inoculation treatments. Lane 1, Q = 0%, T = 0 h, lane 2, Q = 1%, T = 0 h, lane 3, Q = 0%, T = 24 hr, lane 4 Q = 1%, T = 24 hours , lane 5, Q = 0%, T = 0 h, lane 6, Q = 1%, T = 0 hrs, lane 7, Q = 0%, T = 24 hr, lane 8 Q = 1%, T = 24 h (E). Gel electrophoresis of the amplification of the gene encoding the POD. Lane same order. (F)

For expression of the gene encoding the POD, we can see that the inoculation of the pathogen without treatment showed the same expression with and without chitosan (Figure 5). Inoculation treatments, 1.0% chitosan was able to induce gene expression of the POD, showing an increase at 0 and at 24 h compared with controls. This suggests that chitosan acts from the contact with the host through a signal transduction process, thus inducing gene expression and POD. This gene induced by chitosan, could be considered early signaling, because that is active in the early stages of the infection process, since in later stages this gene has a basal state. Reports applied benzotiadiazole to mango fruits, led to the induction of gene expression (POD and PPO), increasing the expression of said enzymes contributing to the activation of defense systems (10).

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Effect Of Edible Coatings Based On Biopolymers And Antimicrobial Agents In The Shelf Life Of Strawberry (*Fragaria ananassa*)

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Introduction

Strawberry (*Fragaria ananassa*) is a highly perishable fruit, has very short shelf life because is a non-climacteric fruit and has a very thin and fragile epidermis, which also very susceptible to attack by microorganisms and to mechanical damage during storage and marketing. Many markets for the marketing of strawberries are quite far from their place of production, why effective management is required to prevent deterioration [16, 12]. As an alternative to this problem, research on edible coatings (EC) and films has increased in recent years. The EC can be prepared from proteins (wheat gluten, zein, whey protein, egg albumin and milk proteins), lipids (waxes, acylglycerol and fatty acids) and polysaccharides (pectin, pullulan and chitosan, among others) [8].The application of these technologies in fruits produce a modified atmosphere in these products, reduce spoilage, delay ripening, reduce water loss, retard discoloration, improve appearance, reduce loss of flavor, reduce the exchange of moisture from fruit pieces, serve as a transport of antioxidants compounds and stabilizers of texture, impart color and flavor, and could serve as carriers of other substances [14]. Our objective was to determine the effects of coating materials and incorporated antimicrobial agents on shelf life of strawberries.

Material and methods

Materials. We used high methoxyl citrus pectin (Danisco), pullulan (90% purity) was donated by Hayashibara Company (Okayama, Japan) and crab shell chitosan (85% degree of acetylation) Sigma-Aldrich. The plasticizer used was glycerol Analytika ® (99.5%). As antimicrobials were used sodium benzoate and potassium sorbate (Piasa SA de CV).

Preparation of coating solutions. Three different coating solutions were prepared: (F1) 3.5% pectin with 0.5% glycerol and 0.05% of sodium benzoate and potassium sorbate; (F2) 6.5% pullulan with 0.5% glycerol and 0.05% of sodium benzoate and potassium sorbate; and (F3) 1.5% chitosan with 0.5% glycerol and 0.05% of sodium benzoate and potassium sorbate in 1% acetic acid. All coating solutions were prepared in distilled water.

Strawberries Coating. Strawberries Albion variety were purchased from Mercado de Abastos Estrella, San Nicolás de los Garza N.L., from California, U.S.A. Strawberries were selected, removed those that presented mechanical injuries and accepted firmly appearance, red uniform, typical pleasant odor and free from molds. Strawberries were disinfected with hypochlorite 250 ppm and subsequently were coated by immersion. Coated strawberries were stored at 4° C and analyzed for each of the parameters (physicochemical, microbiological and sensory) every 3 days for 15 days.

Physicochemical assays. Weight loss was determined by gravimetry, taking the initial weight and final weight of strawberries, the results were expressed as percentage of weight loss (%)[1].Ash and acidity were determined by gravimetry and titration with sodium hydroxide 0.1 N expressed as percentage of citric acid, respectively [2]. Soluble solids was determined by refractometer Extech RF15 (0-32%) at 20° C. The results were expressed as Brix[1]. Furthermore, Vitamin C content was determined by titration with 0.01N iodine solution and expressed as vitamin C content in mg/100g sample [15].

Microbiological assays. The aerobic mesophilic count was determined according to NOM-093-SSA1-1994, total coliforms according to NOM-113-SSA1-1994 with violet red bile agar (RBA) and counts of fungi and yeasts according to NOM-111-SSA1-1994. The results of these analyzes were expressed as colony forming units per gram (CFU/g) [19, 20].

Sensory evaluation. Acceptance of product was assessed by 5 judges untrained, which tested the strawberries coated and control. The variable was evaluated with qualitative values in range of 1-5 (poor to excellent) for each fruit. Decay rate or deterioration symptoms caused by fungi on fruits were evaluated visually according to following scale: 1= no damage, 2= slight damage (<25%), 3= moderate damage (>25% and <50%) 4= severe damage (>50 and <75%) and 5= completely damaged (75-100%) [16].

Statistical analysis. Results of chemical and microbiological assays were subjected to analysis of variance (ANOVA) and Tukey test. Results of sensory evaluations were subjected to Kruskal-Wallis and Mann Whitney test in SPSS 17.0.

Results and discussion

Physicochemical assays. Control of weight loss of fruits and vegetables is one of the primary goals of the edible coating technology. All coatings reduced significantly weight loss ($p<0.05$) of strawberries according to Mali[11] (Figure 1). Coatings based of pectin, pullulan and chitosan decreased weight loss of fresh strawberries until 29.44% (chitosan) compared with control. Coatings form a barrier to O_2 and CO_2, modifying internal atmospheres and slowing down the respiration rate of fruit, which in turn reduced weight loss [3]. Ash content did not change significantly ($p>0.05$) during storage and was not affected by coating application (Figure 2), similar as observed in previous studies [1]. Citric acid is the most abundant acid in strawberries, followed by malic acid, and ascorbic acid, which is biologically important because of its antioxidant activity [11]. During storage, acidity content no showed significant difference ($p>0.05$) (Figure 3), opposite to reported by that referred a decrease in organic acid content during storage of strawberries coated with gelatin [16]. Furthermore, Almenar[1] reported that acidity may vary depending on the treatment conditions, concentrations of O_2, CO_2, temperature, among others. Vitamin C content decreased significantly ($p<0.05$) with the prolongation of the storage period for F2, F3, and control. This reduction may be due to high respiration rate and oxidation process in the product. Furthermore, the vitamin C content of F1 was stable during 15 days of storage, indicating that this formulation present better barrier to avoid oxidation of vitamin (Figure 4). The lowering of vitamin C loss of strawberries treated with edible coatings attributed to slowing down the respiration rate of fruits, as affecting by coating which delays the deteriorative oxidation reaction of vitamin C [3]. Soluble solids content is increase according to the maturity of product. Strawberries coated presented significantly less ($p<0.05$) soluble solids content respect to control during storage (Figure 5). These results showed that control fruits presented a maturation development more pronounced that coated fruits, and this occurred because these fruits were stored without any protection against the external atmosphere [11].

Microbiological assays. Accounts of aerobic mesophilic, molds and yeasts were increased significantly ($p<0.05$) during storage in control, F1 and F2. However strawberries coated presented lower content respect to control (Figure 6, 7 and 8). These results suggest that coatings form a barrier of protection to decrease or avoid the contamination by microorganism, also, addition of antimicrobial agents such as sodium benzoate and potassium sorbate also decreases the growth of microorganisms. Furthermore, formulate based-chitosan (F3) inhibited until 100% the growth of aerobic mesophilic, fungi and yeasts due to the protective barrier and the antimicrobial properties of chitosan [4, 7, 10, 11, 17.]

Sensory evaluation. Acceptance of strawberries decreased significantly ($p<0.05$) during storage mainly in the control (Figure 9). Strawberries coated had higher acceptance compared to the control due to they showed less variation in changes of color and presented better flavor and appearance for panelists [5, 9, 17.] Furthermore, all coating significantly reduced ($P<0.05$) decay rate on strawberries during storage. Strawberries coated showed slightly better retardation on mold growth respect to control. Coating based

chitosan inhibited 100% the mold incidence due to the protective barrier, antimicrobials presents in coating and the antimicrobial properties of chitosan (Figure 10) [17].

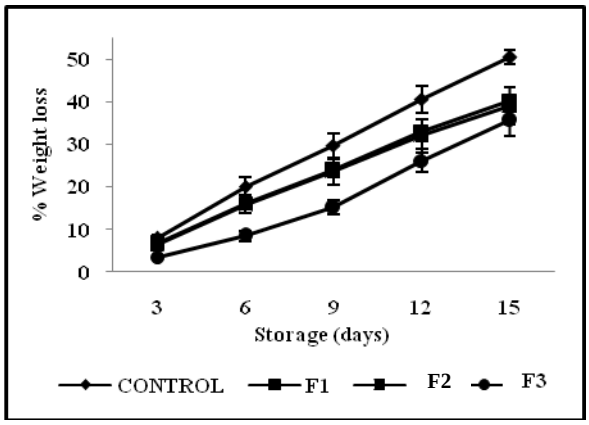

Figure 1. Weight loss of strawberries coated with different polymeric materials and stored at 4° C for 15 days.

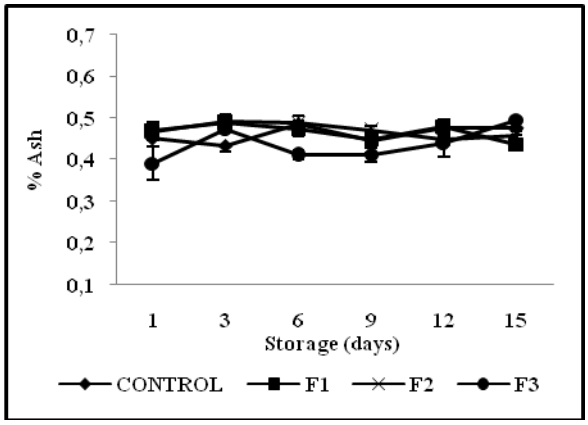

Figure 2. Ash content of strawberries coated with different polymeric materials stored at 4° C for 15 days.

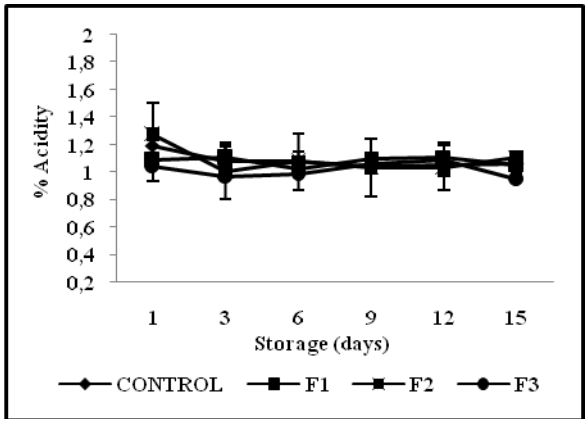

Figure 3. Acidity (citric acid) content of strawberries coated with different polymeric materials stored at 4° C for 15 days.

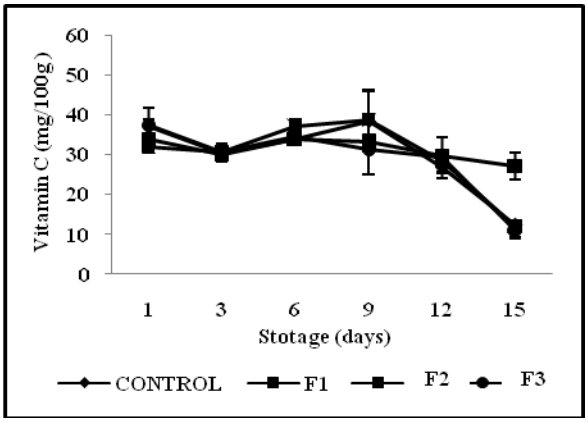

Figure 4. Vitamin C content of strawberries coated with different polymeric materials stored at 4° C for 15 days.

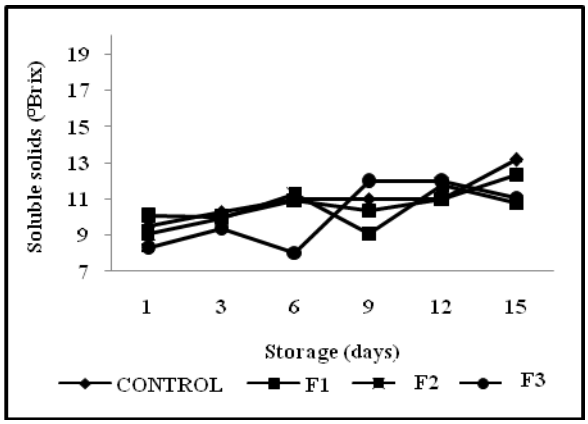

Figure 5. Soluble solids content of strawberries coated with different polymeric materials and stored at 4° C for 15 days.

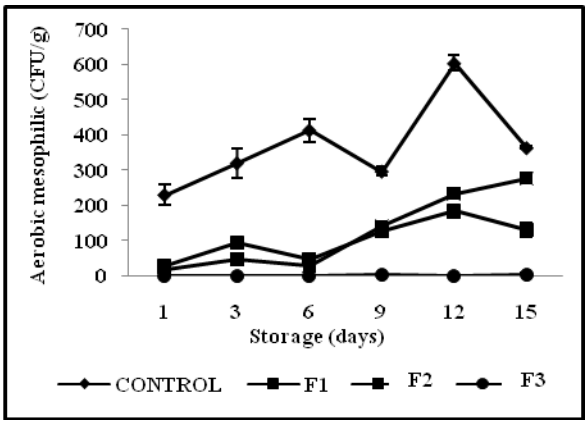

Figure 6. Aerobic mesophilic accounts of strawberries coated with different polymeric materials and stored at 4° C for 15 days.

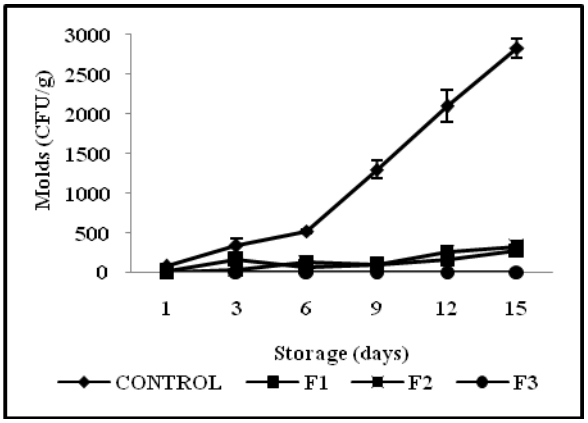

Figure 7. Molds accounts of strawberries coated with different polymeric materials and stored at 4° C for 15 days.

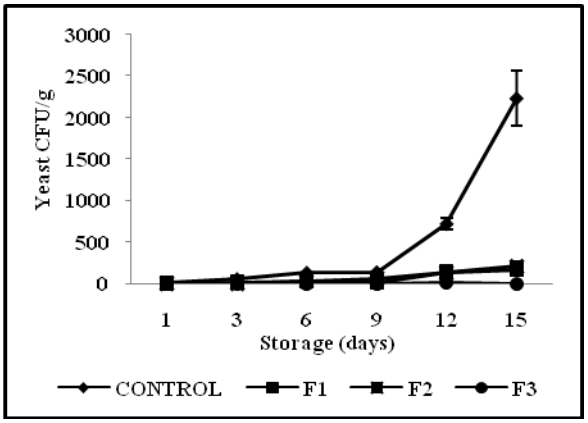

Figure 8. Yeast accounts of strawberries coated with different polymeric materials and stored at 4° C for 15 days.

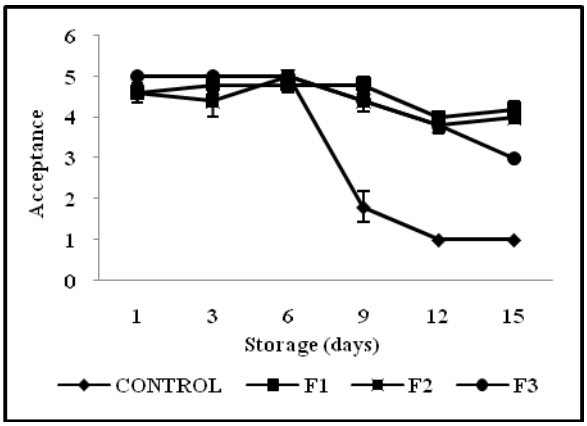

Figure 9. Acceptance of strawberries coated with different polymeric materials and stored at 4° C for 15 days.

Figure 10. Decay rate of strawberries coated with different polymeric materials and the control at 15 days of storage.

Conclusion

Addition of coatings decreased weight lost. Vitamin C content was stable in F1 during storage. Addition of coatings reduced microbial accounts of mesophilic aerobic, molds and yeasts and chitosan coatings inhibed growth of microorganism and reduced decay rate until 100%. Furthermore, the addition of coatings improved sensory properties (color, olor and flavor) of product and increased acceptance by evaluators. Finally, shelf life of strawberries control was 9 days, while the strawberries coated with F1, F2 and F3 was increased 166% (15 days).

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Debaryomyces hansenii As ABiocontrol Agent Of Some Diseases Of Persian Lime (*Citrus latifolia*)

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Abstract
Ability to inhibition *in vitro* growth of *Penicilliumcitrinum*and *Mucorfragilis*, postharvest biological control of mold decay (caused by *P. citrinum*) and population dynamics on Persian lime were evaluated for *Debaryomyces hansenii*. A 100 % inhibition of infected fruits was obtained by alone application of *Debaryomyces hansenii*but treatments with dextrose and Tween 80 promoted infection by *Penicillium citrinum* on Persian lime.
Key words:*Debaryomyceshansenii, Penicilliumcitrinum,* Persian Lime

Introduction
Citrus diseases caused by postharvest fungal cause economic losses throughout the world, in some cases as high as 50% of the total production of fruits. Fungal infection can occur during cultivation, at the time of collection, during processing or packaging, storage, transportation and marketing, and even after being acquired by the consumer (1).The application of biological control agents may allow early colonization of fruit surfaces, thus protecting from infections. This strategy may be an appropriate for fruits and vegetables subject to damage in postharvest handling (2).

The aim of this study was to apply *Debaryomyceshansenii* to assess their ability to inhibit the decay caused by*Penicilliumcitrinum* and *Mucorfragilis*on Persian lime during postharvest.

Materials and methods

Biological material
Persian limeswere harvested in the municipality of San Pedro Lagunillas in the Stateof Nayarit.*Penicilliumcitrinum* and *Mucorfragilis* were provided by the Centro de Investigaciones Biológicas del Noroeste (CIBNOR),La Paz, Baja California Sur. Fungiwereisolated from healthy fruit from the champ and a packingin the Stateof Nayarit.*Debaryomyceshansenii*(the biocontrol agent) originally was obtained from seawater samples collected at a depth of 100m at the Mar de Cortés(Baja California, Mexico) and belonging to the Yeast Collection of the CIBNOR (Centro de InvestigacionesBiológicas del Noroeste, La Paz, B.C.S., Mexico). The yeast strain was conserved at -80ºC in YE broth with 40% glycerol as cryoprotector. Fungi were conserved in PDA plates and reseeded monthly.

Pathogenicity test
In order to discard secondary pathogens or saprophytic fungi from primary pathogens, a pathogenicity test based on Koch Postulates was applied. Fruits were washed and disinfected with 2% sodium hypochlorite solution for 2 min, then rinsed with sterile distilled water and allowed to dry in a laminar flow chamber (10 min). Wound was performed using a sterile punch to artificially infect them with 20 µL of spore'ssuspension (10^3,10^4, 10^5 spores/ml) of the fungus in study (3).The fruits were placed in plastic chambers with elevated relative humidity (60-75%), at25-29 ° C. For fungus reisolation, cuts (1x1 cm) were made in the flavedoof fruits with severe symptoms of disease, area affected (50 %) and healthy surface (50%) (3). Tissue sectionswere superficiallydisinfectedfor 1 min (2% sodium hypochlorite),

thenrinsedwithsteriledistilled water and placed in the middleofPDAagar plates and incubatedat 28 ° C for 8 days (3).

***In vitro* inhibitiontest**

In vitro inhibition for *P. citrinum*and *M. fragilis* was tested in flavedo agar (FA) and albedo agar (AA). 30µl yeast suspension (10^7,10^8 CFU/mL) were added and incubated 24 hat 28ºC, subsequently a 7 mm agar piece with mycelial fungi (7days growth) was replaced in the medium (FA and AA) and incubated at 28ºC. Diameter growth was registered during 7days (4). Germination inhibition was tested in 5 mLPDB, yeast (100µL, 10^8CFU/mL) and fungal spores (100µL, 10^5spores/mL) were added, incubated and germination measured every 2 h for 14 h (5).

***In vivo* inhibitiontest**

Lime fruits were sprayed (2ml/fruit) with the antagonistic suspension: T1(10^8CFU/ml+Tween80+Dextrose), T2(10^8CFU/ml+Dextrose), T3(10^8CFU/mL+Tween80), T4(10^8CFU/mL) and T5 (Sterile distilled water). A fruit center wound was made and a 20µL suspension (10^5 spores/mL) was added. Disease incidence and damaged surface were measured from 20 to 25 day (4).Damaged surface were evaluated with a decay scale (1-5).

Population dynamicsof biological control agent (BCA)

The same treatments applied for *in vivo* test were used to determine the population dynamics of the yeast. Fruitswithout pathogen inoculation were individually immersed in a 500 mLbeaker with 100 mL of distilled water supplemented with Tween 80 (0.05%), stirred for 30 min using a rotary shaker at 110 rpm. An aliquot (100 µl) was inoculated into YPD culture medium then incubated at 28 ° C. Measurements were performed every 4 days for 20 days(1).

Results and discussion

Pathogenicity test

Fruits inoculated with *Penicilliumcitrinum* showed diffuse green spots on the flavedo (day 12) with the subsequent emergence of a spot located (yellow pale) in the area of the lesion with softening. The lesion changedof pale yellow to dark brown and extended to the periphery of the lesion withdamage surface onthe half offlavedo surfaceand absence of mycelium (day 19) (Fig. 1). Fruits inoculated with *Mucorfragilis* showed the appearance of yellow color on the flavedosurface, in the area of inoculation appeared a brown stain that spread to the apical zone of the fruit; at day 19 fruits presented an affectation of the surface without presence of mycelium.Control fruits inoculated with sterile distilled water did not showed damage on the flavedo surface.

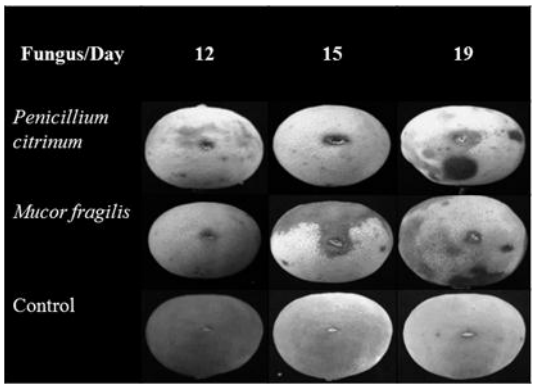

Figure 1. Evaluation of disease symptoms within 19 days

The macroscopic characteristics of *Penicilliumcitrinum*inoculated and reisolated were the same:green fungus with white halo, velvety appearance and yellowish reverse (Fig. 2a).In the case of *Mucorfragilis*,a colony with aerial mycelium sparse, cottony texturecolor white to gray and white reversewas observed, whichdid not correspond to macroscopic characteristics of the inoculated fungus. Microscopiccharacteristics of *Penicilliumcitrinum*are readily recognized by its penicilli, which consist of 3-5 divergent and usually vesiculatemetulae, bearing long well defined columns of conidia (6). These characteristics were observedinreisolatedfungus. However for *Mucorfragilis* the characteristics found in the reisolated fungus did not correspond to the inoculated (Fig. 2b).The pathogenicity of *P. citrinum* was confirmed but not for *M. fragilis*.

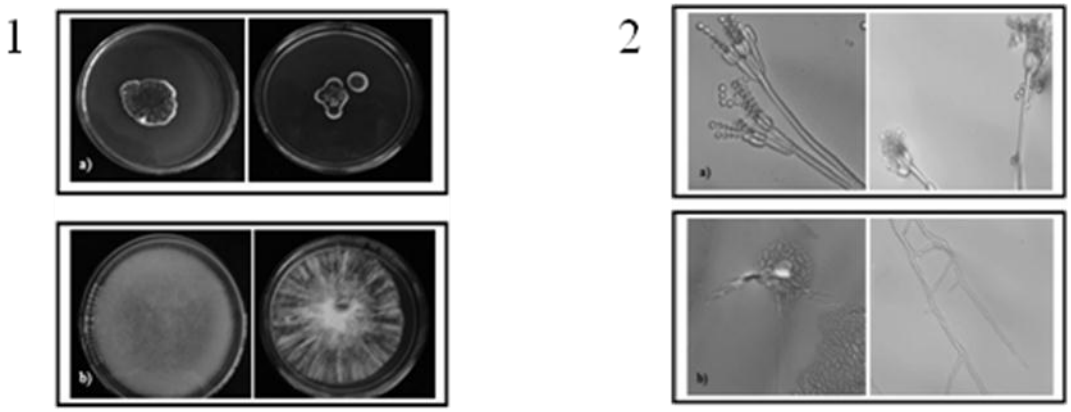

Figure 2. Macroscopic comparison (1),Microscopiccomparison (2)
a) *Penicilliumcitrinum*b)*Mucorfragilis*. Fungus inoculated (left) and reisolated (right).

***In vitro*test**

*M. fragilis*inhibition was 98.6% (AA) and 91.3% (FA) ($p<0.0001$). In the case of*P. citrinum*, the obtained inhibition was of97.4% (AA) and 68% (FA) ($p<0.0001$). The low content of sugars (glucose, fructose and sucrose) in the flavedo and albedo is likely to promote carbon competition between *Debaryomyces hansenii* and *Penicillium citrinum* (4). *In vitro* antagonism of *Debaryomyces hansenii* relates *killer* toxin production (7) wich interacts specifically with the cell wall of the fungus identifying (1-6)-β-D-glucan as receptor. As a consequence, cell death is induced (8). No germination was observed for both fungus in presence of yeast (Data not shown).

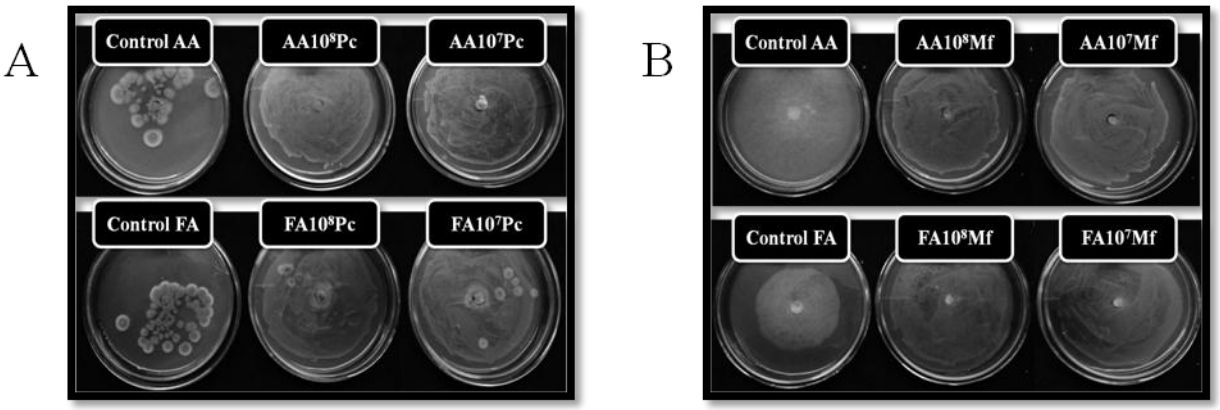

Figure 3. Evaluation of *in vitro* inhibition, A) *Penicilliumcitrinum*(Pc) B)*Mucorfragilis* (Mf)
AA (Albedo agar) and FA (flavedo agar).

***In vivo* test**

A 100% *P. citrinum*inhibition was observed when applying T4 (BCA) (Figure 4B). However,lower inhibition percentages were found with T1, T2 and T3 (Figure 4A). However population dynamics of T4 showed a decline compared to the other treatments ($p<0.0001$).The levels of surface damage were 1.4 (T1), 2.2 (T2) and 1.6 (T3) at the end of the evaluation comparing with 3.2 (control). Production of enzymes β-1,3 glucanase, chitinase and protease may be involved in the *in vivo* antagonism by *Debaryomyces hansenii* (7) causing the disintegration and collapse of hyphae (10).

The level of protection by *Debaryomyces hansenii* against decay of the fruit can be explained by the ability of the yeast to assimilate carbon sources from the wounds of the fruit to establish and multiply (11). The adaptability of an antagonist is an important factor in reducing the colonization of pathogenic fungi (12).

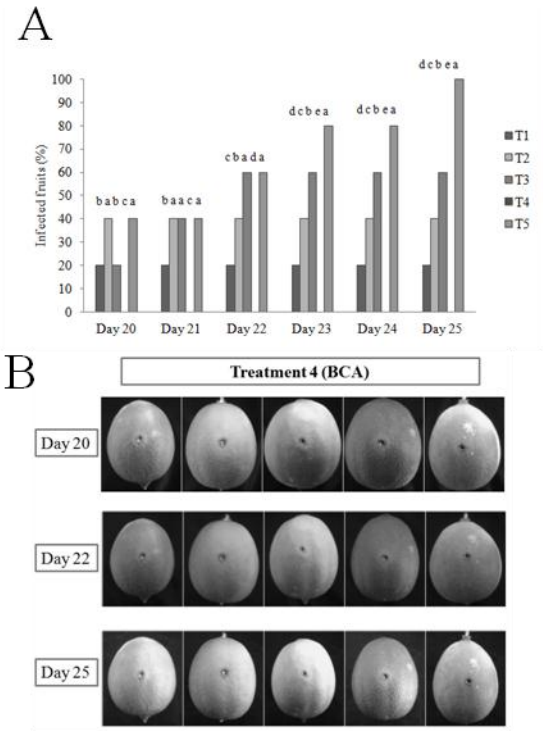

Figure 4. A) Percentage of infected fruits, B) Fruits treated with *D. hansenii*.

Population dynamics

Treatments 1 (BCA+Tween80+Dextrose), 2 (BCA+Dextrose) and 3 (BCA+Tween80) are those who favored the permanency of yeast on flavedo. A decrease in the population dynamics on fruits with treatment 4 was observed. Surfactants (Tween 80) can help overcome the environmental conditions that limit colonization, breaks the surface tension and hold water on the surface to prevent desiccation, likewise form surface films which maintain moisture (9).A simple and effective method to increase the population and survival of biological control agents is the use of adjuvants (dextrose) (10).

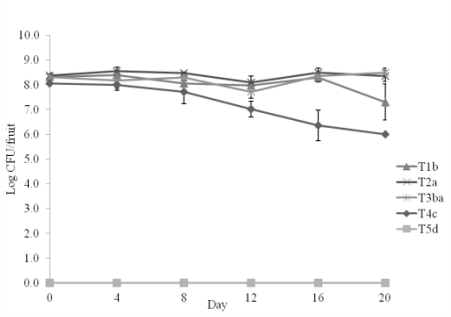

Figure 5. *Debaryomyceshansenii*population dynamics observed with treatments.

Conclusion

A 100% protection against infection by *P. citrinum*was determined. The population dynamics of the antagonist was enhanced with the application of Tween 80 and dextrose on the surface of the fruit, however a lower biocontrol was observed comparing with the sole aplication of *Debaryomyces hansenii.* The application of *Debaryomyces hansenii* as biological control agent can be an alternative of postharvest control of *P. citrinum* in Persian lime.

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Recovery Of Value Added Proteins From Seafood Wastes

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Introduction

After processing fisheries, the sea food industry generates wastes amounting for 30-85%, depending of the species and processes, of the landed production (1). Waste causes technical and environmental problems. On the other hand, waste can be seen as source of of by-products recoverable by suitable processing strategies. Crustaceans, like shrimp, and mollusks, like clam and squid, are important sources of waste materials after processing. In Mexico the shrimp processing industry processes 1,345,000 t from fisheries and 123,000 t from aquaculture (2), which generates a large amount of shell and head waste. The cephalothorax (head portion) of shrimp accounts for approximately 40-45% of the body weight. It also is a cheap and abundant source of materials, such as protein, chitin, and astaxanthin (3, 4).

In the Norwest coast of Mexico, the jumbo squid (*Dosidicus gigas*; d'Orbigny 1935) is becoming an important fishery, distributed in the eastern Pacific from Monterey, CA (USA) to Chile, in South America (5). It is an especially abundant marine resource in the Gulf of California. Squid wastes like viscera also have the potential to be used as secondary material to obtain valuable components like protein or palatants, among others. Squid viscera amounts for 10% of the whole body (6).

The clam, *Megapitaria squalida*, is a commercial species captured and distributed in the Gulf of California and along the Pacific coast from Scammon's Lagoon, Baja California Sur to Mancora, Peru (7). The viscera of clams (the waste) is around 20% of the soft tissue (8) and a source of protein and other potential value-added products like palatants and enzymes.

The aim of the present work was to study some procedures to recover high value protein from wastes of three important fisheries: Shrimp (*Penaeus vannamei*) squid (*Dosidicus gigas*) and clam (*Megapitaria squalida*).

Materials and methods

Three batches of cephalothorax (Sc) from recently killed shrimp were obtained from CIBNOR ponds. All samples were kept in plastic bags and in ice beds until processed. Squid viscera were obtained from 6 fresh *D. gigas* specimens at La Ventana Bay, located 50 km Southeast of CIBNOR, La Paz, B.C.S, Mexico. Immediately after capture, specimens were gutted and the whole viscera were separately stored between ice-beds in an ice chest to limit spoilage and assure freshness until arrival at CIBNOR facilities. Clam (*M. squalida*) viscera were obtained fresh from local sea food retailers in small batches stored in plastic bags, kept on ice from evisceration until arrival to CIBNOR.

All raw materials were kept in ice, ground and sieved into a paste (2-mm mesh, Retsch/Brinkmann grinder, Westbury, NY), and then stored at –20C until used. The protein content of the Sc paste was determined by applying a correction for the nitrogen content in chitin according to: $P=6.25\times(NT - NCh)$, where P is the protein content in Sc paste, NT is the total nitrogen in the sample and NCh is the nitrogen content of the chitin present in the sample. The chitin content in the Sc paste was measured by the method of Synowiecki described in Mizani et al. (8). Protein, ash, fiber and lipids of all materials were determined by standard methods (10). Freeze-dried samples were used for preparing amino acid profiles by the methods described in Vázquez-Ortiz et al. (11). Amino acid analysis was performed only on raw viscera from calm and squid and in the three protein concentrates.

Shrimp cephalothoraxes, Jumbo squid and clam viscera were processed as described in Palafox, et al. (12) to obtain the protein concentrates. Briefly, a protein-solubility curve was prepared using the Biuret method

(13) to determine the iso-electric precipitation pH point and the maximum solubility pH of most of the proteins in the three sources of protein,. The results from the solubility curves were validated by analyzing the solubility of proteins at different pH values for composition and molecular weight using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (14). Samples of containing 20µg protein from each source and pH (from pH 2 to 12) were mixed (1:1) with sample buffer containing dithiothreitol (DTT), heated for 5 min in a boiling water bath and then loaded into a 0.75 mm Acrylamide gel slab (10%T) assembled in a vertical electrophoresis unit (model SE 260, Hoefer, Inc, San Francisco, CA). For pH process, pH=4.0 and pH=7.0 were selected for shrimp cephalothoraxes, pH=4.0 and pH=11.0 for squid viscera and pH=5.0 and pH 11 for clam viscera as the pH values to precipitate and solubilize the proteins respectively.

The procedure to obtain the protein concentrates was as follows, using either shrimp cephalothoraxes, clam or squid viscera: a portion of homogeneous paste was stirred with deionized water (1:3) using a propeller impeller, then the homogenates were adjusted to the maximum solubility pH value for each source of proteins (pH 7 for Sc and pH 11.0 for clam and squid viscera) by adding 5 N HCl or 5 N NaOH at slow but constant stirring. Then the mixture was centrifuged 30 min at 4°C and 10000 x *g* (Eppendorf 5804R, Brinkman Instruments Inc., Hamburg, Germany). The sediment was discarded and the next step was to adjust the pH of the supernatant to the iso-electric point (pH 4.0 for the three sources of proteein) to precipitate the proteins. Then, the suspension was centrifuged 30 min at 4°C at 10000 × *g*. The supernatant was discarded and the sediment constituted the protein concentrate that was mixed with distilled water (1:3) and then dried in a vacuum oven at 50 °C to convert it into a powder. The yield from each source of protein was calculated as % of raw materials in dry basis. The water content of the three protein sources was measured by drying samples until constant weight in a vacuum oven at 110 °C.

All results are reported as the mean of three replicates. ANOVA was carried out when required to compare mean values. Differences were determined at $P < 0.05$.

Results

The solubility of proteins as function of pH from the three sources used is shown in Figure 1. The SDS-PAGE from each protein source were compared with the solubility curves from each protein source. The amount of soluble proteins shown in the electrophoresis gels were in agreement with the amount of soluble protein observed in the data of the solubility curve.

The proximate composition of the raw protein sources and the protein concentrates is shown in Table 1. Shrimp cephalothorax had the less amount of total protein, compared with clam viscera and squid viscera. The clam protein concentrate showed the highest protein content compared to the other two protein concentrates. The relative amount of lipids in the three protein concentrates was higher than in the raw materials. The lowest yield of protein concentrate was for the shrimp cephalothorax (Table 2). Regarding to the essential amino acid content of the three protein concentrates, they contained all (Table 3).

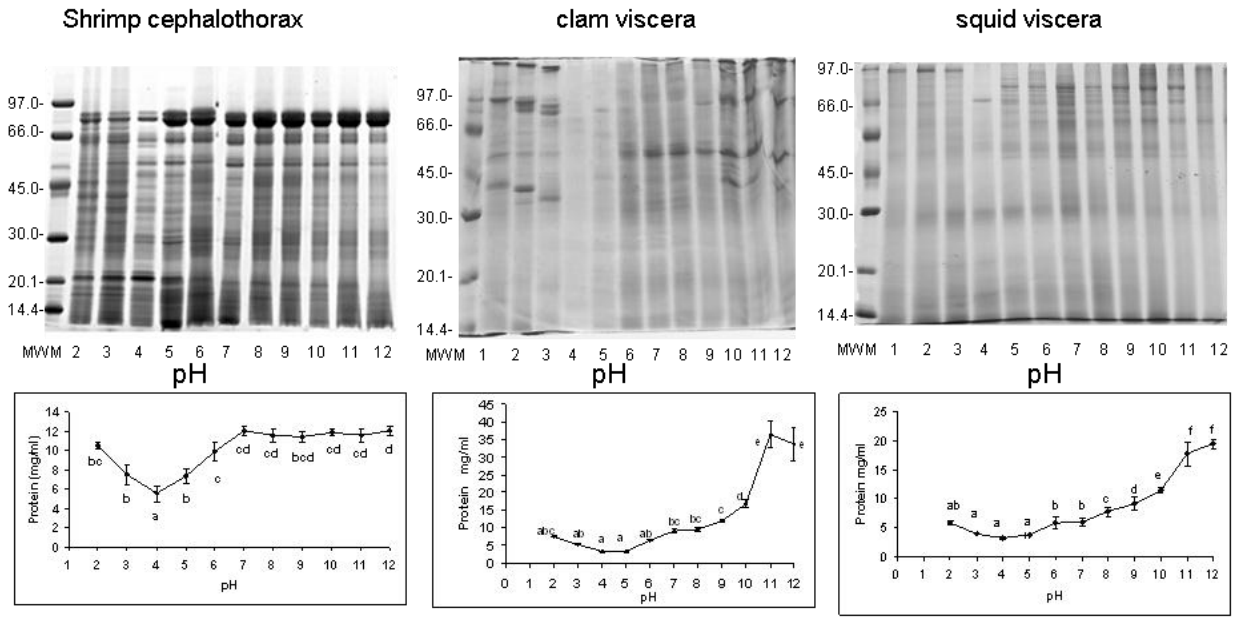

Figure 1. Solubility of proteins of the three protein concentrates compared with proteins in SDS-PAGE at the assayed pH values. Different letters in graphs represent significant differences at $P < 0.05$

Table 1. Proximate composition of the three protein sources and the protein concentrates expressed as dry basis (g/100g) ± estándar deviation.

	[1]R-Sc	Sc-Pc	R-Sq	Sq-Pc	R-clam	Clam-Pc
Protein	*29.8 ± 0.6	58.7 ± 0.3	70.1 ± 0.2	57.8 ± 0.3	72.7 ± 0.2	71.36 ±0.2
Lipids	14.5 ± 0.2	20.6 ± 0.3	8.2 ± 0.1	11.74±0.95	5.7+0.1	9.5 ± 0.2
Ash	14.3 ± 0.1	2.6 ± 0.1	11.29 ± 0.2	15.62±0.2	11.3 ± 0.1	9.5±0.25
Fiber	nd	nd	0.12 ± 0	0.01 ±0	0.03 ± 0	0.01 ±0

[1]R-Sc = Raw shrimp cephalothorax, R-Sq = Raw squid viscera, R-clam = Raw clam viscera, Sc-Pc = shrimp-cephalothorax protein concentrate, Sq-Pc = squid-viscera protein concentrate, Clam-Pc = clam-viscera protein concentrate., nd= non done.
* (Total N-chitin N) × 6.25. See methods section.

Table 2. Yield expressed in g/100 g of dry mater of each protein source.

	Shrimp Cephalothorax	Clam viscera	Squid viscera
Water content (%)	72.87 ± 4.8	83.2 ± 1.1	79.9 ± 3.1
Yield (dry basis, %)	37 ± 6.3	44.8 ± 2.4	45.7 ± 3.6

Table 3. Amino acid content (expressed as mg/g protein).

Amino Acid	**R-Sc	Sc-Pc	R-clam	Clam-Pc-	R-squid	Squid-Pc
Asp	153	69	57	44	57	47
Glu	218	109	52	43	64	54
Ser	n/d	n/d	24	17	22	17
Gly	108	66	46	34	28	20
Arg	48	93	21	10	13	10
Ala	59	118	45	33	44	33
Pro	84	141	27	23	34	27
Tyr	26	59	15	11	16	13
†Thr	28	62	22	17	25	21
†His	8	17	n/d	n/d	n/d	n/d
†Val	2	4	n/d	n/d	n/d	n/d
†Phe	33	76	12	11.	17	15
†Ile	31	67	22	18	30	24
†Leu	55	114	30	23	38	30
†Lys	79	137	40	26	33	18

*n/d = Non done.

**R-Sc = Raw shrimp cephalothorax, R-Sq = Raw squid viscera, R-clam = Raw clam viscera, Sc-Pc = shrimp-cephalothorax protein concentrate, Sq-Pc = squid-viscera protein concentrate, Clam-Pc = clam-viscera protein concentrate.

†Essential amino acids

Discussion

Solubility is an important feature for protein functionality and the solubility of proteins depends on the properties of the protein and the solvent, pH, concentration, charge of other ions, and temperature (15). Differences in solubility of proteins are important to separate individual proteins or groups of proteins. This is an important issue when applying the pH solubility-precipitation process.

Based in the amino acid profiles of the three protein concentrates, they are suitable for animal and human consumption. Amino acid content in all protein concentrates showed similar or higher values than international recommendations (16).

Because the pH-shift process proceeds at low temperature, it is an attractive alternative for processing fishery by-products to separate and concentrate protein. This process also minimizes the potential damage produced by heat to proteins and unsaturated fatty acids when heat-based extraction methods are used. From the discarded material after the solubilizing step, it also should be easy to separates other components, such as oils or pigments that normally are concentrated in the discarded fractions that were not investigated? in the present investigation. This methodology should be considered as a choice to recover protein from fisheries or aquaculture by-products and obtain value added products from waste materials. To make this possible, it is also important to teach seafood processors that the waste material must be appropriately disposed to avoid spoilage and damage to proteins and other biomolecules of high potential value, like lipids, pigments, and enzymes. Additional research is in progress in our research group to recover high value products from low value fisheries resources and waste materials from several fisheries.

Acknowledgements

Projet EP4.0 from the Fisheries Ecology department of CIBNOR.

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Effect Of Hydrogen Peroxide And Chitosan In Corn Seeds Exposed To Pathogenic Fungi

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Introduction

Maize (*Zea mays*) is the staple food of the mexican population. Globally, U.S.A is the greatest producer of this cereal generating 307, 383,552 tons yearly, followed by China which produces 166,035,097 tons. Mexico ranks the third place producing 24,320,100 tons per year (1). Corn is a very important cereal in the world because it is the basis of human and animal feed, being rich in carbohydrates, vitamins and minerals, but is deficient in two essential amino acids: lysine and tryptophan. For this reason, Mexico also cultivates High Quality Protein Maize (QPM) which contains up to 100% more lysine and tryptophan than ordinary maize, equaling the protein quality of milk (2). Nevertheless, this enriched cereal is more susceptible to be attacked by bacteria and fungi than ordinary maize because of the high content of proteins that make it a good source of carbohydrates for microorganism growth.

There are many factors that affect negatively this cereal, being the fungi *Aspergillus flavus* and *Fusarium moniliforme* the greatest threats. *A. flavus* is a plant pathogen and an opportunistic fungus that causes human and animal diseases because of the production of some mycotoxins named aflatoxins, which are carcinogenic, hepatotoxic, teratogenic and immunosuppressive molecules (3). *F. moniliforme* also produces harmful secondary metabolites known as fumonisins, which have negative effects on consumer health (4). Abiotic stresses affect seriously the development of crops too, being drought the most important one (5). Recently, some chemicals have been used to reduce the effect of these pathogens in seeds, for example, Chitosan and Hydrogen Peroxide. Chitosan is one of the most important elicitors in the oligosaccharides group, and it is well known for its antimicrobial properties, making it useful for many uses in different areas like food chemistry by covering fruits and vegetables for their preservation (6). Due to the successful outcome of Chitosan as biocide, it seems to be a promise in seed preservation, because researches had found favorable decrease of pathogenic fungus on *in vivo* and in storage seeds and also in the amount of mycotoxins developed in the grain even under high humidity conditions (7, 8, 9, 10, 11). It is believed that Chitosan has the ability of inducing massive accumulation of fungitoxic substances in application place, and it acts as a barrier to prevent nutrients flow to the pathogen too. Hydrogen Peroxide inhibits the growth of pathogenic fungus and active some signal transmission pathways that induce defense responses (12). That's why the objective of this investigation was to evaluate the effect of Chitosan and Hydrogen Peroxide coatings and sprayings in maize plants over their physical characteristics, their fungal development (against *Aspergillus flavus* and *Fusarium moniliforme*) and the nutritional quality.

Materials and methods

Normal variety and QPM variety seeds with a germination of 100% were used. For the seeds coating, a 2% Chitosan solution (CH) and an 8mM Hydrogen Peroxide solution (H_2O_2) were prepared. For the seedlings spraying, a 0.2% CH solution and an 8mM (H_2O_2) were prepared. In order to establish the biotic stress conditions in maize seeds, *A. flavus* (UNIGRASASP68 strain, aflatoxin-producer specie) and *F. moniliforme* (UNIGRASFUS44 strain, fumonisin-producer specie) were used. Spore suspensions were prepared as described by Gilchrist *et al*. (13), at a concentration of 10,000 spores / mL, applying an aliquot of 10 mL of spore suspension per seed to ensure infection. Drought stress was induced by decreasing the

frequency of irrigation. Sixteen treatments were generated for each variety, as shown in *Table 1*. This assessment was carried out through the measurements of the germinated seeds number and the measurements of leaf length, stem length, stem thickness and total seedling length at 14, 21, 28 and 38 days after planting.

The qualitative assessment of damage severity was done according to Stubbs (14), the days 17 and 26 after sowing, by determining the visually observable percentage for each treatment according to the damage caused by the fungus.

The presence of fungi stressors in plant leaves was evaluated by cutting small portions of leaves and placing them by triplicate in Petri dishes with Potato Dextrose Agar incubating them at 28 ° C during 8 days in order to observe the microbial growth. Finally, some bromatological analysis like content of dry matter (by thermobalance method (15)), proteins (micro-kjeldahl(16)), ashes (general method(16)) and crude fiber (Weende method(17)) were conducted in order to evaluate the nutritional quality of the treated product. For data analysis, the experiment was evaluated by an ANOVA with repeated measurements with a Post Hoc test using Tukey by the program JMP 5.0.1.2 (SAS Institute Inc, USA). Mean values with statistical difference of $P < 0.05$ were considered significant.

Tx	Stress	H_2O_2	CH
1	Drought	Covering	Covering
2	Drought	Covering	Covering and Spraying
3	Drought	Covering and Spraying	Covering
4	Drought	Covering and Spraying	Covering and Spraying
5	*A. flavus*	Covering	Covering
6	*A. flavus*	Covering	Covering and Spraying
7	*A. flavus*	Covering and Spraying	Covering
8	*A. flavus*	Covering and Spraying	Covering and Spraying
9	Without stress	Covering	Covering
10	Drought	Covering	Without CH
11	Drought	Without H_2O_2	Covering
12	*F. moniliforme*	Covering and Spraying	Covering and Spraying
13	*A. flavus*	Covering and Spraying	Spraying
14	*A. flavus*	Spraying	Covering and Spraying
15	*A. flavus* + Drought	Covering and Spraying	Covering and Spraying
16	Without stress	Without H_2O_2	Without CH

Table 1. *Treatments design of the experiment.*

Results and Discussion

Evaluation of the growth of seedlings under the influence of microorganisms inoculated.

In most measurements, both varieties exhibited significant difference in the growth of seedlings according to the treatment applied (Table 2).

	21 days after planting								38 days after planting							
Tx	total height		stem height		leaf height		stem diameter		total height		stem height		leaf height		stem diameter	
	QPM	Normal	QPM	Normal	QPM	Normal	QPM	Normal	QPM	Normal	QPM	Normal	QPM	Normal	QPM	Normal
1	28.90 ab	31.8ab	7.65 ab	7.37ab	21.25 ab	24.43ab	0.33 a	0.32ab	64.76ab	67.2ab	17.31ab	15.71a	47.45ab	51.49ab	0.77bc	0.79a
2	29.03 ab	31.39ab	6.31 ab	7.92a	22.72 ab	23.47ab	0.35 a	0.37a	64.3ab	67ab	15.71ab	16.74a	48.59ab	50.26ab	0.82abc	0.78a
3	27.00 ab	32.28ab	7.49 ab	7.65ab	19.51 ab	24.63ab	0.35 a	0.4a	59.9abc	66.96ab	15.24ab	15.87a	44.66abc	51.09ab	0.78abc	0.82a
4	26.53ab	33.39ab	6.15 ab	6.86ab	20.38 ab	26.53ab	0.32 a	0.37a	62.13abc	70.16a	14.69ab	16.56a	47.44ab	53.6a	0.80abc	0.85a
5	34.78 a	28.06ab	7.96 ab	6.79ab	26.82 a	21.27ab	0.35 a	0.29ab	62.93ab	59.81ab	17.67ab	14.19a	45.27abc	45.63ab	1.01ab	0.75a
6	28.23 b	27.65ab	7.60 ab	7.64ab	20.63 ab	20.01ab	0.33 a	0.31ab	60.3abc	60.04ab	18.81ab	14.49a	41.49abc	45.55ab	0.92abc	0.73a
7	21.07 b	24.96b	5.83 b	7.1ab	15.23 b	17.86b	0.25 a	0.35ab	47.13c	48.99b	13.94b	13.81a	33.19c	35.17b	0.69c	0.86a
8	28.74 ab	29.59ab	6.78 ab	7.9ab	21.97 ab	21.69ab	0.29 a	0.33ab	62.16abc	61.03ab	16.84ab	15.28a	45.31abc	45.75ab	0.92abc	0.71a
9	24.64 ab	25.75ab	4.78 b	6.38ab	19.86 ab	19.37ab	0.32 a	0.26ab	48.9bc	63.7ab	13.2ab	15.22a	35.7bc	48.48ab	0.7bc	0.84a
10	28.41ab	35.09ab	7.48 ab	8.35a	20.93 ab	26.74ab	0.33 a	0.41a	65.74ab	71.6a	17.79ab	17.63a	47.95ab	53.97a	0.91abc	0.84a
11	30.06ab	32.67ab	8.07 ab	7.32ab	21.99 ab	25.35ab	0.37 a	0.42a	70.11a	65.42ab	18.84a	14.98a	51.27a	50.44ab	0.92abc	0.78a
12	34.49 a	24.83b	9.17 a	4.7b	25.31 a	20.13ab	0.39 a	0.17ab	66.66ab	51.37ab	19.07ab	13.02a	47.59ab	38.35ab	1.04a	0.67a
13	31.77 a	37a	8.14 ab	9.24a	23.63 a	27.76a	0.36 a	0.41a	65.93ab	65.22ab	16.96ab	16.35a	48.97ab	48.87ab	0.96abc	0.85a
14	30.09 ab	32.39ab	8.29 ab	7.88a	21.80 ab	24.51ab	0.37 a	0.36a	56.03abc	62.3ab	16.03ab	17.54a	40abc	44.76ab	0.92abc	0.93a
15	28.22 ab	35.95ab	7.00 ab	8.65a	21.22 ab	27.3a	0.32 a	0.42a	64.67ab	60.14ab	18.62ab	14.58a	46.04abc	45.56ab	0.95abc	0.87a
16	21.07 ab	28.42ab	5.17 ab	7.38ab	16.43 ab	21.04ab	0.30 a	0.36ab	59.77abc	71.54ab	16.03ab	17.64a	43.73abc	53.9ab	0.77abc	0.88a

Table 2. Measurement of seedlings at 21 and 38 days after planting (Levels not connected by same letter are significantly different, p=0.05)

In the first days of growing, the seed covering with H_2O_2 favored the seedling growing. The Normal variety presented a minor resistance to *F. moniliforme* despite of being treated with the two coverings. QPM variety was favored by the use of the CH and H_2O_2 treatments when compared with the control.

Seedlings development during the day 14 and 21 of growth was aided by the use of CH and H_2O_2 coverings, since they acted as a source of carbon and nitrogen nutrients that enabled the rapid development of the seedlings when compared with the control. However, this effect was diminished after this time; this might be because the seedlings began to develop defense mechanisms when exposed to CH and H_2O_2 sprayings.

In the day 21, it was observed that the use of CH and H_2O_2 covers has different effects for each variety, being less effective for Normal variety than for the QPM ones under *F. moniliforme* infection. Normal variety under *A. flavus* infection grew the most under H_2O_2 treatment.

Qualitative assessment of damage severity

In day 17, all treatments showed curled leaves in both varieties. In day 26, it was observed under stress conditions by *A. flavus* and drought, QPM variety was the most resistant, with an 11% of seedlings without damage, so, under these conditions, the CH and H_2O_2 treatments favour the seedlings health.

Qualitative microbiological evaluation

Although only one group was infected with *F. moniliforme,* this fungus was present in almost all the seedlings, so we can assure that this specie was contained in corn seeds, and it developed easily because of the greenhouse temperature (25-30ºC). In treatment 12, it was observed that the use of CH and H_2O_2 in covering and spraying inhibits the development of this fungal burden in both species. In general, seedlings from QPM species presented more fungal resistance tan the Normal variety (*Table 3*).

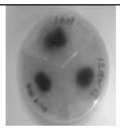
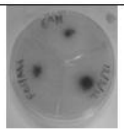
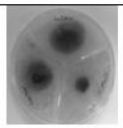
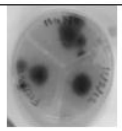
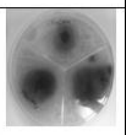
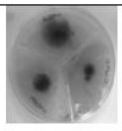
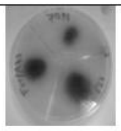
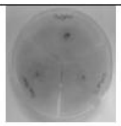
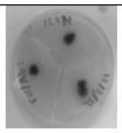
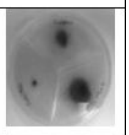
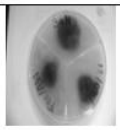
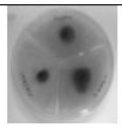
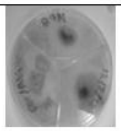
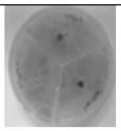
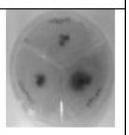
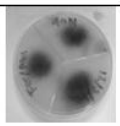
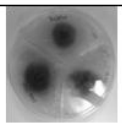
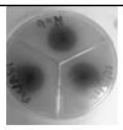
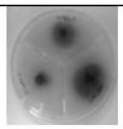
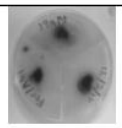
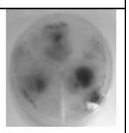
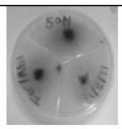
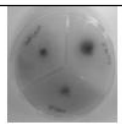
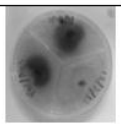
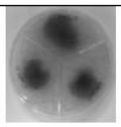
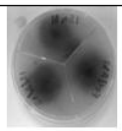
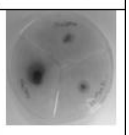
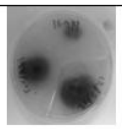
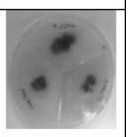
TX	NORMAL	QPM	TX	NORMAL	QPM	TX	NORMAL	QPM
Drought Covering Seed H_2O_2-CH			*A. flavus* Covering Seed H_2O_2-CH Leaf CH			Drought Covering Seed CH		
Drought Covering SeedH_2O_2-CH Leaf CH			*A. flavus* Covering Seed H_2O_2-CH Leaf H_2O_2			*F. moniliforme* Covering Seed H_2O_2-CH Leaf H_2O_2-CH		
Drought Covering Seed H_2O_2-CH Leaf H_2O_2			*A. flavus* Covering Seed H_2O_2-CH Leaf H_2O_2-CH			*A. flavus* Covering Seed H_2O_2 Leaf H_2O_2-CH		
Drought Covering Seed H_2O_2-CH Leaf H_2O_2-CH			Covering Seed H_2O_2-CH			*A. flavus* Covering Seed CH Leaf H_2O_2-CH		
A. flavus Covering Seed H_2O_2-CH			Drought Covering Seed H_2O_2			*A. flavus* Drought Covering Seed H_2O_2-CH Leaf H_2O_2-CH		
						Control		

Table 3. Microbiological evaluation

A. flavus presented more resistance to CH and H_2O_2; this was observed in Treatment 8 (*A. flavus*, covering with H_2O_2-CH, spraying with H_2O_2-CH), observing a significant development of this fungi in both varieties. This could happen because of the humidity given by the application of coverings, because *A.*

flavus overcome the humidity levels generally tolerated by other fungal species (18). It is considered that CH can induce the massive accumulation of fungitoxic substances en the application site, or even a barrier can be built, impeding the nutrients flux towards the pathogen. This last consideration is supported in deterioration signals that frequently show the fungal cells exposed to CH, for example, the abnormal formation of deposits rich in chitinase between the plasmatic membrane and the cell wall (19).

Nutritional Evaluation

Prior to evaluation, seedlings were dried in an oven at 30-35°C for 15 days, reaching moisture percentages of about 2.5-8 for Normal variety and 5-10% for QPM variety.

According to protein content, the QPM variety did not increase the protein content when compared with the control, being the Treatment 5 (*A. flavus*, covering with H_2O_2, spraying with CH, %N= 1.4432813, p=0.0001) and Treatment 9 (covering with H_2O_2, spraying with CH, %N= 1.4031146, p=0.0001) which presented the minor protein content. Unlike QPM, Normal variety showed a higher protein content, being the treatments 4 (Drought, covering with H_2O_2-CH, spraying with H_2O_2-CH, %N= 3.0538419, p=0.0001), 5(%N=2.9644818, p=0.0001) and 9 (%N=3.4213018, p=0.0001) (mentioned above), those which presented a significant difference respecting to the control (%N=2.2148904). This shows that seed covering with H_2O_2-CH favors the nutritional value of the product in the normal variety. In Treatment 3 (Drought, covering with H_2O_2-CH, spraying with H_2O_2, %N= 1.6499999) and 15 (*A. flavus*, covering with H_2O_2-CH, spraying with H_2O_2-CH, %N=1.6452880), it was observed a decrease in the protein content, being the different stresses the possible factors that affect this behavior. The increase in the protein content can be because CH induces defense reactions in plants, being more sensible for answering quicker to pathogens attack, favoring the induction of some substances like phytoalexines, pathogenesis related proteins and protean inhibitors (19). In case of CH, there are purposes that this sensitization occurs because it presence stimulates some known defense mechanisms, like the chitinases and glucanases production. This induction, is not only used for plant protection, but also is used to enhance the yield of some important compounds in the product quality (19).

Respecting to fiber, QPM variety showed significant difference when compared with the control(%N=24.159967, p=0.0001); this means that any of the treatments increase the fiber content, while in Normal variety, the use of CH or H_2O_2 did not represent a significant difference when compared with the control. Best QPM results were found when seed was covered, that is the case of Treatment 3 (Drought, covering with H_2O_2-CH, spraying with H_2O_2, %N=30.053048), Treatment 5 (*A. flavus*, covering with H_2O_2-CH, %N=32.056855) and Treatment 15 (*A. flavus*, drought, covering with H_2O_2-CH, spraying with H_2O_2-CH, %N=29.154248).

According to the Results presented above, the use of CH and H_2O_2 as coatings and sprayings, benefit the seedlings growing in the first development stages, acting as a nutrients font for them; moreover, CH and H_2O_2 help to fight against fungal damage by activating defense mechanisms. CH and H_2O_2 favor the nutritional quality of the product by increasing principally its nitrogen (proteins) content.

Acknowledgments

To PAPIIT Program IT 220411 for the financial support.

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Physicochemical Characterization And Sensory Evaluation Of Two Varieties Of Blackberry: Tupy And Brazos Coated With Nopal Mucilage (*Opuntia ficus-indica)* And Sodium Alginate

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Currently the interest in natural and nutritionally healthy food has increased significantly. It is shown that high consumption of fruits and vegetables is considered inversely proportional to the incidence of various degenerative diseases. Blackberry fruit, has been considered one of the most valuable nutraceutical fruit, its components are associated with the prevention of chronic degenerative diseases.The aim of this study was to analyze the effect of a composite coating on the physicochemical properties of blackberry fruits (control sample,MC) in contrast with blackberry fruits coated with two different polymer matrices, the first based on cactus mucilage 100% w/w alginate + 4% w/w_mucilage (MA), and the second basedcactus mucilage 100% w/w alginate + 4% w/wmucilage and glycerol 5% w/w mucilage (MAG). Two varieties of blackberries were used, Tupy and Brazos, from the town of Ziracuaretiro, Michoacán. The fruits were stored and refrigerated at 4 °C ± 1 °C and analyzed the percentage of weight loss over a period of eleven days, there was a greater decline in the percentage of weight loss in the samples MC with respect to the MA and MAG. Furthermore, the tritable acidity (TA), pH and soluble solids (SS) were evaluated. Was carried out colorimetry of fruits, allowing to appreciate that the luminosity (L) is significantly affected by the factors of storage and the type of coating the fruit, while the coordinate a *, degrees Hue (º F) and color saturation (C) were affected by the type of coating. Furthermore it is concluded that the type of coating and the storage time are significant factors important to the properties of the fruit. Also, conducted a sensory evaluation of fruit samples where MC and MA have a higher acceptability starting the experiment, while at the end the fruits coated with the formulation MAG are the most accepted and best evaluated against other samples. Therefore, the coating contributes to improving the properties of the fruit within the storage time.
Keywords: Blackberry, *Opuntia ficus* indica mucilage, sodium alginate, edible film.

Introduction

In recent years, edible films has received more attention, due to the growing interest for reducing environmental pollution caused by synthetic materials, the need to extend the shelf life of foods, and the increasing demand for healthier foods. Many polysaccharides have been studied for the development of edible films; however, *Opuntia spp* mucilage a hydrocolloid belonging to Cactaceae family could represent a good option for the development of edible films constituting a processing alternative for nopal, and a resource for communities of low income [1]. The growing demand for nutraceuticals is paralleled by an increased effort in developing natural products for the prevention of human diseases. According to several studies demonstrating cladode yielding high values of important nutrients, such as betalains, amino acids, minerals, vitamins, as well as further antioxidants, the cactus pear (*Opuntia spp.*) appears to be an excellent candidate for the inclusion in food. In this regard, the blackberry also has recently received a lot of attention for improving overall well-being, as well as in the preservation of health. Both have been reevaluated and recognized as valuable sources of nutraceuticals. The aim of this work was to evaluate the effect of mucilage - alginate based film on the color, tritable acidity, soluble solids and sensory profile of blackberry fruits stored at refrigeration temperature.

Materials and methods.

Materials. Mucilage obtained from fresh cladodes of *Opuntia ficus indica* cultured at Instituto Tecnológico de Celaya, Celaya, Guanajuato, Mexico, and stored at 4 ◦C until mucilage extraction. Harvesting of cladodes was carried out at 12 pm. Glycerol ACS (Fermont Lot: 818333, Monterrey México), sodium alginate (Sigma Aldrich. Viscosidad 20.000 - 40.000 cps).

Mucilage extraction. *Opuntia ficus indica* cladodes were crushed for 5 min at 25 ◦C. The mixture was centrifuged (4000 × *g*, 15 min, at 25 ◦C) in a Hermle Z-383 (Hermle-Labortechnik, Wehingen, Germany) centrifuge. Supernatant was recovered and precipitated was discarded.

Film-forming dispersion. Dispersion consisted of mucilage, sodium alginate, and glycerol, at a ratio of 91: 4 : 5 % w/ w, respectively. Dispersion was heated for 20 min at 70 °C (to prevent enzymatic action). This procedure ensures an homogeneous dispersion. The pH of the film forming dispersion was adjusted with sodium hydroxide solutions (0.1 N), to obtain a pH values of 8,3. Film-forming dispersion was maintained 2 h under magnetic stirring and stored overnight at room temperature (25 ◦C). Film-forming solutions were subjected to vacuum for 20 min to eliminate trapped air.

Coating of Blackberries. Both Tupy (T) and Brazos (B) blackberry varieties were sprayed with the coating solution and drained on plastic screens for 30 minutes until drying with a flow air velocity of 3.5 m/ s. Blackberries were packed into clamshell boxes and stored at 4º C during 11 days. Samples were defined as: MC (uncoated), MA (coated with mucilage and alginate) and MAG (coated with mucilage: alginate : glycerol)

Characterization of blackberry coated with *Opuntia ficus* indica mucilage – sodium alginate films.

Fruit color surface measurement. Color values of the blackberries covered with mucilage films were determined using a Chroma Meter CR-400 (KONICA MINOLTA Camera Co., Osaka, Japan). The Chroma meter color scale was used for measuring color values: L*, the lightness variable; a*, from green to blue, b*, from yellow to red, C*, chroma and °h, hue angle. Readings were obtained in the CIELAB scale; hue angle (h∗) value was calculated by tan−1(b∗/a∗), and chroma (C∗) by (a∗2 + b∗2)1/2. Surface color was determined as means of 5 measurements at different locations for three fruits from each replicate.

Total Soluble Solids (TSS), Tritable Acidity (TA) and pH Determinations. **Three fruits from each replicate were squeezed in a hand press, and the juice was analyzed for TSS, TA and pH. TSSA was determined at 25º C using a portable refractometer (ATAGO, ATC-1E, Japan). TA was determined by titrating 3 mL of blackberry juice mixed with 3 mL of distilled water to pH 8.2 with 0.1 N NaOH (AOAC 932.12). pH was measured with a pH-meter (OAKTON, Singapour). Three replicates were carried out for all the measurements.**

Weight loss. Weight loss of fresh blackberries during storage was measured daily by monitoring the weight and size changes of six fruits from each treatment.

Statistical Analysis

Significant differences were evaluated by ANOVA and Tukey (HSD) multiple comparison tests means at 0.05% significant level with Statgraphics® computer program (Statgraphics Software, Inc., Centurion XV.I, USA).

Results and discussion

One of the major parameters to determine the fruit senescence is loss of texture, which is dependent on both cell wall degradation and loss of turgidity of the tissue (Ribeiro *et al.*, 2007). As a consequence a significant amount of water vapor is lost and fruits get dehydrated easily. The water vapor transference, is mainly responsible for this loss of weight, occurs generally through the hydrophilic part of the film. During 11 days the weight loss of the varieties Tupy and Brazos blackberries were evaluated. Blackberry Tupy samples containing alginate and glycerol (TMAG) showed a weight loss of 12.63% compared to their initial weight, even it can be seen that weight loss in this treatment is lower than that presented

uncovered Tupy sample (TMC), and Tupy covered with mucilage and alginate (TMA) samples with 18.19% and 14.15% respectively (Fig. 1). Blackberry Brazos fruits covered with mucilage, alginate and glycerol (BMAG) had a weight loss of 23.63%, while in the control samples (BMC) was 21.79% and the sample with alginate(BMA) was 24.84%. Results obtained previously in our laboratory by Arredondo showed a 25% weight loss compared with the present study.

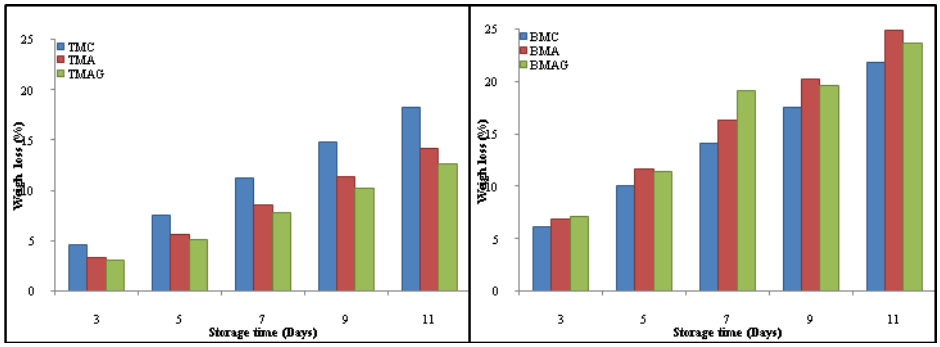

Figure 1. Effect of coating treatments on weight loss (%) in relation to the storage time (days) and blackberry variety. Samples were defined as: T (Tupy); B (Brazos); MC (uncoated); MA (coated with mucilage and alginate) and MAG (coated with mucilage: alginate : glycerol)

Commonly color changes in fruit color are used as an indicator of maturation (Ribeiro *et al.*, 2007). In this work there were significant differences in C values among the samples during the storage period. There was a slight increase in the measurement of fruit surface color lightness (L) (Table 1) and hue angle during the eleven days of storage period. Coordinate a* and b* presented variations among treatments. In this research the blackberries were cultured in Ziracuaretiro, Michoacán, many other factors affected the conditions of the fruit.

Table 1. Effect of coating treatments on color attributes of two blackberry varieties after 11 days of storage at 4° C. Samples were defined as: T (Tupy); B (Brazos); MC (uncoated); MA (coated with mucilage and alginate) and MAG (coated with mucilage: alginate : glycerol)

	L*	a*	b*	C*	hº
TMC	39.06±3.88	1.63±1.07	-0.02±0.40	1.68	231.86
TMA	40.20±4.26	2.59±1.04	0.35±0.34	2.64	77.92
TMAG	38.65±4.88	2.33±0.75	0.27±0.28	2.69	128.95
BMC	40.80±2.69	2.25±1.10	0.18±0.43	2.28	101.56
BMA	41.11±4.14	3.37±0.78	0.52±0.24	3.34	60.12
BMAG	38.47±3.14	2.86±0.65	0.42±0.24	2.90	52.94

Fig. 2 shows the results of physicochemical properties of the samples. pH of refrigerated blackberries reached the highest values of pH. Tritable acidity (TA) values in all the samples studied were between 0.5 and 0.7. These values were close to the values cited in the literature [3]. The relation between acidity and sweetness increased during storage period in all the samples.

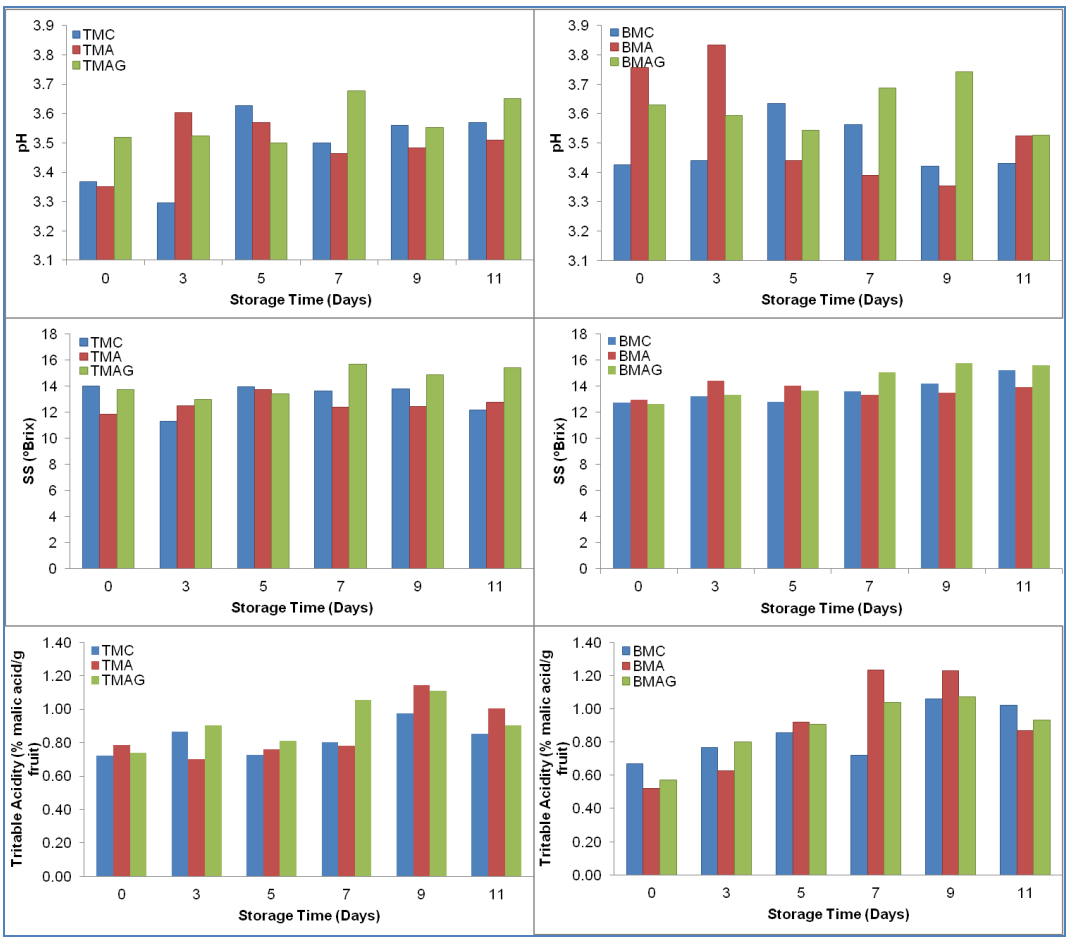

Figure 2. Effect of coating treatments on the physicochemical properties in relation to the storage time (days) and blackberry variety. Samples were defined as: T (Tupy); B (Brazos); MC (uncoated); MA (coated with mucilage and alginate) and MAG (coated with mucilage: alginate : glycerol)

Change in texture is a consequence of the natural process of senescence and also of the atmosphere in which the fruit is stored [4]. Sensorial analysis showed that odor, color, firmness and flavor were affected by the day of storage as well as the coating; moreover there was an interaction among the day of storage and the treatment of the blackberry. Although low temperature increases the shelf-life of the fruit it does have some detrimental effects on the fruit's structure as changes in structural integrity and off flavor development due to cell walls insides the blackberry break down during cooling process.

Values of sensory attributes increased during storage period in all the samples, especially with the variety Brazos. This behavior is similar to different berry fruits corresponding to fruit softening as a result of postharvest senescence [5]. It has been well established that fruit texture is closely related to cell wall structure and composition and fruit softening is the consequence of disassembly of primary cell wall and middle lamella structures. Ripening-related softening of fruit tissues is generally related with a decrease in the strength of cell-cell adhesion. As a result, tissue fracture increasingly occurs by cell separation as ripening process.

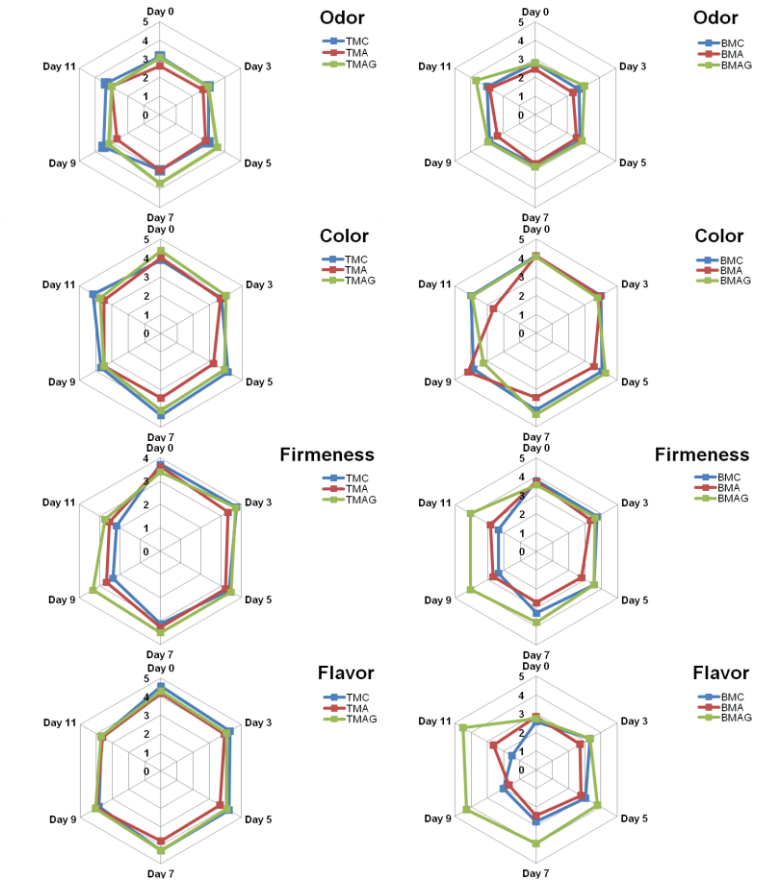

Figure 3. Effect of coating treatments on sensory attributes of two blackberry varieties storage at 4°C during 0 to 11 days. Samples were defined as: T (Tupy); B (Brazos); MC (uncoated); MA (coated with mucilage and alginate) and MAG (coated with mucilage: alginate : glycerol)

Conclusions

Opuntia ficus indica mucilage is able to form a continuous edible film. The coating contributes to improving the physicochemical and textural properties of the blackberries within the storage time.

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Effect Of The Type Of Packaging On The Quality Factors Of White Prickly Pear (*Opuntiaalbicarpa*)

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Abstract

Prickly pear is a fruit with delicate flavor, short shelf-life and short harvest period (June - July). The aim of this research was to evaluate the effect of different packaging condition on the shelf-life of prickly pear. White prickly pears, without physical and microbiological damages, were sanitized using a chlorine solution (150 ppm) during one minute. Fruits were packaged at different conditions: a) high density polyethylene bags with perforations, b) high density polyethylene bags with vacuum, c) low density polyethylene bags with passive atmosphere, d) low density polyethylene bags with vacuum. Fruits without package were used as control. Prickly pears were stored at 4°C and analyzed in total soluble solids, pH, color, weight, texture, phenolic compounds, antioxidant activity ($ABTS^+$ assay), and moisture content every seven days during 49 days. All results were analyzed by ANOVA using the Minitab 16 program. It was observed that total soluble solids and pH significantly ($P<0.05$) decreased during the storage time in all prickly pears. Fruits stored in bags had the lowest moisture and weight losses. Prickly pears stored with vacuum conditions presented the highest total change in color (ΔE) in the pulp and skin with maxima values of 28.7±4.8 and 15.9±4.5, respectively. The texture of fruits was similar for control, fruits packaged in high density polyethylene bags with perforations, and fruits packaged in low density polyethylene bags (vacuum). After 49 days of storage, the phenolic compounds content and the antioxidant activity were significantly higher ($P<0.05$) in fruits stored in low and high density polyethylene bags with values of 30.94±0.4 mg of Gallic acid/100 mL of juice and 77.61±0.5 mg of trolox/100 mL of juice, respectively. The packaging of white prickle pears in low and high density polyethylene bags could be an alternative to offer to consumers prickly pears with good quality and appearance for longer periods.
Keywords: Prickly pear, packaged, antioxidant activity, phenolic compounds.

Introduction

White prickly pear (*Opuntiaalbicarpa*) is an exotic fruit native from México and until now is the most commercially of the cactaceae. Is a very sweet fruit whit a lot of seeds and have a great attribute like phenolic compounds, ascorbic acid, and antioxidant activity (1).The principal problem of prickly pears is the short shelf life (10 days at room temperature) and the short time of harvest (June-July). Therefore, is important to work in finding alternative to enhance the shelf life and offers to the consumers a prickly pear whit a great quality.Some operations of postharvest that could enhance the shelf life of prickly pear were gathering, cleaning, disinfection, storage, refrigerated transport and the possible application of modified or controlled atmospheres (2).

The Modified atmosphere packagingis a technologyused to extend the shelf life offresh fruits and vegetables, and has also been used for prepared food for busy consumers. Fruits and vegetables continue breathing after being cut from their source of nutrients (3). Therefore it is important to consider the type of package, atmosphere condition, and temperature of storage to each kind of fruit and vegetable. The aim of this research was to evaluate the effect of different packed condition on some quality characteristics of white prickly pear.

Materials and method

Prickly pear.White prickly pear (*Opuntiaalbicarpa*) were sorted free from physical and microbiological damages. Fruits were washed, disinfected in a hypochlorite sodium solution (150 ppm) during 1 minute, and dried with absorbent paper. Fruits were packaged at different conditions: a) high density polyethylene bags with perforations (HDPBP), b) high density polyethylene bags with vacuum (HDPBV), c) low density polyethylene bags with passive atmosphere (LDPBPA), d) low density polyethylene bags with vacuum (LDPBV). Unpackaged fruits where used as control (WOP). The fruits were storage during 50 days at 5°C.

Figure 1. White prickly pear (*Opuntiaalbicarpa*)

*Weight loss (Wl).*Prickly pears were weighed before (*Wi*) and after (*Wf*) each storage period. The weight losses were calculated as $Wl=[(Wi-Wf)/Wi]x100$.

*Physicochemical characteristics.*The pulp of the prickly pear was used for determining pH, total soluble solids, phenolic compounds and antioxidant activity. Total soluble solids and pH were determined using the 932.12 and 981.12 AOAC official methods (4), respectively.

Color.The *L*, *a*, and *b* color parameters was measure in whole and pulp prickly pear using a Gardner Colorgard®System05 (Geretsried, Germany) colorimeterin the reflectance mode.

Texture.The penetration test was performed in whole prickly pear using a TA-XT2 texture analyzer (Stable Micro Systems, Haslemere, England). The prickly pear was penetratinguntil reaching a deep of 5 mm using a needle of 1 mm in diameter and a speed of 1 mm/s.

Moisture. The moisture of prickly pear skin was performance accordance of the 934.06 of the AOAC (2000) method.

*Phenolic compounds.*2 mL of distilled water were placed in an amber glass tube with 200 µL of the Folin and Ciocalteu´s phenol reagent (Sigma-Aldrich, Toluca, Mexico) and 100 µL of prickly pear juice. This mixture was homogenized. After 3 min (25°C), 1 mL of 20 % (p/v) Na_2CO_3 was added and thoroughly mixed. This blend was incubated for 1 hour at room temperature in a dark environment. The absorbance was measured at 765 nm using a UV-visible spectrophotometer model 2800H (UNICO, NJ, EUA). Astandard curve of Gallic acid was prepared to assessed total phenolic compounds as follow: GA= [(A-b)/m]*100. Where *GA* is the Gallic acid content (mg Gallic acid/100 mL), *A* is the absorbance of the sample, *b* is the intercept (abs) and *m* is the slope (abs/mg GA/100 mL of juice). The standard curve was $y = 4.108x-0.010$ ($R^2 = 0.989$).

*Antioxidant activity.*The antioxidant activity was performance according to the Kuskoski*et al*. (5) methodology. The $ABTS^+$ radical was obtained placing 5 mL of distilled water, 3.3 mg of potassium persulfate (Sigma-Aldrich, Steinheim, Germany), and 19.4 mg of the ABTS(Sigma-Aldrich, Steinheim, Germany) reagent into an amber glass flask. Reagents were totally mixed and let stand for 16 hours in a dark environment. Afterward, absolute ethanol was mixed with the $ABTS^+$ radical (ABTS radical solution) until reaching an absorbance of 0.70 ± 0.02 at 754 nm. Eighty µL of prickly pear juice were

mixed with 3,920 mL of the ABTS radical solution, and the initial absorbance was measure. The antioxidant activity was calculated using a trolox standard curve.

Statistical analysis. The results were analysis by ANOVA using the Minitab 14 program. A p value of 0.05 was used for deciding significant differences among averages.

Results and discussion

*Physicochemical characteristics.*pH and total soluble solids of white prickly pear stored in different packaging decreased during the time of storage. pH decreased from 6.0 to 4.9 and 4.8 in prickly pears packed in LDPBPA and WOP, respectively. Prickly pear in bags (HDPBV, LDPBPA, and LDPBV) showed the highest loss of total soluble solids. The decreases in pH and total soluble solids was probably due to senescence of fruits, several researchers have reported that pH and total soluble solids decreases with the time of storage.

*Weight loss.*Figure 1 show the weight loss of prickly pearstored at different packed. It was observed that unpackaged prickly pear (WOP) presented the higher weight loss, followed by the fruits packed in HDPBP due to perforations. The fruits packed in HDPBV, LDPBPA, and LDPBV did not presents weight loss significant (P>0.05) during the time of storage. One of the mainfunctions of the packaging is to delay the dehydration of the fruits and vegetables because is one of the factors that can cause postharvest losses.

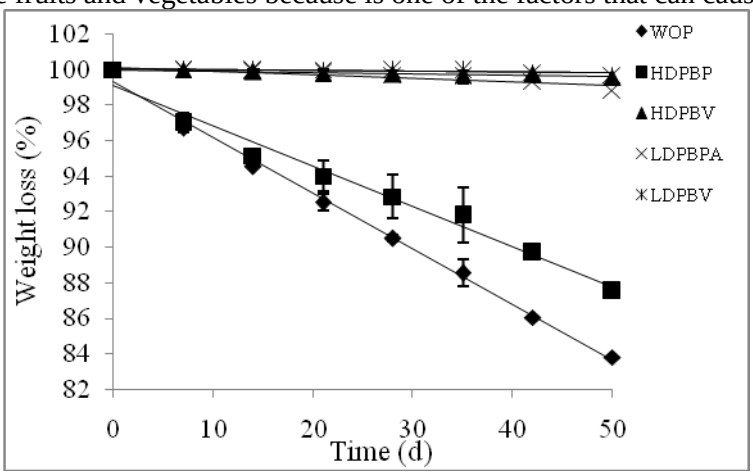

Figure 2.Weight loss of white prickly pear stored in different packaging.

*Color.*Figure 3 presents the total color change in the pulp of prickly pear stored in different packaging. Prickly pears stored under vacuum condition (HDPBV and LDPBV) had the highest total color change with values of 28.0±0.6 and 28.7±4.8, respectively. This change in color is evident to the naked eyes, and color change from pale green to crystalline green. The fruit packed in HDPBP and WOP had the lowest color change.

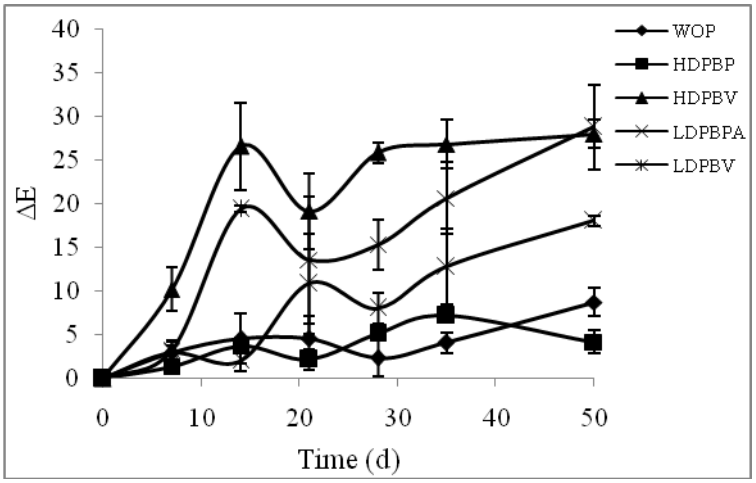

Figure 3. Total color change in pulp of white prickly pear stored at different packaging.

Figure 4 shows the total color change in prickly pear peel stored at different packaging. At the end of the storage time, the fruits packed in polyethylene bags with passive atmosphere (LDPBPA) had the lowest ($P < 0.05$) color change with a value of 7.6 ± 0.6. Unpackaged prickly pear presented similar values to the prickly pear packed with passive atmosphere; however, at the end of the storage time, unpackaged prickly pear showed an increase in the total color change probably due to dehydration which causes a brown color.

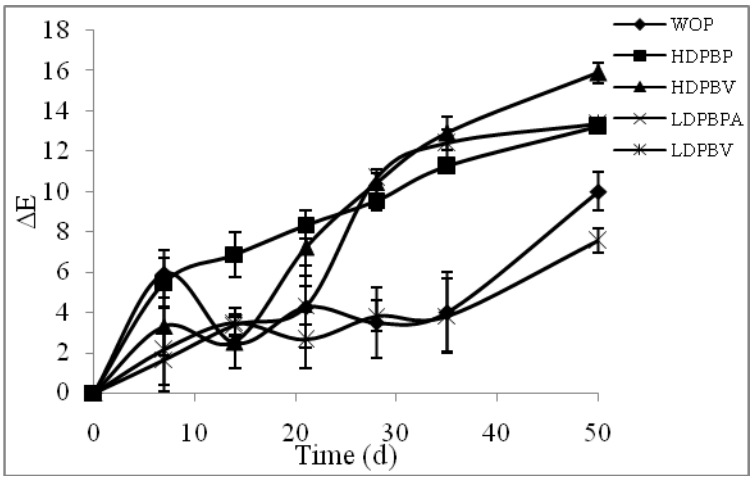

Figure 4. Total color change in prickly pearpeel stored at different packaging.

*Moisture.*Moisture on the prickly pear peel is presents in figure 5. Unpackaged prickly pear (WOP) and fruits packed in high density polyethylene bag with perforation (HDPBP) showed the lower moisture at the end of storage (5°C). This is because the interaction between the fruit and the atmosphere that causes the higher loss of water. The package is capable to maintain the moisture of the fruit during the time of storage.One of the main factors of spoilage of fruits and vegetables is moisture loss, which can be reducing with suitable package.

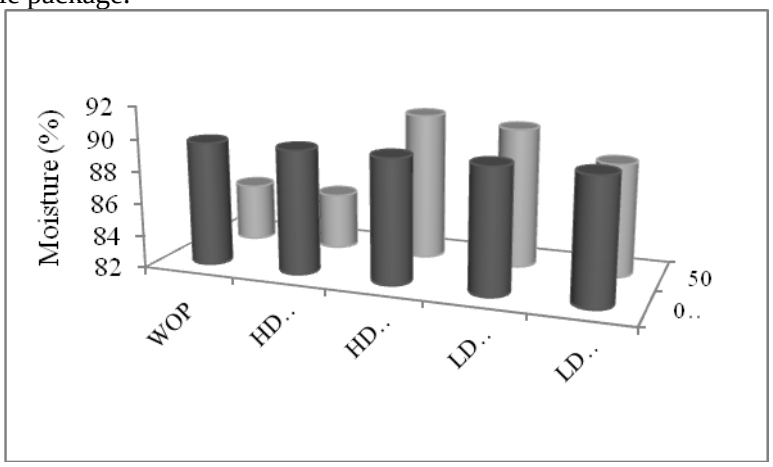

Figure 5. Moisture on the prickly pear peel stored at different packaging.

Firmness. The firmness is one of the most important physicochemical characteristics of fruits and vegetables. Figure 6 shows the firmness (penetration) ofprickly pear stored at different packed. Unpackaged prickly pear showed an increase of the resistance to the penetration, probably because the loss of water that increases the rigidity. Fruits packed in HDPBV and LDPBPA shows the greaterloss of firmness; however, did not exist significantly difference ($P < 0.05$) in all treatments.

*Phenolic compounds and antioxidant activity.*Table 1 and 2 shows the phenolic compounds and antioxidant activity of prickly pear stored at different packed, respectively. In all treatments phenolic

compounds and antioxidant activity decreased during the time of storage. However, the fruits that hadcontact with the air (WOP and HDPBP) shows the greatest loss of phenolic compounds; this is probably becausethe air could act as substrate for the oxidases enzymes that degrade the phenolic compounds. As a result of the phenolic compounds reductions it was observed a decreases in the antioxidant activity. Piga*et al*.(6)informed that phenolic compounds are mainly responsible of the antioxidant activity of prickly pears. Unpackaged prickly pear (WOP), prickly pear packed with perforation (HDPBP) and packed in low density polyethylene with vacuum (LDPBV) presents the lower antioxidant activity.

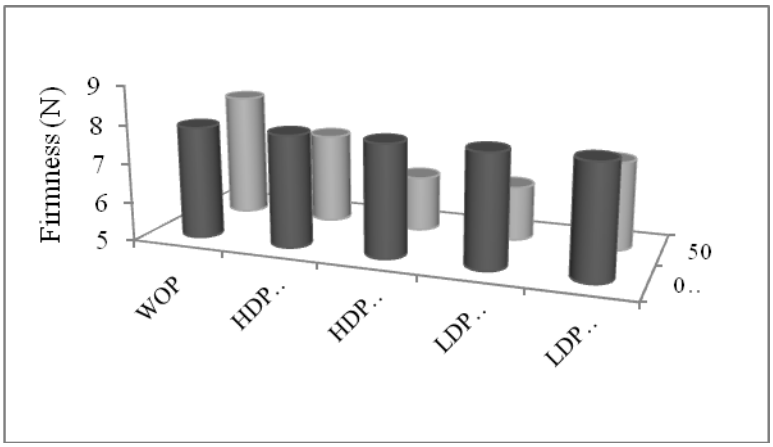

Figure 6. Firmness in white prickly pear stored at different packaging.

Table 1. Phenolic compounds in prickly pear storage at different condition package.

Time (d)	WOP	HDPBP	HDPBV	LDPBPA	LDPBV
	(mg of gallic acid/100 mL of juice)				
0	$39.6\pm2.3_a$	$39.6\pm2.3_a$	$39.6\pm2.3_a$	$39.6\pm2.3_a$	$39.6\pm2.3_a$
14	$32.3\pm0.5_b$	$35.3\pm0.7_a$	$35.4\pm0.9_a$	$31.8\pm0.9_b$	$34.9\pm0.5_a$
28	$36.7\pm0.9_a$	$37.5\pm3.8_a$	$44.9\pm0.2_a$	$39.8\pm0.2_a$	$36.7\pm0.4_a$
35	$27.2\pm1.9_b$	$25.1\pm0.9_b$	$39.6\pm0.8_a$	$29.4\pm0.8_b$	$39.6\pm2.3_a$
50	$29.9\pm1.9_b$	$29.7\pm0.5_b$	$35.8\pm0.8_a$	$32.7\pm0.9_b$	$32.6\pm1.5_a$

Table 2.Antioxidant activity in prickly pear storage at different condition package.

Time (d)	WOP	HDPBP	HDPBV	LDPBPA	LDPBV
	(mg de trolox/100 mL of juice)				
0	$135.3\pm7.3_a$	$135.3\pm7.4_a$	$135.3\pm7.5_a$	$135.3\pm7.6_a$	$135.3\pm7.7_a$
14	$126.2\pm6.7_{ab}$	$122.3\pm8.2_{ab}$	$127.7\pm7.8_a$	$108.2\pm7.6_b$	$124.8\pm2.7_b$
28	$116.6\pm2.8_{ab}$	$118.4\pm8.9_{ab}$	$138.3\pm8.7_a$	$110.6\pm7.8_b$	$114.4\pm2.4_b$
35	$109.6\pm6.3_{bc}$	$113.7\pm3.6_{ab}$	$90.8\pm9.2_b$	$118.2\pm2.5_b$	$110.2\pm3.0_{bc}$
50	$102.2\pm5.5_{bc}$	$103.5\pm9.7_b$	$103.4\pm0.8_b$	$115.3\pm2.2_b$	$100.3\pm0.2_c$

Conclusions

The packaging is a good way to enhance the shelf life of white prickly pear. After 50 days of storage it was observed that prickly pear packed in low density polyethylene bags with passive atmosphere showed high moisture and low weight loss, an acceptable color change in pulp, the lower color change in the peel of the prickly pear, these factors are very important to the consumers.Phenolic compounds were higher in prickly pear packed than in the prickly pear in contact with air. The prickly pear packages in the other conditions did not show better characteristics than unpackaged prickly pear.

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Elaboration Of A Carboxymethylcellulose/Gelatin Based Edible Coating For Application In Minimally Processed Golden Delicious Apple And Cantaloupe Melon.

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Introduction

An edible coating is defined as a thin layer of material applied on the surface of food products. Edible coatings may contribute to improve quality and shelf life of food by acting as a semipermeable barrier to control the mass transfer between the food and the storage environment. They also have a potential to act as vehicles of additives such as antioxidants, antimicrobial agents, colorants, flavors, nutrients, spices (1).

Edible coatings can be produced from polysaccharides, proteins, lipids or mixtures of biopolymers. The carboxymethylcellulose (CMC) is a cellulose derivative used in many foods by its viscosity, water-binding capacity and solution clarity. The gelatin is a hydrocolloid obtained by acidic or basic hydrolysis of the collagen and used in the food, pharmaceutical and photographic. Both components have been effective in extending the shelf life of fruits like cherries, strawberries, mango, banana and apple (2).

Apple and melon are among the most important fruits in Mexico with 7.18 and 3.94 kg, respectively in consumption per capita; furthermore, Golden delicious apple and Cantaloupe are the most produced varieties, 50% and 51% respectively of the national production (3).

In Mexico, 75% of the fruit is consumed fresh and only 0.60% is consumed as minimally processed fruit. However, the current trend of healthy food consumption and change in lifestyle, can lead to increase consumption of minimally processed fruit, not only for consumption on individual presentations, but also for marketing in restaurants, hotels and foodservice (4).

However, minimally processed fruits deteriorate faster than whole fruit due to damage suffered during the preparation process, therefore, is more difficult to market. Edible coatings can be an alternative to preserve minimally processed fruits to reduce moisture loss, migration of solutes, gas exchange, respiration and oxidation reactions.

Therefore, the aim of this study was to develop an edible coating based on carboxymethylcellulose / gelatin and evaluate its effect on the physicochemical characteristics of minimally processed fruit (Golden delicious apple and cantaloupe).

Methods and materials

Materials

The apples and melons were purchased at Toluca's *Central de Abastos* market at commercial maturity. Edible coating was elaborated with food grade CMC (WALOCEL^{TM}CRT 10000 y CRT^{TM} 30000, Down Wolff Cellulosics, Germany) and gelatin type A with bloom of 250 (Gelita, Mexico).

Preparation of edible coating solutions

Coatings solutions were prepared with different concentrations of CMC/gelatin as shown in Table 1, these concentrations used ***were*** based on previous work in whole fruit. . CMC was dissolved in distilled water at 75 ° C and stirring until completely dissolved. Similarly, gelatin was dissolved in distilled water at 60 ° C with constant agitation until complete dissolution. Then CMC and gelatin solutions were mixed and heated to 80 ° C for 10 min on hotplate with constant agitation. Finally, the solutions were cooled to room temperature.

Table 1Composition of coating solutions

Solución	CMC (%)	Grenetina (%)
B[1]	50*	50
C[1]	75*	25
D[2]	25	75
E[2]	50	50

1: used in Apple, 2: used in melon, *CMC CTR 10000

Sample preparation

Apples and melons were washed and sanitized by immersion in Citrus21® (7gotas/l) for 15 min, rinsed and dried prior cutting operations. Then, apples were cut into wedges, 8 from each apple. Melons were cut into 8 slices and each slice was cut into 6 pieces. Apples and melons pieces were dipped into coating solutions for 2 min and residual solutions was allowed to drip off for 1 min, in control sample no treatment was applied. Finally, 4 wedges of apple or 50g of melon were packed into Ziploc plastic bags and storage during 14 days at 4°C and 7 days at room temperature. Analyses were carried out in duplicated at 0, 4, 7, 10 and 14 days at 4°C or 0, 4, 7 days at room temperature. The whole study was performed in triplicate.

Water loss

Weight loss of apple and treatment of melon was monitored weighing an apple wedge and a piece of melon. A BOECO BBC22 digital balance (BOECO, Germany) was used. The percentage of weight loss was determined by difference from the first determination.

Firmness evaluation

The firmness evaluation was performed using a Shimadzu EZ Test texture analyzer (Shimadzu Scientific Instruments, Japan) by measuring the maximum penetration force required for a 3 mm diameter probe to penetrate into an apple cylinder of 12 mm diameter by 10 height to a depth of 5 mm at a rate of 1 mm.s-1. In the same way, a cylinder of 21 mm diameter by 15 height to a depth of 7.5 mm at a rate of 1 mm.s-1 was used for melon.

Color evaluation

Color was directly measured with a CR-400 Minolta chroma meter (Minolta, Inc., Japan). L (lightness, 0 black to 100 white), a (chromaticity -60 green to 60 red) and b (chromaticity -60 blue to 60 yellow) were measures in Hunter scale. The color was measured in an apple wedge and melon piece, in both fruits in 6 different points. Color change in apple was determined by L and the browning index (BI) obtained by Eq. 1 and 2 (5). The browning index represents coffee color purity and is an important parameter in enzymatic browning processes. For cantaloupe change in color was determined by L and chroma obtained by Eq. 3 (6). Chroma value (scale 0 to 60) represents the color intensity, a higher value of this equates to a more brilliant orange color for Cantaloupe melon.

$$\mathbf{IP} = \frac{\mathbf{100(x - 0.31)}}{\mathbf{0}}\mathbf{.172} \quad \text{(Eq. 1)} \qquad \mathbf{x} = \frac{\mathbf{a + 1.75L}}{\mathbf{5.645L + a - 3.012b}} \quad \text{(Eq. 2)}$$

$$\mathbf{Croma} = [(\mathbf{a})^2 + (\mathbf{b})^2]^{\frac{1}{2}} \quad \text{(Eq. 3)}$$

Determination of soluble solids, titratable acidity and pH

The soluble solids, titratable acidity and pH were measured in the fruit juice. The juice was extracted from apple wedges or melon slices using a standard extractor Turmix (Turmix Mexico, Mexico). Soluble solids were measured with a digital refractometer Atago Palette PR-201α (CO ATAGO., E.UA) and expressed as ° Brix at 20 ° C. The acidity was determined by titration with 0.1N sodium hydroxide according to the NMX-FF-011-1982. The acidity is expressed as mg of malic acid/100ml in apple and mg of citric acid/100ml in melon. The pH was determined according to the NMX-F-317-S-1978 using a digital potentiometer Conductronic (Model PC18, Mexico).

Data Analysis

An analysis of variance (ANOVA) was performed to compare means values for different coatings and control samples and Tukey's test was applied at a 5% significance level in SPSS software (Version 15.0, SPSS Inc. Chicago, IL).

Results and discussion

Water loss

An increase of weight loss over time of storage was observed in both fruits, showing no significant difference in apple between the treatments applied at both temperatures (Table 2). However, in apple was observed that E treatment had the lowest weight loss (6.78%) when compared to control (7.52%). In contrast, in melon there was significant difference ($p < 0.05$) between control and treatment C (75% CMC/25% gelatin) under refrigeration as shown in Fig. 1. At room temperature no significant differences were observed.

Table 2 Weight loss in apple at 4°C (%)

Days	Control	D	E
0	0.00	0.00	0.00
4	1.34	1.79	3.35
7	4.23	4.94	4.95
10	5.88	5.73	4.98
14	7.52	7.55	6.78

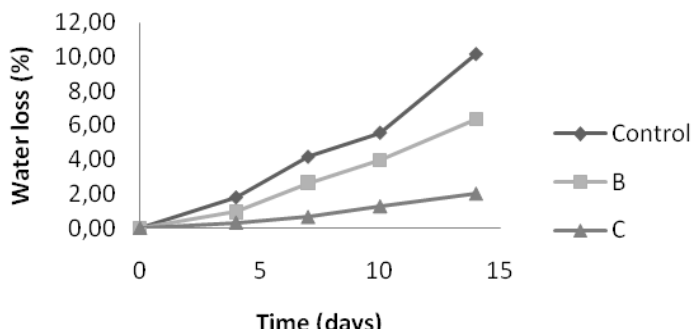


Fig. 1 Water loss in Cantaloupe melon at 4°C

The loss of water in the fruits is given mainly by transpiration due to the gradient between the fruit and the environment. Minimally processed fruits have no outer protective cover that regulates weight loss in whole fruits making them more susceptible to this. Edible coatings can act as a semipermeable barrier to water vapor and gases, impacting the flow of water and metabolic processes. The lowest weight loss treatment C (75% CMC/25% gelatin) can be attributed to the higher CMC content; it has been shown that the CMC has a better water barrier than gelatin, probably due to its highly crystalline nature (7). In 2012 Ghanbarzadeh et al. found that the addition of CMC to a film of starch decreased the permeability to water vapor (8).

Firmness

In apple, firmness decreased in all treatments, both room and refrigerated temperature, without significant difference between treatments. Figure 2 shows the firmness changes in coated and uncoated melon. Significant difference ($p < 0.05$) was found between the coated and uncoated refrigerated melon and treatment C was the most effective in maintain firmness with 5.20 N compared to control (0.71N).

The loss of the fruit texture is mainly due to the structural change of cell wall components, for example the solubilization and depolymerization of pectin (9). The minor loss of texture on the coated melon can be attributed to the ability of edible coatings to act as selective barriers to gases as O_2 and CO_2, consequently reducing the metabolism. Raybauidi-Massila et al. in 2008 reported that an alginate coating improved texture and decreased ethylene production in minimally processed melon (6).

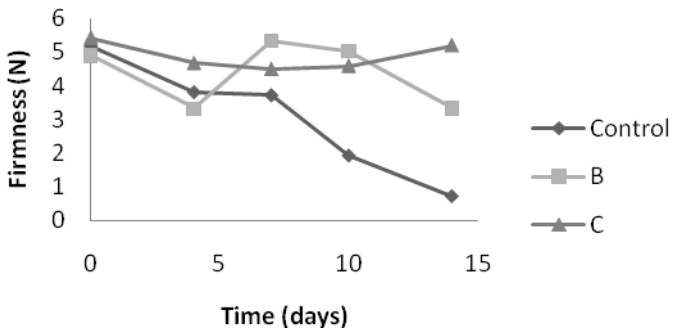
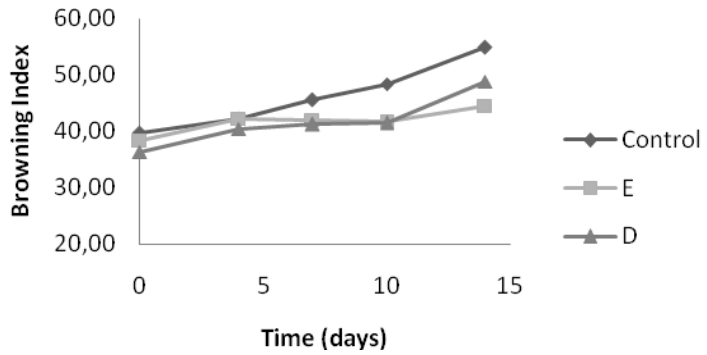


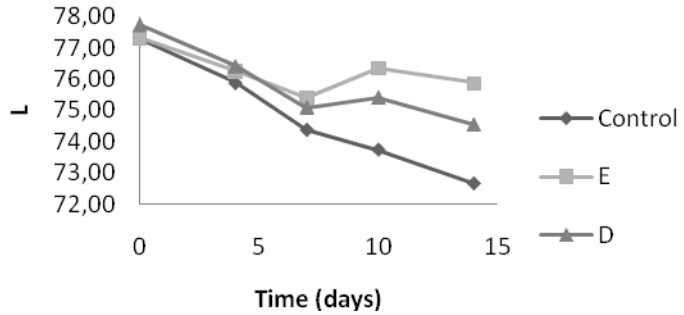
Fig. 2 Firmness in Cantaloupe melon at 4°C

Color

Edible coating had a significant ($p < 0.05$) effect in L and BI in coated apple at 4°C (Figure 3). Treatment E had the less increase in L (75.86) and less decreased in BI (48.61) compared to control (72.66 and 60. 96). Enzymatic browning is the main cause of color changes in apples. The higher L value and lower IB in coated fruit due to the semipermeable barrier formed, which limits oxygen availability in browning reactions. Le Tien, et al in 2001 found that protein-based coatings decreased enzymatic browning in apples and potatoes. No significant differences were found in apple and melon at room temperature and melon at 4°C (10).



A)



B)

Figure 3 Color changes in apple: A) Browning index B) Lightness

In contrast to the control (59.62), melon coated had a higher L value at the end of storage (55.72 and 57.71), in control samples water-soaked areas was observed as result of the loss of tissue integrity. The higher content of CMC produced a better result probably because of micro modified atmosphere formed and subsequently a slower metabolic process. For example, in 2012 Azarakhsh *et al.* reported that a gellan edible coating reduced respiration rate in pineapple minimally processed (11)

Soluble solids, titratable acidity and pH

Edible coating had no significant effect in soluble solids, titratable acidity and pH at 4°C and room temperature in both fruits. However, a decrease in soluble solids was found, which could be attributed to the consumption of sugars as source of energy during the respiration process (12).

Conclusions

In general, CMC/gelatin based edible coatings helps to maintain physicochemical characteristics in melon when storage at 4°C for 14 days, mainly water loss and firmness. On the other hand, in apple only help to slow enzymatic browning.

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Postharvest Quality Of Fruits Of *Opuntia*: Their Potential As Genetic Resources In Arid And Semiarid Regions Of Jalisco

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Abstract

Mexico is considered the origin center of *Opuntia*, even though the genus is cultivated in other American countries. Different species of non-cultivated *Opuntia* are grown in Jalisco, Mexico; characteristics of these *Opuntia* fruits are not described, and in most cases they are only consumed locally. The objective of this research was to evaluate morphologic and postharvest characteristics in fruits of *Opuntia* species from two different regions in Jalisco, Mexico. Morphologic (size, fresh total weight, edible portion weight, peel thickness, seed number), postharvest characters (ascorbic acid, ashes, total acidity, °Brix), and pigment concentration (betaxanthins and betacyanins) were analyzed and compared in 15 varieties belonging to 8 *Opuntia* species from two different regions in Jalisco, Mexico: Ojuelos, an arid region; and Lagos de Moreno, a semiarid region. A completely random experimental design was used considering 10 to 15 fruits of each variety were evaluated. Data were subjected to analysis of variance (ANOVA). Means and standard deviations were calculated and mean comparison was performed using Tukey's Test ($\alpha = 0.05$). Results showed statistically significant differences *($P<0.05$)* in characters among species from the two regions analyzed. *Opuntia ficus*-indica fruits from the arid region of Ojuelos presented the highest values for size (10.5±0.3 cm), total fresh weight (234±9 g) and edible portion weight (124±10 g); and the lowest values for peel thickness (0.2±0.3 mm) compared with fruits from the semi-arid region of Lagos de Moreno. Pigment concentration varied from 0.20 to 0.42 mg/100 g of betaxanthins and from 0.020 to 0.038 mg/100 g of betacyanins in flesh of *O. robusta* and *O. ficus*-indica fruits, respectively. The highest values for ascorbic acid, °Brix and ashes were registered in fruits of *O. ficus*-indica and *O. leucotricha* from Ojuelos, Jalisco, Mexico. The results show the high variability in morphologic and postharvest characters in fruits of *Opuntia* species from different climatic conditions; this information is important and necessary in order to make utilization and conservation proposals for those species and highlight the value of the studied species as phytogenetic resource.

Keywords: *Opuntia*, Cactaceae, fruits, postharvest quality.

Introduction

Mexico is considered the origin center of genus *Opuntia*, which represents an important Cactaceae group including a diversity of species with a variety of special characteristics. *Opuntia* fruits, commonly known as "prickly pear" and "tunas", are ovoid shape; the weight varies from 100 to 200 g, with thick peel, juicy flesh and lots of hard seeds. Two subgenera come from *Opuntia*: *Cylindropuntia*, generally without economic importance; and *Platyopuntia*, which groups species of economic, medicinal and nutritional importance (1,2). Streptacantha is an interesting *Opuntia* group with arborescent characteristics, big yellow to orange flowers, and edible fruits (3,4). In Mexico, different species of non-cultivated *Opuntia* grow abundantly, especially in the highlands, including Zacatecas, San Luis Potosí, Aguascalientes, Durango, Guanajuato and Jalisco, the cultivars include shrubby plants like *O. robusta* ("nopal tapón") (Fig. 1A), *O. cantabrigiensis* ("nopal cuijo"), *O. rastrera* ("nopal rastrero"), *O. lindheimeri* ("nopal cacanapo"), *O. leptocaulis* ("nopal tasajillo"); woody vegetation such as *O. leucotricha* ("nopal duraznillo"), *O. streptacantha* ("nopal cardón") (Fig. 1B). All these cacti, together with agaves and mesquite, had represented a source of work and livelihood for the indigenous natives since prehispanic times. Some of these species continue being utilized, mainly in local and regional markets. Many species, including *O. streptacantha*, *O. cantabrigiensis*, *O. leucotricha*, *O. rastrera* and *O. robusta*, are widely

distributed, and present morphological variability (3). *Opuntia* species constitute key organisms in biotic communities from "Altos Norte", located in the highlands of Mexico in the state of Jalisco; they also constitute an important economic resource, because of its potential use in food industry. The objective of this research was to evaluate morphological and postharvest characteristics, as well as pigments content in fruits of *Opuntia* species from two different regions in Jalisco, Mexico, in order to make utilization and conservation proposals for those species; and highlight the value of the studied species as phytogenetic resource.

Figure 1. **A**) *Opuntia robusta* Wentd, **B**) *Opuntia streptacantha* Lemaire

Materials and methods

Mature and healthy fruits of 15 cultivars belonging to eight *Opuntia* species: *O. ficus-Indica* (L.) Mill, *O. cantabrigiensis* Lynch, *O. leucotricha* De Candolle, *O. robusta* Wentd, *O. streptacantha* Lemaire, *O. chavena* Griffiths, *O. undulata* Griffiths, *O. icterica* Griffiths were collected in two different regions of Jalisco, Mexico: Lagos de Moreno (21° 31´ N, 101° 41´ W, 2,100 masl) a semiarid region; and Ojuelos (21° 33´ N, 103° 09´ W, 2,100 masl) an arid region. All the fruits (10 to 15 of each variety from both collecting places) were carefully selected, cleaned and prepared for morphological characters determination.

Fruit size, equatorial diameter, peel thickness, pulp (edible portion) size were determined using a Mitutoyo digital caliper. Fresh total weight and edible portion weight were determined using an analytical scale (Precisa Gravimetrics, AG. XT220A). After registering of morphological characters, fruits were stored at -80°C, until postharvest characters and pigments determination. Analyses of moisture, ashes and soluble solids (°Brix) were carried out according to the procedures of Association of Official Analytical Chemists (5). Total acidity was determined by titration and results were expressed as citric acid percentage (6). Ascorbic acid quantification was done according to Tillman´s method, based on the reduction of 2,6 dichlorophenol-indophenol (7). Betacyanins and betaxanthins were quantified using the sepectrophotometric method described by Cai *et al*. (8). Previously, pigments were extracted using 80% ethanolic solution. Pigments were determined spectrophotometrically at 474 and 538 nm, for betaxanthins and betacyanins, respectively, and dividing by molar extension coefficient (4.8×10^4 for betaxanthins, and 6.16×10^4 for betacyanins).

Statistical analysis

A completely random experimental design was used considering 10 to 15 fruits of each variety. Data were subjected to analysis of variance (ANOVA). Means and standard deviations were calculated; means comparison was performed using Tukey's Test ($P<0.05$) with the Statistical Analysis System, version 9.1 (9).

Results

Lagos de Moreno, the semiarid region, presented higher diversity of cultivars, 12 of the 15 cultivars analyzed were from this region. Only three cultivars (*O. cantabrigiensis* Lynch "memela"; *O. streptacantha Lemaire* "cardona"; *O. chavena Griffiths* "cascarona") were present in both regions. Results showed statistically significant differences *(P<0.05)* in characters among species from the two regions analyzed. *Opuntia ficus* fruits from the arid region of Ojuelos presented the highest values for length (10.5±0.3 cm) and total fresh weight (234±9 g) (Fig. 2).

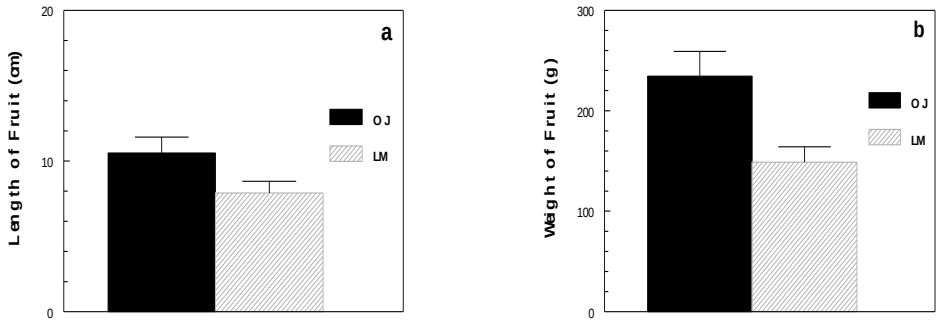

Figure 2. Comparison of morphological characters of *Opuntia* fruits from two regions: Ojuelos (OJ) and Lagos de Moreno (LM), Jalisco, México. a) Length of fruits, b) Weight of fruits. Bars represent the mean + standard deviation.

Morphological characters presented wide variability; *O. ficus* from both collecting places presented the highest dimensions of edible portion (Table 1). While *O. chavena* "cascarona", presented the highest values of peel thickness and their edible portions represented less than 50% of total weight.

Moisture content in all samples analyzed was in the range of 80 to 90%, in accordance to the usual content of moisture in fresh fruits (Table 2). Ashes content varied from 0.35 to 1.0% in Ojuelos' cultivars; and from 0.26 to 1.04% in Lagos de Moreno's cultivars. *O. ficus* "manza-naranja", from Lagos de Moreno, presented the highest value for soluble solids (15.54 °Brix), while, from Ojuelos, *O. streptacantha* "cardona" presented the highest value for soluble solids (14.0 °Brix). The lowest sugar content was determined in *O. chavena* "loba" (7.55°Brix). In general, all the samples presented low acidity levels, from 0.01 to 0.51% of citric acid. The highest values for ascorbic acid were registered in fruits of *O. ficus* from Lagos de Moreno and Ojuelos.

Table 1. Comparison of quantitative morphological characters in fruits of *Opuntia* species cultivars from Ojuelos (OJ) and Lagos de Moreno (LM), Jalisco, Mexico.

	Species	**Cultivar**	**Peel thickness (mm)**	**Pulp weight (g)**	**Pulp size (mm)**	**Pulp diameter (mm)**
	O. ficus	Burrona –blanca	2.4 ^{a}	43.1 ^{a}	47.5 ^{a}	32.5 ^{a}
	O. ficus	Burrona –naranja	2.0 ^{a}	55.4 ^{b}	58.0 ^{b}	42.0 ^{b}
OJ	*O. cantabrigiensis*	Memela	2.9 ^{a}	20.6 ^{c}	44.8 ^{c}	25.2 ^{a}
	O. robusta	Tapona	3.3 ^{b}	47.6 ^{a}	41.0 ^{c}	39.8 ^{b}
	O. streptacantha	Cardona	3.9 ^{b}	29.5 ^{c}	41.5 ^{c}	31.1 ^{b}
	O. chavena	Cascarona	6.0 ^{c}	23.6 ^{c}	35.3 ^{d}	31.1 ^{b}
		Mean	3.4	36.6	44.7	33.6
		CV (%)	41.9	38.5	17.2	18.5
	O. ficus	Manza-naranja	3.2 ^{b}	72.9 ^{d}	56.2 ^{b}	43.2 ^{b}
	O. ficus	Manza-blanca	5.7 ^{d}	103.5 ^{e}	64.7 ^{e}	54.4 ^{c}
	O. ficus	Calabazona	2.2 ^{a}	82.7 ^{f}	70.5 ^{f}	42.7 ^{b}
	O. cantabrigiensis	Memela	4.1 ^{e}	26.3 ^{c}	38.0 ^{d}	31.1 ^{b}

	O. streptacantha	Cardona	3.1 ^{b}	27.4 ^{c}	37.1 ^{d}	30.7^{b}
	O. chavena	Cascarona	9.4 ^{f}	24.4 ^{c}	38.5 ^{d}	32.7 ^{b}
LM	*O. chavena*	Chaveña	4.6 ^{de}	24.1 ^{c}	38.5 ^{d}	29.9 ^{b}
	O. chavena	Loba	4.4 ^{e}	21.8 ^{c}	32.8 ^{d}	28.1 ^{b}
	O. undulata	Sangre de toro	3.6 ^{b}	45.7 ^{a}	55.3 ^{b}	39.9 ^{b}
	O. ictérica	Larguita	3.5 ^{b}	20.1 ^{c}	41.8 ^{c}	26.2 ^{b}
	O. ictérica	Jaraleña	2.9 ^{a}	21.3 ^{c}	35.6 ^{d}	27.2 ^{b}
	Opuntia spp.	Redondilla	4.3 ^{d}	17.8 ^{g}	31.9 ^{d}	27.7 ^{b}
		Mean	4.3	40.7	45.1	34.5
		CV (%)	43.9	71.8	29.0	25.2

Different letters on each column indicate significant statistical differences among cultivars (Tukey, P< 0.05).
CV = Coefficient of variation.

From the 15 cultivars analyzed, only two cultivars ("burrona blanca" and "manza blanca") presented green or white fruits, which shows absence of betalains. Pigment concentration presented wide variability among orange and red cultivars (Table 3); higher concentrations were determined in fruits from the semiarid region (Lagos de Moreno). Betaxanthins varied from 0.020 to 0.052 mg/100 g, while betacyanins ranged from 0.001 to 0.041 mg/100 g of in flesh fruits. *O. chavena* "cascarona" was the cultivar with the highest content of total betalains.

Table 2. Postharvest characters of *Opuntia* fruits from Ojuelos (OJ) and Lagos de Moreno (LM), Jalisco, Mexico.

	Cultivar	**Moisture (%)**	**Ashes (%)**	**Soluble solids (ºBrix)**	**Total acidity (%)**	**Ascorbic Acid g/100g**
	BB	88.05 ± 1.08^{ab}	0.70 ± 0.07^{a}	11.06 ± 0.47^{a}	0.01 ± 0.001^{c}	1.17 ± 0.38^{a}
	BN	86.11 ± 0.63^{ab}	0.35 ± 0.09^{a}	13.21 ± 0.59^{a}	0.12 ± 0.01^{abc}	3.98 ± 0.44^{bacef}
OJ	MEM	85.28 ± 0.46^{a}	0.75 ± 0.37^{a}	12.3 ± 0.35^{a}	0.04 ± 0.005^{b}	0.12 ± 0.01^{c}
	TAP	80.69 ± 2.47^{b}	1.00 ± 0.32^{a}	13.74 ± 0.88^{bad}	0.08 ± 0.006^{bc}	2.00 ± 0.46^{dce}
	CAR	86.77 ± 0.27^{ab}	0.39 ± 0.08^{a}	14.00 ± 0.16^{cda}	0.01 ± 0.002^{c}	0.75 ± 0.07^{e}
	CAS	83.47 ± 0.99^{a}	0.38 ± 0.03^{a}	11.16 ± 0.14^{d}	0.02 ± 0.003^{c}	1.88 ± 0.66^{fc}
	Mean	85.06	0.59	12.57	0.054	1.65
	CV (%)	3.0	44.3	10.2	75.0	81.2
	MN	83.55 ± 0.48^{ab}	0.69 ± 0.12^{b}	15.54 ± 0.25^{d}	0.13 ± 0.03^{a}	2.34 ± 0.37^{c}
	MB	85.86 ± 0.56	0.30 ± 0.03^{a}	12.33 ± 0.23^{a}	0.05 ± 0.02^{a}	1.69 ± 0.18^{d}
	CAL	81.99 ± 4.29^{ab}	0.25 ± 0.02^{a}	12.78 ± 0.31^{a}	0.07 ±0.006^{a}	2.30 ± 0.37^{c}
	MEM	82.83 ± 1.27^{ab}	0.26 ± 0.04^{a}	12.2 ± 0.32^{a}	0.03 ± 0.006^{a}	0.11 ± 0.01^{a}
	CAR	85.12 ± 0.33^{ab}	0.57 ± 0.05^{b}	13.43 ± 0.47^{b}	0.01 ± 0.002^{a}	0.73 ± 0.09^{b}
LM	CAS	79.54 ± 0.98^{d}	1.04 ± 0.21^{b}	12.85 ± 0.41^{a}	0.09 ± 0.01^{cb}	2.91 ± 0.32^{c}
	CHA	88.24 ± 0.48^{c}	0.46 ± 0.06^{c}	9.17 ± 0.40^{e}	0.16 ± 0.003^{ca}	1.11 ± 0.16^{d}
	LOB	90.68 ± 0.90^{b}	0.34 ± 0.07^{a}	7.55 ± 0.42^{f}	0.51 ± 0.01^{bac}	0.64 ± 0.12^{a}
	SDT	86.11 ± 0.41^{ad}	0.52 ± 0.08^{c}	13.43 ± 0.29^{b}	0.05 ± 0.003^{a}	3.01 ± 0.18^{e}
	LAR	87.49 ± 0.74^{ad}	0.52 ± 0.08^{c}	13.66 ± 0.32^{b}	0.01 ± 0.002^{a}	2.50 ± 0.31^{c}
	JAR	83.26 ± 0.53^{ab}	0.43 ± 0.04^{c}	12.74 ± 0.52^{a}	0.01 ± 0.001^{a}	0.97 ± 0.08^{d}
	RED	85.49 ± 0.55^{ab}	0.57 ± 0.06^{c}	13.87 ± 0.66^{b}	0.04 ± 0.004^{a}	1.11 ± 0.17^{d}
	Mean	85.01	0.50	12.46	0.10	1.62
	CV (%)	4	44	17	143	60

Data represent mean ± standard error. Different letters on each column indicate significant differences (Tukey, P<0.05). Cultivars: BB ("burrona blanca", n = 12); BN ("burrona-naranja", n = 12); MEM ("memela", n = 10); TAP ("tapona", n = 12); CAR ("Cardona", n = 15); CAS ("cascarona", n = 12); MN ("manza naranja", n = 10); MB ("manza blanca", n = 10); CAL ("calabazona", n = 12); CHA ("chaveña", n = 15); LOB ("loba", n = 15); SDT

("sangre de toro", n = 12); LAR ("larguita", n = 15); JAR ("jaraleña", n = 15); RED ("redondilla", n = 15). CV = Coefficient of variation.

Conclusions

The results show the high variability in morphologic and postharvest characters in fruits of *Opuntia* species from different climatic conditions. *Opuntia ficus* cultivars characterized for presenting the highest sizes of fruit, the highest percentage of edible portion, as well as high soluble solids content, which could contribute to consumers' preference. The results presented are important in order to make utilization and conservation proposals for those species.

Table 3. Betaxanthins, betacyanins and total betalain content in fruits of *Opuntia* species cultivars from Ojuelos (OJ) and Lagos de Moreno (LM), Jalisco, Mexico. [A]

	Cultivar	Betaxanthins[B]	Betacyanins[B]	Total Betalains[B]
OJ	BN	0.011 ± 0.002	0.002 ± 0.0008	0.013
	MEM	0.021 ± 0.005	0.020 ± 0.005	0.041
	TAP	0.020 ± 0.004	0.020 ± 0.002	0.040
	CAR	0.007 ± 0.0006	0.005 ± 0.001	0.012
	CAS	0.004 ± 0.0005	0.004 ± 0.0008	0.008
	Mean	0.013	0.010	0.023
	CV (%)	60.6	81.6	71.3
LM	MN	0.003 ± 0.0005	0.001 ± 0.0001	0.004
	CAL	0.012 ± 0.003	0.001 ± 0.0004	0.013
	MEM	0.022 ± 0.003	0.023 ± 0.004	0.045
	CAR	0.042 ± 0.005	0.031 ± 0.007	0.073
	CAS	0.052 ± 0.006	0.041 ± 0.006	0.093
	CHA	0.024 ± 0.003	0.033 ± 0.001	0.057
	LOB	0.005 ± 0.0006	0.004 ± 0.0006	0.009
	SDT	0.023 ± 0.003	0.011 ± 0.002	0.034
	LAR	0.034 ± 0.005	0.037 ± 0.005	0.071
	JAR	0.039 ± 0.008	0.038 ± 0.006	0.077
	RED	0.033 ± 0.004	0.028 ± 0.002	0.061
	Mean	0.026	0.023	0.049
	CV (%)	59.1	68.8	62.0

[A]Data represent mean ± standard error. [B] Cultivars: BN ("burrona-naranja", n = 12); MEM ("memela", n = 10); TAP ("tapona", n = 12); CAR ("Cardona", n = 15); CAS ("cascarona", n = 12); MN ("manza naranja", n = 10); CAL ("calabazona", n = 12); CHA ("chaveña", n = 15); LOB ("loba", n = 15); SDT ("sangre de toro", n = 12); LAR ("larguita", n = 15); JAR ("jaraleña", n = 15); RED ("redondilla", n = 15). [A] = mg/100g fresh pulp. CV = Coefficient of variation.

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Physical And Rheological Characterization Of Edible Coatings Formulated With A BWP (biopolymer - wax - polyol) System

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Introduction

In the most recent years, food and packaging industries have been joining efforts to reduce the amount of food packaging materials. This attitude is closely connected with the growing importance of the environmental issues at the consumer level, which are being translated to increasingly restrictive policies by the governments of many countries worldwide. As an answer to these issues, the commercial use of bio-based primary food packaging materials is being implemented, based on the research efforts which are being made by many groups all over the world. Related with the concerns for a safer and healthier environment, consumers also demand for high quality food, without chemical preservatives, and with an extended shelf life, leading to additional efforts to get new natural preservatives and antimicrobials[1].

An emulsion consisting of two immiscible liquids (normally, water and oil), one of which is dispersed as spherical droplets in the other. In most foods, the diameters of the droplets usually have a sequence between 0.1 y 100 µm [2,3]. The emulsions may be conveniently classified according to the distribution of oil and water phases. A system in which oil droplets are dispersed in an aqueous phase emulsion is called oil-in-water emulsion O/W (Oil in Water).

The behaviour of oil-in-water emulsions in foods is defined by the three parts of the system; the fat or oil that is in the interiors of the emulsion droplets; the interfacial material between this lipid material and the aqueous phase; and the aqueous phase itself. Each of these 'phases' may be chemically complex. The lipid may be partly or wholly crystalline and it may be subject to chemical change such as lipolysis or oxidation. The interfacial material can be composed of proteins or of small emulsifiers such as monoglycerides, esters, or phospholipids, or mixtures of a number of these components. Finally, the aqueous phase may contain ions, which may destabilize the emulsions, or macromolecules such as polysaccharides, which may exert stabilizing or destabilizing effects. Therefore, to understand the functional properties of the emulsions, it is necessary to understand the properties of these three parts, individually and collectively [4].

The objective of this work was to evaluate the physical and rheological properties of several coatings formulated with a BWP system, a mixture of polysaccharide as biopolymer, candelilla wax and glycerol as polyol.

Materials and methods

Preparation of edible coating:forcoating formulation (patent requested).polymers were used pectin, gum (arabic and xanthan) andglycerol was used as plasticizer. The components of edible films were mixed in water following the reported by Saucedo-Pompa[5].Mixture was heated at 80°C and homogenized at 15000 rpm in a homogenizer (WiseTis Homogenizer HG-15D, USA).

Density: Pycnometer was used to measure the density of each emulsion. Pycnometer was weighed without sample, the pycnometer filled and closed, it was proceeded to weigh the pycnometer with the emulsion to evaluate.

Density was calculated using the equation

$$d_L = \frac{W_{pi} - W_p}{V_p}$$

Where
d_L = Density (g/ml)
W_{pi} = Finalweight
W_p = Empty weight
V_p = Exact known volume

Surface tension(σ): represents a force through a line on a surface divided by the line length of the element. Surface tension is the free energy divided by the area at which energy is applied[6], the method of capillary rise (liquid – gas), In which a liquid ascends a capillary tube and wets the walls of the tube, due to it, manner a meniscus on the surface by the pressure difference between the liquid in the container and the liquid pressure inside the capillary. This pressure difference causes a rise of fluid within the capillary is stopped at the time that the pressures reach equilibrium.
Rheological Fluid Model: The viscosity of the emulsions was determined using generated of a Rheometer modular and compact swingMOD Physica MCR 301, was measured to 20° C.
The power law model ofOswald ofWeale (equation (1)), pseudoplastic fluids can be modeled, in this case the viscosities decrease with increasing velocity gradient; just beginning to flow is applied shear stress(τ > 0).

$$\tau = K\left[\left(\dot{\gamma}_o\right)^n\right] \quad (1)$$

Where
n: flow Rate (n<1)
K: Consistency index
$\dot{\gamma}_o$: Velocity gradient

Experimental design
All determinations were carried out in triplicate and data were subjected to variance analysis,analysis of variance was performed using the ANOVA procedure. Statistical analyzes were carried out according to the SoftwareSAS® System 9.0.

Results and Discussion
The systems generated with biopolymerspectin, gum (arabic and xanthan) -candelilla wax, showed a similar pattern in the parameters, density and surface tension, however in the rheological was detected a difference between formulations.
In the table 1 shows the density and surface tension of the three coatings generated under the same conditions and with constant candelilla wax and glycerol.

Table1 Biopolymers effect on physical properties and colligative

Variable en los sistemas	Densidad N/m	Tensión superficial N/m
Pectina	0.986±0.005 a	59.163±0.103 a
Xantana	0.983±0.013 a	59.31±0.194 a
Arabiga	0.990±0.005 a	59.160±0.038 a

Means the columns followed by different letters are significantly different(P≤0.05).
Density: Formulations with pectin and xanthan have a very similar density (0.986 and 0.983 g/mLrespectively), for the case of the formulation with gum arabic a density presented of 0.99 g/mL.This can occur because the generated solution with gum arabic-candelilla wax contains a total of4.65 % of dissolved solids in water, being these 2.40 % higher solids content in the coating produced with pectin-

candelilla wax and 2.95% higher solids content in the coating produced with gum xanthan-candelilla wax. The coating with pectin-candelilla wax and gum xanthan-candelilla waxalthough minor amount of solids present density decreases to this may be due to these two biopolymers which are moregelling gum arabic. Recent studies mention that with increasing pectin edible films decreases in density in the formulation[7,8].

Surface tension: the formulation with xanthan gum-candelilla wax presented 59.31 N/m of Surface tension. being numerically greater than the formulations of arabic gum-candelila wax and pectin-candelilla wax (59.163 and 59.160 N/m respectively). The coatings were oil in water (O/W). The surface tension of the liquid can be affected by the addition of surfactants because they decrease the surface tensionthe liquid to concentrate on the water-air interface.In this manner, the surfactant concentration at the interface is greater than the dissolution and this excess surfactant concentration of the surface excess is called Gibbs[9].

rheological Parameters: From the curves obtained the parameters are estimated*n* and *K* (index of the power law and consistency index). The equation (1), for the three cases presented mean square deviation related to each trend line; according to the results shown in Table 2.

Table 2 Rheological parameters obtained and mean square deviation

N°	Parámetros	Biopolímero		
		arábiga	xantana	pectina
1	Visc. aparente	1.781	2.078	1.961
2	K (Pa · S)	0.001	0.022	0.001
3	n (Adim)	1.098	0.653	1.088
4	R^2	0.998	0.997	0.997

Analyzing the values shown in table 2, it can be noted that using gum arabic and pectin have a value close to 1, which is for Newtonian Fluid and xanthan gum with a value 0.65, be a value less than 1and is indicative that a pseudoplastic. In all results the value of R^2were higher than 0.99; ($P \leq 0.0001$),what is considered as acceptable results for independent variables.

The coating generated with arabic gum and pectin showed characteristics of Newtonian fluidswhich is stable behaviorand this coincides with Oza*et al*., [10] they concluded that pectin can be used as thickeners or agents gellingstabilizing oil/water. SimilarlyAdachi *et al*., [11] reported that the presence of a hydrocolloid such as maltodextrin or arabic gum in the external aqueous phase is essential for the emulsion water/oil/water to be converted into microcapsules by spray drying [12].

Conclusions

The physical parameters of the biopolymers evaluated (density and Surface tension) showed no significant difference between them. Formulated coatings with arabic gum-candelilla wax and pectin-candelilla wax exhibit a Newtonian fluid behavior, That it is indicative to a fluid with stable behavior. The coating generated with xanthan gum-candelilla wax presented a bigger apparent viscosity (2.079) but being a pseudoplastic fluid no stability.

Acknowledgements

Emilio Ochoa thanks the CONACYT by the scholarship to postgrade studies in Food Science and Technology. The authors thank the Sector Fund CONAFOR-CONACYT project financing C01-2008-91633.

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Ultrastructural Analysis Of The Effect Of Chitosan On *Alternaria alternata* Isolated From Fruits Of Mango C.V. Tommy Atkins

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Introduction

Post-harvest decay caused by *Alternaria alternata*represents a major limitingfactor in the long-term storage of fruits and vegetables. Currently, fungicides constitutethe primary means of controlling post-harvest diseases *[11]*. However, because of thewithdrawal of key fungicides, a critical need exists for alternatives. Different, promisingapproaches, including the use of antagonistic micro-organisms and intensification ofhost resistance mechanisms, have been advanced as alternatives to synthetic fungicides*[4, 6, 12, 13]*.

Recently, the potential of chitosan, andβ-l,4-glucosamine polymer, as an antifungalpreservative has been shown in several post-harvest commodities *[5, 6]*. At aconcentration of 10 mg ml -x, chitosan coating was shown to delay ripening by actingas a selective barrier to diffusion of gases and to reduce the incidence of decay *[5, 7]*.

The inhibitory effect of chitosan is believed to originate from the combination of itsantifungal and eliciting properties. This is supported by the observation that chitosanactivates defense responses in plant tissue and interferes with the growth ofpost-harvestpathogens *[6, 8]*.

Although there is considerable literature on the biological activity of chitosan [1, 3, 9, 10], the basis of the mechanisms that underlie its ability to increase host resistance and thereby prevent lesion development has not been fully elucidated. In order to maximize its potential use as an antifungal preservative, an understanding of how chitosan exerts its effect on disease development is necessary. So far, little has been published regarding its effect on disease development in plant [2]. In the present study we report some aspects of the cytological changes that occur during the infection of chitosan-treated black rotfruit by*Alternaria alternata*.

Materials and methods

Pathogen

Alternaria alternata, causal microorganism of black rot fruit, was isolated by the Institute technologic de Tepic in laboratory the Food biotechnology. The pathogens were removed from the one-week-old PDA, discswere takenand inoculatedin Petri dishesthat containedchitosanconcentrations(0.5,1 and 1.5% w / v). Sampleswere taken to theGraduate CollegecampusMontecillo forthe micrographsbyscanning electron microscopy and The UNAM Faculty Sciences by Transmission electron microscopy.

Scanning Electron Microscopy (SEM)

Agar plates were made with the sample present and given vacuum for 2 min. To remove bubbles present and allow penetration glutaraldehyde, washing was performed with buffer (Solution A + Solution B) for 7 min. On three occasions after washings were performed with graded concentrations Ethanol (30-100% v / v) for 50 min. And for the concentration of 100% three times for 20 min. Samples were taken at a critical point drying with CO2 for 40 min. To then mount the samples in sample holder plate and coated with gold in a metal ionizer FINE COAT by 15 minutes. The samples were examined using a scanning electron microscope Carl Zeiss DSM 940 operating at 30 kV.

Transmissionelectron microscopy (TEM)
Agar plates were taken with the sample present and were fixed with 2.5% glutaraldehyde in PBS buffer is washed*n*for 30 min. And fixed with osmium tetroxide for 1h at 1%, were washed 4 times for 5 min. With PBS, the samples were then dehydrated with graded concentrations of ethanol (70, 80, 90, 96 and 100% v / v) for 10 min. 100% each time as 3 washes were performed for 5 min. then placed in 3 changes of propylene oxide and epoxy resin for 16 h. at room temperature, and then be covered by resin at 60 ° C for 15 hours for polymerization, subsequently obtained semi thin of 250 microns using a Leica Ultracut microtome R brand, to scan the sample, then thin sectioned 70 microns and were compared in membranes copper uranyl bath for 20 min. And lead acetate for 10 min. were dried and observed with TEM electron microscope JEOL-1010 at 60 kV.

Results

Scanning Electron Microscopy (SEM)
SEM microscopy allowed appreciate that the diameter of themycelium was variable, it showed some swelling and others seemed apparent thinner, in contrast with the hyphae developed chitosan free medium, showing amore healthy appearance which is not observed in chitosan treatments, as can be seenin the figure 1.

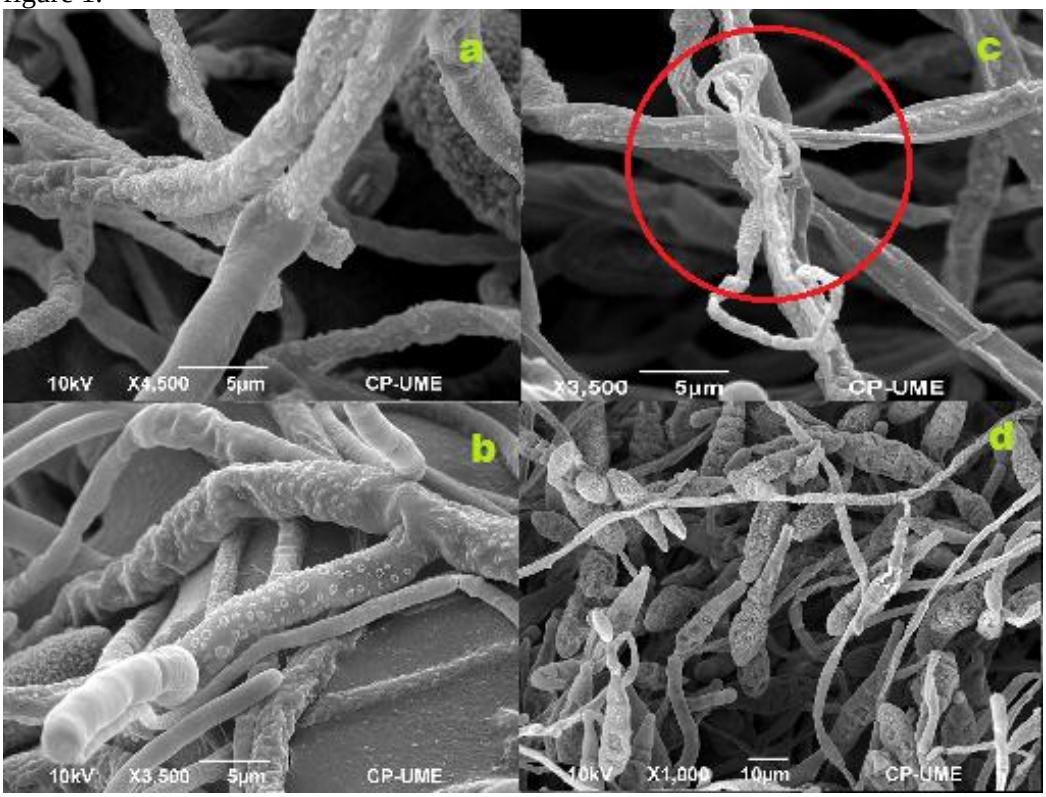

FIGURE 1. Micrograph of *Alternaria alternata* mycelium isolated from Tommy Atkins mango fruit treated with different concentrations of chitosan. a) chitosan mycelium absent, b) mycelium treated with 0.5% chitosan, c) mycelium treated with 1% chitosan, d) mycelium treated with 1.5% chitosan of low molecular weight.

Transmission electron microscopy (TEM)

Were analyzed by TEM micrographs best illustrated the effects of chitosan on the mushroom structures, with reference to those obtained for the control, to contrast, carrying out the analysis and interpretation observed damages of the same ultrastructural level on cells Alternaria alternata.
A micrograph of the control sample, then some of the spore structures and well defined with no degree of

damage, unlike what is appreciated in subsequent images by the effect of chitosan BPM at 0.5, 1 and 1.5%.

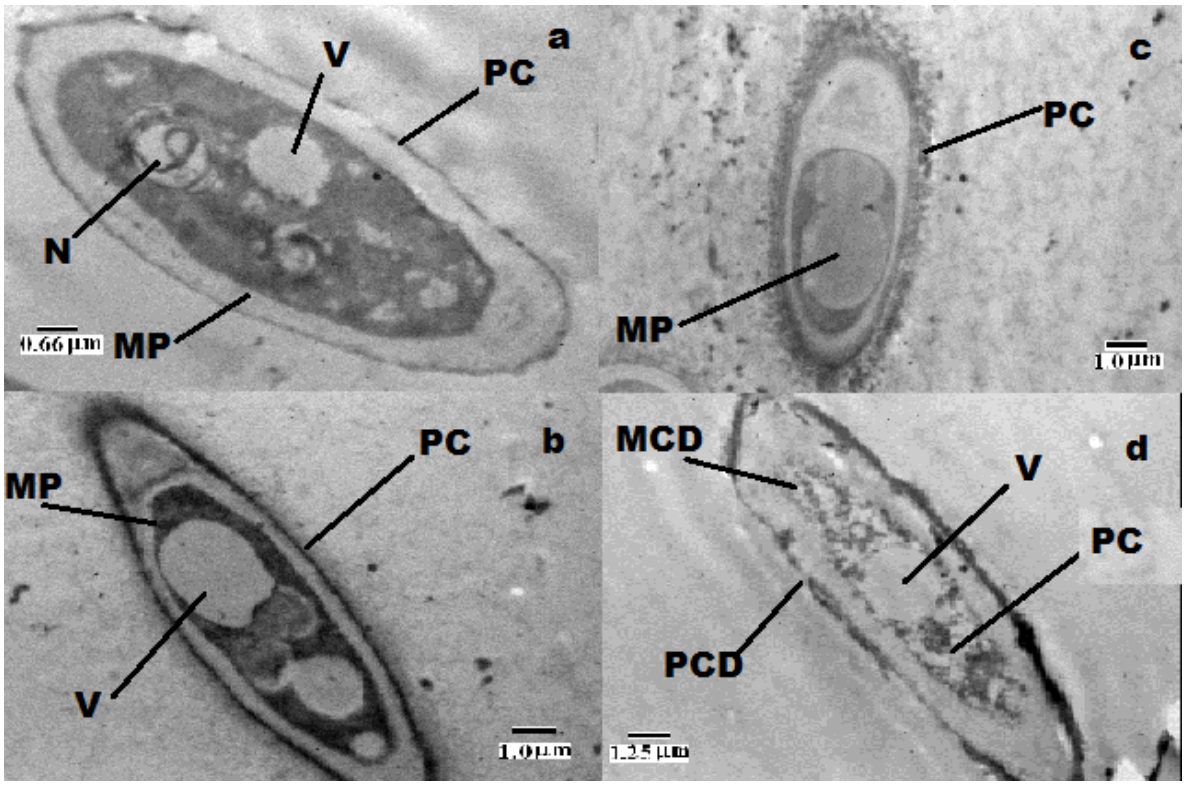

FIGURE 2. Transmission electron micrograph of the fungus A. alternata isolated mango cv. 'Tommy Atkins'. a) Spore with absence of chitosan, b) spore treated with 0.5% chitosan, c) treated chitosan spore 1%, d) spore treated with 1.5% chitosan BPM, V = vacuole, PC = cell wall MP = plasma membrane, N = nucleus, cell membrane MCD = disintegrated.

The damage observed in the spores and mycelium were detachment and / or cell wall disintegration, total disruption of the fungal cells, the cytoplasm vaculizacion observed that as the concentration was increased BPM chitosan damage was more severe on the spore.

Discussion

In recent years there has been increased interest in the use of chitosan in agriculture and identified several of its biological potential. The results obtained in this study indicate that inhibition of the fungus can be attributed to some antifungal properties of this compound are well known and have been demonstrated against various phytopathogenic fungi (Allan and Hadwiger, 1979; Benhamou, 2004; Bautista-Baths 2006 , Palma-Guerrero et al. 2008).

The SEM and TEM study allowed us to observe the serious condition that causes chitosan in fungal cells at the morphological and structural information that helps us understand the phenomena observed for fungicidal and fungistatic effects of chitosan on the fungus *Alternaria alternata*. According to what observed in microscopies can establish a connection to say that the conditions are the cause morphological level direct fungistatic effect and ultrastructural level conditions are caused by the fungicidal effect of chitosan, so noted, the generated damage caused the collapse of the fungal cells, either mycelium or spores, which leave no room for the survival of the pathogen. However this is a physical explanation, since the deep explanation of this phenomenon occurs, is different levels as the physiological, biochemical

and molecular.
The fungus Alternaria alternata treated with chitosan is susceptible, because in the comments under different Microscopies was damaged and several morphological alterations, which have been reported in other fungal pathogens (3, 7).

The fungal cell changes observed by SEM at morphological processing for different chitosan concentrations where the more severe the 1.5% chitosan were hyphae size reduction, sprains and distortions and excessive presence of spores which This matches ElGhaouth et al. (1992), Benhamou, (1992), Benhamou, (2004), Chea et al. (1997), Barka et al. (2004),ElHassini et al, (2004) who in phytopathogenic fungi *R. stolonifer; F. oxysporiumf.sp. lycopersici, S. sclerotiorum, B. cinerea, F. oxysporium f. sp. albedinis* subjected to the action of chitosan, found effects and / or similar defects which can be understood and explained fungicidal and / or fungistatic of this biopolymer.
In TEM microscopy observations were deformation and / or disintegration of the cell wall, cytoplasm disorganization and vacuolation, and the collapse of the spores and mycelia, evidence that the effect of chitosan obtained in other studies of phytopathogenic fungi as *R. stolonifer, P. digitatum, B. cinerea* (3, 7).

Some alterations made were reported in old fungal cells and deprived of nutrients (Campbell, 1970). Perhaps it is that being a polycation chitosan and filmogénicas properties, can act as a barrier to the flow of nutrients to the outside and therefore affects the establishment of nutrients between host and pathogen, although this has not been proven (7).
The morphological and ultrastructural effects are probably present in part caused by the biopolymer power has antifungal and which has been evidenced in research, such as polycationic character and positively charged free amino groups, amid acid residues interacting with macromolecules negative exposed in the wall of the mold, changing the permeability of the plasma membrane and altering the essential functions of this (3), this may explain the interaction of the membrane, was a constant alteration was made in the mycelium and spores treated with chitosan.

Conclusions

According to the results obtained the use of chitosan in different concentrations morphologically and structurally affects the growth of the fungus *Alternaria alternata*, showing that increasing the concentration the more severe effects are according to this microscopies used, and reports currently available, so that chitosan is an alternative that has gained momentum, to control postharvest diseases, perhaps in the near future eradicated the use of chemical fungicides.

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Plant Growth Promoting Bacteria In The Nutraceutical Quality Of Mexican Strawberry Fruits

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Introduction

Strawberry is an important crop in Mexico, primarily for the state of Michoacán because it is the largest producer, generating an increase in foreign exchange through export (25). It is a plant belonging to the family Rosaceae and the genus *Fragaria* (23), its fruit is distributed worldwide due to its great commercial demand, attractive both fresh and processed, for its qualities of color, aroma and flavor. Besides being rich in vitamins A and C (7), contains certain antioxidant compounds such as anthocyanins (24). It has been documented that such compounds found in strawberry help reduce the risk of cardiovascular events, improve endothelial function and decrease vascular thrombosis. Additionally, it has been shown in different experimental systems, the anticancer activity of extracts of strawberries and blocking initiation of carcinogenesis, and suppressing the proliferation of tumors (23).

Moreover, global trends in human feed in recent years indicate that consumers have become more demanding and concerned not only with the sensory attributes but also with the contents of nutritional and nutraceutical compound in foods, as well as production technologies (8). That is why farmers look for new options for improving both culture conditions and quality of final products.

One of the alternatives being studied recently is the application of biofertilizers like *Bacillus subtilis*, considered a plant growth-promoting bacteria (PGPR), as it improves plant development, roots associated with bacteria have a better structure and thus facilitate the assimilation of nutrients (2, 3, 5, 15) and is also used for biological control (17, 26). Additionally, it is known that some microorganisms used as biofertilizer or biocontrol agents, help to increase the content of certain nutrients in fruits (22). Recently, it has been reported that these bacteria can improve the quality of different agricultural products. In this regard, it has been documented that a strain of *Bacillus subtilis* had a positive effect on the size and texture of tomato fruits (20), also found that the combination of PGPR and arbuscular mycorrhizal fungi (AMF) increased lycopene content and antioxidant activity in tomato (21).

Considering the above information, the objective of this study was to determine the effect of *Bacillus subtilis* on the nutraceutical quality of Mexican strawberry produced in greenhouses.

Methods and materials

Biological material

Mexican strawberry plants Fragaria x ananassa var Jacona) acquired with a producer in the region-Jacona Zamora Michoacan, were used. The strains of *Bacillus subtilis* DN and *Bacillus subtilis* MZA were provided by the Laboratory of Ecological Biochemistry of CINVESTAV unit Irapuato. DN and MZA grown for 12 and 14 h respectively at 25 °C in potato infusion and the bacterial suspension was adjusted 1 X 10^7 CFU / ml (13).

Greenhouse experiment

The experiment was conducted in the greenhouse-type Chapel of CIIDIR-IPN unit Michoacán in Jiquilpan, Michoacan, Mexico. This is located at an altitude of 1560 m, bounded by the coordinates 20

°03’02’’ and 19°52’54’’ N latitude and 102°39’33’’ and 102°56’16’’ length W. The experiment was established in 3500 cm^3 pots, containing 5 kg of soil mixture lama, ground sheet, "Shunshine Mix 3" (SUN GRO Horticulture), perlite and vermiculite (1:2:3:1:1 v / v) previously sterilized at 121°C for 1 h for 3 days. A randomized complete block design was used. Ten plants were experimental unit, there were 3 experimental units.

The treatments were: 1) *Bacillus subtilis* DN, 2) *Bacillus subtilis* MZA and 3) control without inoculum. The bacterial suspension was inoculated (15 ml) at transplanting directly in holes made at the center of each pot (about 8 cm deep). Subsequently, microorganisms were inoculated to plants each 15 days throughout the experiment. The plants were watered daily with Steiner nutrient solution (pH 5.5-6.0), using a drip system (fertigation).

Quantification of phenolics compounds, flavonoids and anthocyanins

For quantification of phenolics compounds 10 g sample was macerated in mortar with 20 ml of aqueous methanol (80:20 v / v), the extracts were allowed to stand at 4°C for 24 h, then filtered and the filtrate of each sample were added 20 ml of aqueous methanol and placed at 4°C for 24 h. 2 more filtrations were performed, leaving a volume of 80 ml per sample. Quantification of phenolics compounds was carried out by triplicate using the method of Folin-Ciocalteau. 50 µl of crude extract were mixed with 200 µl of distilled water and 250 µl of Folin-Ciocalteu reagent (50% v/v), stirred vigorously vortexing for 3 min, then 500 µl of Na_2CO_3 (7.5% w/v) were added and vigorously mixed. Samples were incubated in the Thermomixer for 15 min at 45°C and 500 rpm. Absorbance was measured at 765 nm using a blank aqueous methanol (80:20 v / v) in a UV-Visible spectrophotometer (Cary 3E). The results were expressed in mg of gallic acid in 100 g of fresh weight (28).

For extraction of flavonoids, one gram of sample was macerated and homogenized with 10 ml of aqueous ethanol (80:20 v/v) in mortar with pestle, and subsequently sonicated in a digital ultrasonic bath (Branson 1200) for 30 min at 40°C and centrifuged at 2000 rpm for 10 min. The supernatant was placed at 4°C for 16 h with 3 replicates per sample. After this time, 100 µl of crude extract was added 200 ml of solution of potassium acetate and 200 µl of 1M aluminum nitrate at 10% was diluted to 1 ml with aqueous ethanol (80:20 v/v). Mixtures were allowed to stand 40 min and then absorbance was measured against a blank of aqueous ethanol (80:20 v/v) at 415 nm in the UV-Visible spectrophotometer (Cary 3E). The results were expressed as mg of quercetin in 100 g of fresh weight (6).

For anthocyanins determinations, extractions were performed by taking one-gram samples which were macerated and homogenized with 5 ml of acidified ethanol (ethanol and 1N HCL; 90:10 v/v), the solutions were stirred vigorously in vortex and pH was adjusted to 1. The extracts were allowed to stand at 4°C for 24 h, then, filtered, filtrates were diluted to 25 ml with ethanol acidified (1N HCl and ethanol, 90:10). Absorbance was read against a blank of acidified ethanol at 535 nm in a spectrophotometer (UV-Visible CARY 3E). The cyanidin 3-glucoside was used as standard (1).

Discussion and results

Several studies have evaluated the content of anthocyanins in strawberry; contents ranging between 55 and 145 mg/100 g (14) and 11.4 and 32.8 mg/100 g (27) have been reported. These ranges are variable because the content of anthocyanins may be influenced by the cultivars, since they differ genotypically one of each other. Related to Mexican varieties, there is a report in which their content of anthocyanins was compared with that in foreign varieties, values fluctuated between 10.89 to 40.62 mg/100 g, and the content presented in variety Jacona was 25.34 mg/100 g (6), so that, data presented in this study are consistent with those values. Regarding the application of PGPR, it was observed that the treatment of *Bacillus subtilis* DN applied in the Jacona variety promoted a significant increase of anthocyanin content by 56% compared to non-inoculated control (Table 1).

It has been previously reported that PGPR have exerted a significant effect on quality parameters of strawberry fruits, such as the total soluble solids content and vitamin C (10), whereas in other studies conducted on strawberry, PGPR treatments had no significant effects on these parameters (11).

It has been shown that the content of total flavonoids in the Jacona variety was of 122 mg/100 g fw (6). Additionally, in regards to PGPR application, *Bacillus subtilis* MZA presented flavonoids content above this value. Therefore treatment of *Bacillus subtilis* MZA increases 2 times the total flavonoids content compared to control (Table 1).
In relation to the phenolics compounds, the fruits from plants treated with *Bacillus subtilis* DN, had an increased content of phenolics compounds in 27% compared to control (Table 1). It has been previously reported the total phenolics content of strawberry in ranges of 185 to 196 (4) and 491 to 1884 mg gallic acid/100 g fw (18). In the present study, we obtained total phenolics content of 315.80, which are consistent with previous reports (208.83 to 378.75 mg gallic acid/100 g fw) (6).

Table 1. Nutraceutical Quality strawberry variety Jacona inoculated with *Bacillus subtilis* under greenhouse conditions.

	TREATMENTS		
PHENOLICS COMPOUNDS	Control	MZA	DN
Total anthocyanins (mg cyanidin-3-glucoside/100g fw)	22.2 b	25.443 b	**34.65 a**
Total flavonoids (mg de quercetin/100g fw)	117.52 b	**248.96 a**	141.38 b
Total phenolics (mg of gallic acid/100g fw)	248.66 b	214.85 b	**315.80 a**

Presents the means separated by Tukey's test. Means followed by different letters within columns differ significantly ($P \leq 0.05$, n=3

Furthermore, it has been reported that inoculation of tomato with PGPR and arbuscular mycorrhizal fungi (AMF), lycopene content increases, the main antioxidant fruit (21). Additionally, in a study with AMF in chili, it was noted that these fungi increased concentrations of carotenoids and xanthophylls (1.4 times and 1.5 times respectively) when compared to the control (19). The mechanisms by which these microorganisms increase the content of pigments in fruits, might relate to the acquisition of nutrients, however, in this study all plants had the same fertilization regime, and it was assured they would not suffer nutritional stress. Other possible mechanism involved in the reported effects, is biostimulation, since PGPR produce phytohormones which promote plant growth (9, 12.16). However, more research is needed to determine the mechanism by which PGPR promote fruit quality.

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Application Of Artificial Intelligence Techniques In Sensory Evaluation: Association Of Tongues' Physical Characteristics And Sensitivity To Bitter Compounds

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Introduction

Fungiform papillae density can be used in a variety of circumstances as an indicator of the function of taste. A high number of fungiform papillae on the dorsal surface of the tongue are commonly found in people who are classified as bitter substances supertasters, specifically the 6-n-propylthiouracil (PROP) and phenylthiocarbamide (PTC), compared with non-tasters and tasters (15, 12).

For purpose of counting the fungiform papillae, non-invasive procedures have been used as videomicroscope and digital camera, both with similar results, however videomicroscope use is limited to laboratory, and it requires 30 to 60 minutes to obtain a high quality image of an individual (8).

Also, digital cameras are faster, portable, flexible, and of lower-cost; they have been used successfully to identify small regions in the first three centimeters of the tongue, which provide reliable measurements of the density of fungiform papillae that highly correlated with the total number of fungiform papillae on the tongue (15, 8).

Moreover, the food industry has long recognized the potential of artificial vision so now ranks among the ten industries that more utilize this technology. Recent advances in hardware and software have helped to expand it, by providing low-cost solutions, cost effectiveness, consistency, speed and accuracy (7).

That is why we have been interested to propose using digital images analyzed with artificial vision to count taste papillae, with significant savings in time and cost reduction. Furthermore, this technique would not invasive and would be relatively simple its implementation on the evaluation of taste function.

Finally it should be noted that this methodology would seek to benefit the food industry in shaping tasting panels. In the area of medicine and research would be film outside laboratories, clinics, in the bed of patients for typical taste loss monitoring and recovery processes during chemo and / or radiation therapy, to name a few (15).

Material and methods

We have selected 31 women and 22 men of the School of Nutrition of the University of Veracruz, through a questionnaire which included: general data, suffering from diseases like cancer, diabetes, neurological disorders, controlled drug use, smoking; that cause degeneration or loss of taste due to the decrease in the number of papillae (1, 15, 8).

The judges selected performed a sensitivity test at 6-n-propylthiouracil. This was carried out with a filter paper impregnated with 1.6 mg of 6-n-propylthiouracil (Sigma-Aldrich brand). The filter paper was placed on the tongue of the panelists, who recorded the perception of bitterness in the Green scale, which ranging from barely detectable, even the most unimaginably strong (3).

It took three to six photographs of each person's tongue (SONY Cyber-Shot DSC-W310 12.1 mega pixels digital camera), and of these there were chosen the best quality image considering sharpness, focus, brightness and contrast. The image analysis was performed in Matlab (R2010a 7.10.0.499), with software that was developed under the following methodology: was defined interest region in a rectangular subregion, including the first centimeters of language, once defined , proceeded to learning color regions,

for which the program was shown, a representative of this area, "background", "skin", "tongue-no-papillae", "fungiform papillae".

It transformed the image of the RGB color space to the space L * a * b * in order to decrease the sensitivity to the changes of illumination and to obtain greater uniformity. Average color was determined for each of the regions shown. Each of the pixels of the image were analyzed, previously defined, and classified according to the nearest neighbor. To calculating the proportion of area of the fungiform in comparison with the area of the tongue, it was used the following equation: Fp = Ap / (Ap + A), where Ap is the area of the fungiform, A is the area of the tongue (not including fungiform papillae) and Fp = proportion of fungiform papillae (Figure 1).

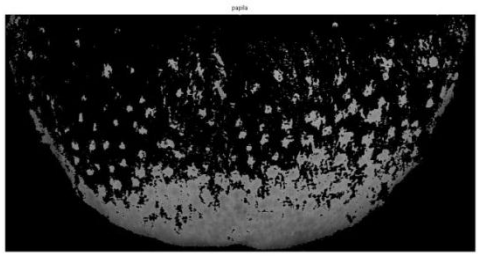

Figure 1. Pixels classified as fungiform papillae.

To evaluate the software accuracy a "manual" counting of fungiform papillae was performed for this, blue dye was applied to the middle of the tongue, dividing by the natural brand presented. The image was digitized and divided into quadrants using a filter paper 1cm as pattern (Figure 2). This occasion, the total count of the quadrants 3 and 4, as these are representative of the total amount present in fungiform tongue (15).

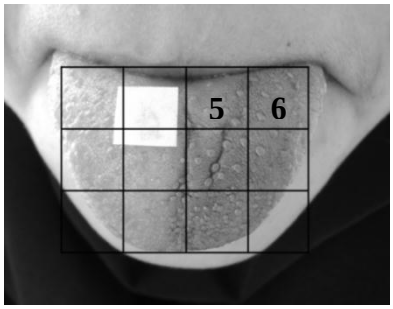

Figure 2. Tongue stained.

Results and discussion

Of the 53 panelists who were administered the test PTC / PROP, we obtained an age range between 18 and 57 years. The distribution of taster status is presented as follows: 35% non-tasters, 44% and 21% supertasters. This differs about reported in caucasians for supertasters with 25% (2) and non tasters, 50% (5), while for the mexican population, 10.4% reported no tasters (13). These differences may be due to the sensitivity PROP / PTC varies for each race and for each region as some research papers report a range of 1.3% to 66% non-tasters (13, 9.10).

A higher percentage of women were classified as supertasters (24%) compared to men (16%), which is consistent with several published works that women have greater PROP sensitivity and are more likely to be supertasters because which have on average a greater number of fungiform papillae and taste pores, which is directly correlated with sensitivity to propylthiouracil (4, 14, 8, 9).

Number of fungiform papillae.

There is a statistically significant positive linear correlation ($r = 0.481$, $p < 0.001$) between the number of fungiform papillae of the quadrants 3 and 4 and the sensitivity of bitterness, which is consistent with previous work, which states that the number of taste buds and the sensitivity is proportional to PROP / PTC (4). However, to describe the bitterness expected value according to the amount of taste, adjusted a nonparametric regression model with lowess estimator, which is one of the most widely used approaches for non-parametrically adjusted regression models.

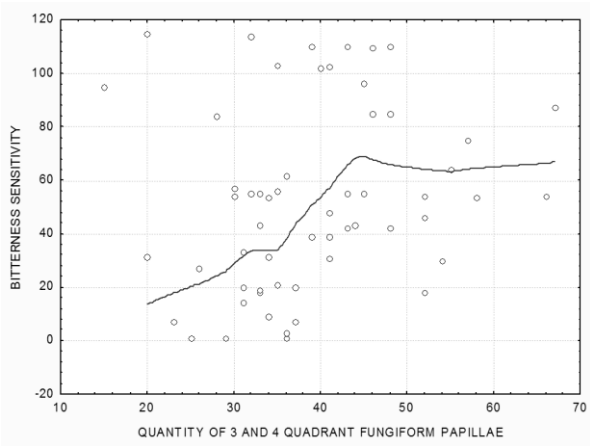

Figure 3. Nonparametric regression model between the amount of fungiform papillae of the quadrants 3 and 4 and the sensitivity of bitterness PROP / PTC.

Figure 3 shows that in principle bitterness sensitivity increases as the amount of fungiform increases; from 30 papillae shows a further increase in the sensitivity of bitterness and finally from 45 papillae

approximately shows a slight decrease in the sensitivity of bitterness in relation to the amount of fungiform papillae of the quadrants 3 and 4.

Subsequently, only the quantity correlated of papillae of quadrant 3 with bitterness sensitivity, obtaining a positive linear trend again significant (r = 0.268, p = 0.057). However, once the quadrant 4 correlated with bitter sensitivity, it can observe a similar correlation coefficient (r = 0.456, p <0.001) than that obtained when including quadrants 3 and 4, this is likely because quadrant 4 has a greater amount of fungiform papillae.

Proportion of fungiform papillae

There is a weak positive linear trend between intensity for bitter tastes (PROP and PTC) and the proportion of fungiform papillae, yielding the following equation:

Sensitivity bitterness = 29.8244 +0.6838 * x, with a Pearson correlation coefficient = 0.309 (p = 0.025).

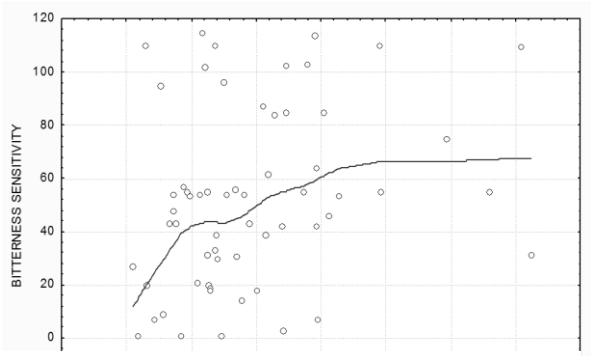

Figure 4. Nonparametric regression model between the proportion of fungiform papillae of the quadrants 3 and 4 and the sensitivity of bitter PROP / PTC.

Figure 4 shows the regression model describing parametric sensitivity of bitterness, but now as a function of the proportion of papillae. It can be seen basically the same behavior as in Figure 3.

Also, to compare manual counting and software correlates the number of papillae of the quadrants 3 and 4 with the proportion of fungiform papillae obtained with machine vision program. As can be seen, there was obtained a correlation coefficient of r = 0.310, p = 0.027, again there being a linear trend, positive weak but statistically significant. This can be explained, because the papillae area in the case of supertasters, not necessarily associated with the sensitivity of bitterness, since supertasters have smaller fungiform papillae, but have a greater amount of taste buds (6, 2).

So, to continue the research, we propose a change in the image analysis process, introducing new features to it for a count of papillae with greater accuracy, since the methods developed so far to determine the phenotype are difficult to implement correctly, and expensive in terms of time and labor (11).

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New Foods Neophobia And Sensory R-Index Of Sausages With Orange Peel And Maguey Leaf Flours As Source Of Dietary Fiber

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Introduction

Due to the recent demand for crops for human consumption, agro-industrial products form fruit processing, which are primarily employed as animal feed, biofuels or compost, represent a new source of dietary fiber and other bioactive compounds. Fruit processing residues (about 70%, peels and seeds, is considered a residue) are promising products due to its high content of insoluble fiber that can be used a functional ingredient.

Dietary fiber plays an important role in human health. Diets in dietary fiber are associated with the prevention, reduction and treatment of diseases such as coronary heart disease. Today, the consumption of healthy foods has increased mainly due to public concern for the prevention of certain diseases. People look for foods that are the closest thing to natural or products to incorporate in their formulation ingredients from natural sources will also provide health benefits. For example, organic compounds in fruits and vegetables are very important in preventing cancer, as several studies link the consumption of flavonoids reduces the risk of cancer (1). This booming market supplements that contain compounds capable of inhibiting or inactivating free radicals, since these compounds have been linked to chronic and degenerative diseases such as cancer, neuronal diseases and cardiovascular diseases. By increasing the consumption of dietary antioxidants may collaborate with the balance between these and oxidant agents (2).

Agroindustrial wastes refer to wastes generated by plants and animals, such as plant fibers, leaves and shells. Although not classified as hazardous waste, waste produced from cereal crops constitute a large volume of waste materials. Traditionally, these waste materials are used as bedding for animals and livestock feed, or additions to the soil as green manure. Recent studies have documented other uses of these agro-industrial wastes such as soil conditioners and fertilizers, biofuels, thermoplastics, activated carbon, and components of other materials. However, the potential use of this waste as a source of dietary fiber in foods has not been fully examined (3), neither its sensory effects nor the consumers acceptability for products with these new ingredients.

Neophobia is defined as the natural human resistance and other omnivorous animals to eat and/or avoidance of new foods, because they have an adaptive value, serving a protective function in the food environment (4).

Orange peel is an important source of fiber that can be recovered from agrifood wastes, the same as Maguey (Agave) leaves. There are few plants that provide the man home, clothing, livelihood and health. For these reasons, the Agave L. has been rated as exceptional. Several scholars agree that Mexico is the center and origin of dispersion of the *Agave* L., since in this vast territory there in the wild agaves less evolved forms, and as many varieties.

Therefore, the objective of this work was to explore sensory effect of the addition of fiber from orange peel and maguey leaves flour in sausages using R-Index and to estimate if consumers have either neophobia or willingness to try these new healthy foods.

Materials and methods

Flour from orange peel and maguey leaf.

The orange peel flour was obtained from oranges collected of market at Iztapalapa area, subjected to a washing by cutting into smaller pieces and then to dehydration at 70 °C until complete dehydration. Is proceeded to a grinding and sieving to obtain a flour with a homogeneous particle size (5), then, samples were packing at vacuum in bag of polyethylene and storage at room temperature.

For the preparation of the maguey leaf flour, maguey leaves were collected previously used for the manufacture of barbecue of market the weekend at Mexico City. Were subjected to a previous washing and were subsequently dried in the sun, to be finally subjected to dehydration at 70 °C. Dehydrated samples were grind and sieve to obtain a homogeneous particle size (5).

Meat batter elaboration

The meat batter was prepared from the commercial formulation of Vienna sausage type (6), adjusting the percentage of wheat flour, orange peel or maguey leaf flours. Pork and pork back fat was obtained of market at Taxqueña area. The meat was ground and mixed with salt, sodium and phosphates, half the required amount of ice in a mini cutter. Later was added the back fat, orange peel or maguey leaf flours respectively, the remaining ice and phosphates, homogenizing to obtain the meat batter. The paste obtained was stuffed into cellulose casings and then cooked to an internal temperature of 70° C. The sausages were vacuum-packed and stored at 4°C until analysis.

Surveys on the consumption of healthy foods

A total of 150 experiment in supermarket located at south in Mexico City were performed. Were chosen men and women between the age ranges 30-50 years, were on the consumption of healthy foods.

Experiment for neophobia scale

150 surveys were conducted with the same characteristics as those of healthy foods in these surveys are presented first to the respondent a card containing information about the characteristics of a functional food, dietary fiber on flour and orange peel of maguey leaf.

Sensory Analysis

A survey to a panel consisting of 30 untrained consumers was made by rating and ranking tests, where they were given two reference samples, categorized as an "S" which was the sausage control, i.e. one that had no added anything, and another called "N" which had been added 2.5% of flour of orange peel or maguey leaf, as appropriate. They also were given a series six coded samples, three belonging to the control and three more with the flour of orange peel or maguey leaf. In the first part of the test, the panelists had to identify the sample flavor, indicating if every coded sample belonged to "S" or to "N", and if they were sure or not sure of the answer according to the following four options: S-sure, S-not sure, N-not sure and N-sure. For the second part, the panelists had to rank the samples according to texture (firmness). From these data R-index was obtained for both flavor and texture.

Results and Discussion

Experiment of Healthy foods

Figure 1 shown the results obtained in experiment of healthy foods. Today people care and give great importance to the food they eat are healthy, taking into account that are low in fat is also rich in vitamins and minerals that do not increase your cholesterol levels, that is, people are looking for natural foods that bring these benefits to your health as well as products in their formulations that incorporate ingredients from natural sources (7).

Determination of index of neophobia

Figure 2 shows the results of surveys of sausages samples with orange peel flour or maguey leaf flour, as seen in the questions asked, nothing is questioned about adding orange peel flour or maguey leaf flour to a meat batter, but as mentioned above. Card was provided to respondents with information about the orange peel flour or maguey leaf flour and its health benefits, then were given the survey to be answered. As the graph shows the results, half of respondents are willing to try new foods, foods from other countries, to try new foods not previously consumed and to consume food in local restaurants from different countries the

other half of respondents saying the opposite. Previous studies show similar results, where says that one of the many variables that have some influence on the level of food neophobia is socioeconomic status, gender, age, education and the living area (urban and rural) (8). They also found that exposure to different cultures and higher socioeconomic status may increase the knowledge of a wide variety of stimuli, including food, and negatively associated with food neophobia. On the other hand Tuorila et al. (9) indicated that women are less neophobics to food than men, with older subjects showed higher levels of food neophobia and subjects who have a higher level of education is less neophobics. MacNicol et al. (10) indicated that low socioeconomic status is associated with a greater tendency to have a picky or demanding attitude toward food thereby causing increased levels of food neophobia.

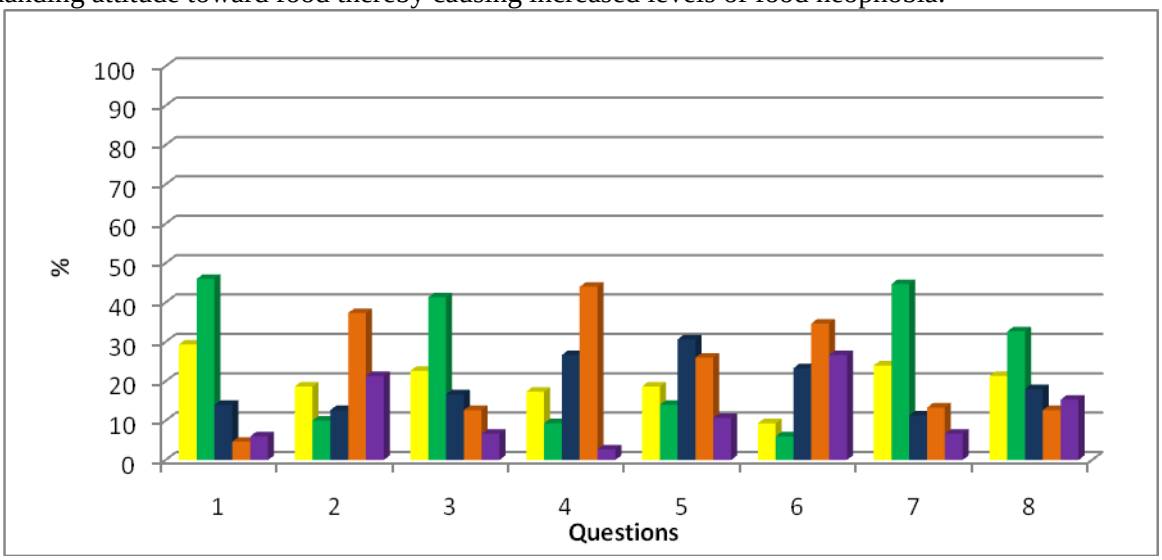

Figure 1. Conduct survey for the consumption of healthy foods, each color represents a) completely disagree, b) disagree, c) neither agree nor disagree, agree and e) completely agree

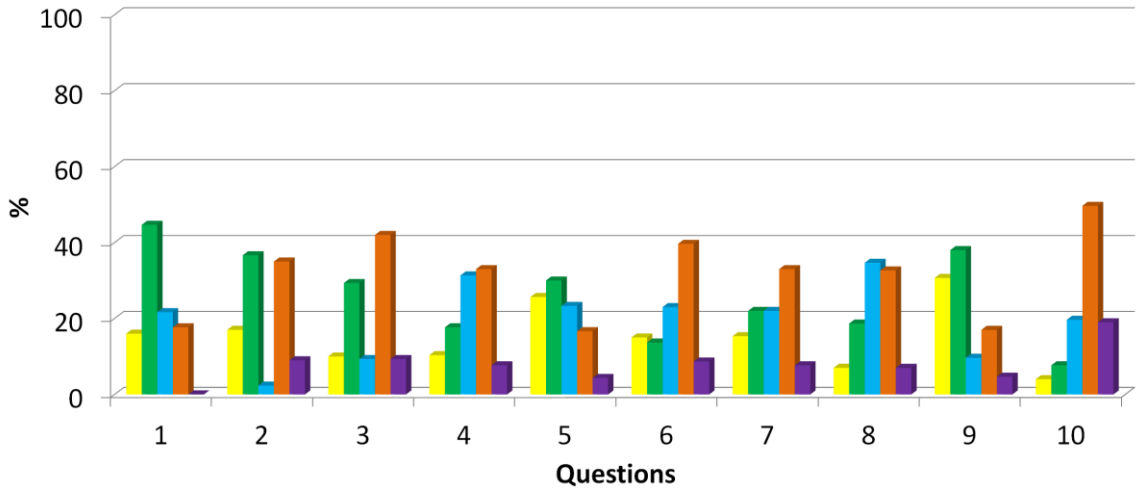

Figure 2. Conduct surveys to determine the index of food neophobia. Each color represents a) completely disagree, b) disagree, c) neither agree nor disagree,

agree and

e) completely agree

Sensory Analysis

Below are the results of sensory evaluation carried out 30 panelists. For sausages added with orange peel flour, Table 1 shows an R-index for flavor of 83%, which means that 80% of the panelists were able to identify which coded sample belonged to "N" and to "S". The other 20% were unable to detect the added stimulus. For texture, 87.5% of respondents were able to detect consistent difference between firmness of the samples; therefore, this parameter was also affected by the addition of the flour of orange peel.

For the sausage added with maguey leaf flour, results showed an R-index of 82% for flavor that indicates that the judges detected the presence of flour of maguey leaf in the meat batter. However, R-index for texture did not show differences between samples with or without maguey leaf flour.

Table 1. R Index for the flavor and texture of orange peel and maguey leaf

Samples	R Index "Flavor"	R Index "Texture"
Orange peel	83.5%	87.5%
Maguey leaf	81.9%	56.3%

Opposed to that, objective results (Texture Profile) showed that there were significant differences between control and a supplemented with orange peel or maguey leaf flours.

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Development Of Fluffy Bread With Amaranth, Rice And Oats Flours, And Its Sensory Evaluation

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Introduction

Nutritional content values amaranth flour, rice and oats, individually are very important because each emphasizes different aspects. The protein content of amaranth is 13 to 17%, is higher than other cereals, besides having a proper balance of essential amino acids, containing all 8 essential amino acids in sufficient quantities so that none of them is considered as limiting amino acid. According FAO and WHO, considering a value of 100 protein, amaranth has 75, 72 cow's milk, soy 68, wheat 60 and maize 44, as seen in this comparative protein amaranth very close to the ideal protein in the diet of man, because of the quality and bioavailability of the amino acids that compose (1). Oats contains a soluble fiber which is beneficial for the reduction of blood cholesterol, besides being one of the major cereal starches, like rice, making them beneficial for bread-making (2, 3).

For functional characteristics in the nutritional aspect and rheological combining amaranth flour, rice and oats are attractive for developing bread that offers a high-fiber, and vegetable protein of high biological value at higher rates. Today's consumers are discerning, demanding and more knowledgeable about food and expect products which are safe, good value and of high sensory quality. Therefore, knowing consumers' preferences and perceptions of the sensory characteristics of food products is very important to food manufacturers and retailers alike. The aim of this work was the development of fluffy bread with amaranth, rice and oats flours, and its sensory evaluation.

Materials and Methods

A literature search on the functional characteristics and rheological properties from amaranth, rice and oats, was conducted. In order to find a suitable formulation that meets fluffy features typical of traditional bread; the development of two formulas using different quantities of amaranth, rice and oat flours (F1 bread and F2 bread) and the control formula (FC bread) with amaranth and wheat flours. The flours were weighed and mixed according to the formulations set forth, as shown in Table 1. All ingredients were obtained from a wholesale food market supplier in Guadalajara, other ingredients used, beside flours, were eggs, oil, sugar, milk and baking powder. Then mixed with the remaining ingredients (egg, sweetener, leavening, milk and fat phase) for later baking, cooling and unmold.

Table 1. Distribution of the ingredients in the formulations

CONTROL	**FORMULATIONS**	
	F 1	**F2**
Wheat Amaranth	Rice Oat Amaranth	Oat Amaranth

To calculate the nutritional contribution of breads, were used Mexican Equivalent System Tables, according to the ingredients of each formulation and the final weight of the breads.

The sensorial evaluation of the bread was carried out with a participation of 40 volunteers untrained judges; the evaluated attributes were: appearance, flavor, texture and fluffiness, using a hedonic scale of 5 levels (4), the panel of judges evaluated all formulations under the same conditions. Acceptance testing used to determine how much each sample was liked based on a 5-point hedonic scale for a set of attributes where 5=really like. 4= like, 3= neater like no dislike 2=dislike, and 1=dislike a lot (5).

Three samples were served to each consumer in monadic order. The first sample served was removed before the consumer received the second sample. Serving order was randomized, Samples were served on white, paperboard plates, and each sample plate was identified by a three-digit random number.

Analysis of variance was conducted on the sample means for overall appearance, flavor, texture and fluffiness. Statistically significant attributes were further analyzed to see where mean differences existed using Tukey's test at the 95% confidence interval ($P< 0.05$).

Results

The products obtained FC were light yellow, fluffy and with predominantly wheat taste; F1were yellow characteristic amaranth, very fluffy bread like bread rice, and F2 were very light yellow, almost withe, less fluffy than others, the taste of amaranth and oat was equal.

The breads had a weight of about 50 grams per piece, the nutritional contribution of the product was calculated based on the Mexican Equivalent System and shown in Table 2. F2 provides higher protein content and lower calorie content.

Table 2. Nutritional composition of bread (pieces of 50 grams).

Feature	**FC**	**F1**	**F2**
Protein (g)	5.7	6.7	9.1
Carbohydrates (g)	28.1	29.0	28.1
Fat (g)	16.2	14.3	12.8
Kcal	281.0	271.5	264.0

*F1.- Formulation with amaranth, oat and rice flours
F2.- Formulation with oat and amaranth flours
FC .- Control bread, with wheat and amaranth flours

Control bread scored the highest percentage of acceptance, almost, for all attributes (appearance, flavor and texture), except the attribute of fluffiness, where F2 had the highest acceptance rate (Table 3) and was second in the other attributes, F1 had the lowest percentages of acceptance in the evaluation of all attributes.
The comparison of the means of the attributes evaluated, showed no statistical significant difference between F1 and F2, compared to F1 and F2 *versus* control showed a difference significant.

Table 3. Percentages of acceptance by attribute

Attribute	Really like (%)	Like (%)	Neither like nor dislike (%)	Dislike (%)	Dislike a lot (%)
Appearance	C 35[a]	C 50[a]	C 8[b]	C 2[b]	C 5[a]
	F1 25[b]	F1 25[b]	F1 17[a]	F1 3[b]	F1 5[a]
	F2 25[b]	F2 53	F2 15[a]	F2 7[a]	F2 0[b]
Flavor (taste and aroma)	C 43[a]	C 40[a]	C 13[b]	C 4[b]	C 0[b]
	F1 38[b]	F1 35[b]	F1 15[b]	F1 12[a]	F1 0[b]
	F2 33[b]	F2 43[a]	F2 21[a]	F2 1[b]	F2 2[a]
Texture	C 35[a]	C 42[a]	C 13[b]	C 3[c]	C 7[a]
	F1 33[a]	F1 25[b]	F1 35[a]	F1 18[a]	F1 2[b]
	F2 22[b]	F2 40[a]	F2 15.5[b]	F2 8[b]	F2 2[b]
Fluffiness	C 42[a]	C 40[b]	C 15[a]	C 3[b]	C 0[b]
	F1 20[b]	F1 42[b]	F1 17[a]	F1 15[a]	F1 2[a]
	F2 23[b]	F2 62[a]	F2 8[b]	F2 5[b]	F2 2[a]

a, b, c.- Means with the same letter show no statistical difference

Discussion

The frequency analysis of the acceptance of all attributes, consistently indicated that most were due to accumulative categories I like and I like both for control and for F1 and F2. By accumulating the percentages for the two categories below: I dislike / I dislike a lot far the worst assessment was for F1 (14%), while F2 had the lowest level of displeasure (5%), bread obtained a 7% FC . In order of preference leaned judges first by the pan control, followed by F2 and last place preference was the F1 (Table 3).

Conclusion

F1 and F2 were evaluated in the same manner, by the judging panel showing a good level of acceptance, although the greater acceptance and preference was for the control bread.

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Effect Of Drying Temperature, Formulation And Apple Variety On The Sensory Attributes Of Apple Leathers

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Abstract
A good choice for the consumption of fruits whit low commercial value are known as "fruit leathers", because they are a source of nutrients and can be used by-products of fruit processing. The objective of this study was to evaluate the effect of drying temperature, formulation and type of apple on the sensory attributes of "fruit leather" made of apple. Two varieties of apples (*Golden delicious* and *Red delicious*) were used and three formulations were developed (natural, chili and sugar). Two drying temperatures (60 and 70°C) were evaluated in a tray dryer. The color of the leathers was determined and the preference was evaluated using the ranking test to select the best products. In a later step was applied a sensory quantitative descriptive analysis (QDA) with a trained panel of 15 persons and was evaluated the acceptability of the leathers through the focus groups method whit 13 consumers. The results of measurement of color showed that parameter b* of the leathers varied significantly with the drying temperature. The attributes of apple like flavor, sweetness, hardness, and acidity were significantly different between samples. The values related to chewiness between the treatment groups showed no statistically significant difference.
Keywords: Apple, Leather, Sensory Attributes

Introduction
The Apple production in Mexico in the 2010 was of 584,655.18 Ton, of which 47,794.72 were from the state of Durango, which placed as the second apple producing state at national level (1). The small fruits or with mechanical damage are usually discarded or sold at a low price, whereby the known "fruits leathers" are a good choice for processing the apple of low commercial value.
The fruits leathers are fruit pulp plates of soft and elastic consistency, sweet flavor, whose color depends on the raw material used. Almost all fruits can be used in the preparation of leathers; however, the most used are the tropical fruits (2). These leathers can be prepared from the puree or fruit pulp, extended in layers on a flat surface whose thickness may vary and are subjected to a drying process by convection or by exposure to sunlight (3).
Mango leathers have been prepared stable in storage of six months by a combination of low pH and water activity (4). Also been evaluated the quality of guava leathers where showed a significant effect on the acidity of the leathers using five different cultivars (5). Furthermore, has been studied acceptability and quality of jaca leather comparing sun-dried leathers with leathers dried in cabin (6). The texture and acceptability of the leathers is affected by the temperature of drying, the final moisture content, the type of fruit used and the addition of other ingredients. Thus the aim of this work was to evaluate the effect of drying temperature, formulation and apple variety in the sensory attributes of "fruit leather" made from apples of low commercial value.

Materials and methods
For the elaboration of the apple leathers used two apple varieties (*Golden delicious* and *Red delicious*) from Canatlán, Dgo., México. Citric acid, chili, sugar and purified water were obtained from local stores.
Sample preparation.

The apples were washed and the stem, leaves, seeds and bone were removed. Then proceeded to divide and weigh the apple to determine yields. One that was fractionated and weighed, was added a 1% solution of citric acid. Then the apples were liquefied using citric acid solution in a blender brand "Osterizer". The formulation ingredients were added, obtaining twelve different leathers according to Table 1. Heat treatment was applied to the formulations to a temperature of 75 ° C. After reaching temperature, the mixture was cooled to 40 ° C. To spread the mixtures were used aluminum trays of 20x30 cm for which previously were placed cellophane in the bottom. The puree was spread along the tray reaching a uniform thickness of approximately 3 mm. The drying was performed in a tray dryer with an air velocity of 5m/s to temperatures 60 or 70 ° C. The leathers were removed from the trays with cellophane base, with which were rolled and packed in sealed bags Zi-ploc until the day of sensory evaluation.

Table 1. Different apple leathers produced.

Sample	Characteristics	Sample	Characteristics
M1	*Red delicious*, natural, 60°C	**M7**	*Red delicious*, natural, 70°C
M2	*Red delicious*, azúcar, 60°C	**M8**	*Red delicious*, azúcar, 70°C
M3	*Red delicious*, chile, 60°C	**M9**	*Red delicious*, chile, 70°C
M4	*Golden delicious*, natural, 60°C	**M10**	*Golden delicious*, natural, 70°C
M5	*Golden delicious*, azúcar, 60°C	**M11**	*Golden delicious*, azúcar, 70°C
M6	*Golden delicious*, chile, 60°C	**M12**	*Golden delicious*, chile, 70°C

Color measurement. The determination of color on the apple slices was carried out with the aid of a Hunter Lab colorimeter, QC Match Easy, using the scale L*, a*, b*.

Sensory analysis. In step one was used a ranking method to select the best eight formulations. In the questionnaire the panelist ordered the leathers in descending order according to their preference. Was involved a panel of 15 people between the ages of 22 and 28 years, students of the Postgraduate in Biochemical Engineering of the Instituto Tecnológico de Durango. The selection criteria included availability of time and interest in participating pleased to consume the product. In this sensory evaluation was used untrained panel, for each panelist was served samples consisting of 12 strips (4x2cm), encoded with a three-digit number at random. Panelists received two cookies (HABANERAS) to reverse the effect of taste between samples, and rinsing the mouth with water.

Once the eight samples were selected according to the preference, was conducted the stage two where applied the descriptive-analytical method (QDA) and the method of focus groups. Was first conducted analytical-descriptive method (QDA) with 15 trained panelists that evaluated the following attributes: apple flavor, sweetness, chewiness, hardness and acidity. Was employed 7-point scale categorized, where 1 indicated the lowest intensity attribute and 7 the highest intensity. Then was applied the method of focus groups with 13 consumers to evaluate the trend in the overall acceptability of the eight samples and commenting on their perception of the product (7).

Statistical Analysis. The means of color assessment were subject to analysis of variance of one-way and the Tukey's test to verify significant differences ($\rho<0.05$) for Statistica 5.0. The sensory analysis data obtained by the ranking test, were subjected to the nonparametric Friedman test. To carry out the analysis of variance in the data obtained in the quantitative-descriptive test (QDA) and acceptability to focus groups, was used Sigma Stat software version 3.5 and the Sigma Plot Version 10 with a confidence interval of 95% ($\rho <0.05$).

Results and discussion

Color. The color parameters values were affected by the drying temperature and the formulation therefore that there was a significant effect ($p<0.05$). The Table 2 shows that natural and sugar leathers dried at the temperature of 70 °C became more yellow-orange. Moreover, the formulation was crucial to the color; those that had chili presented a reddish color.

Table 2. Apple leathers color.

	Color parameters		
Samples	**L**	**a**	**b**
M1	32.92±(0.01)a	15.98±(0.07)a	22.96±(0.04)a
M2	35.83±(0.01)b	21.21±(0.03)b	23.96±(0.17)a
M3	21.55±(0.04)c	19.45±(0.12)c	13.42±(0.12)b
M4	33.70±(0.02)d	17.14±(0.01)d	31.36±(0.09)c
M5	35.38±(0.02)e	12.17±(0.02)e	19.95±(0.13)d
M6	23.64±(0.04)f	23.39±(0.05)f	20.33±(0.20)d
M7	29.23±(0.01)g	19.21±(0.03)g	35.06±(0.55)e
M8	38.08±(0.04)h	16.45±(0.05)h	27.30±(0.15)f
M9	24.47±(0.03)i	29.07±(0.13)i	30.34±(0.47)g
M10	32.79±(0.03)j	17.48±(0.03)j	27.23±(0.09)f
M11	42.12±(0.05)k	15.38±(0.04)k	28.99±(0.07)h
M12	24.14±(0.01)l	25.29±(0.07)l	20.09±(0.27)d

Means Within a row with the same letters are not significantly different (Tukey, p> 0.05).

Ranking test. The Friedman analysis of variance showed that the perception of preference among the samples was significantly different (ρ<0.05). It is observed in Figure 1 that the sample M2 (*Red Delicious*, sugar, 60 ° C) shows the lowest score, therefore the highest preference. On the contrary the sample M4 (*Golden delicious*, natural and 60 ° C) obtained the highest score, winning the last place preference. Samples with highest scores in the total sum were M1, M4, M7, and M11 therefore they were on the last places in preference, were discarded for the subsequent evaluations.

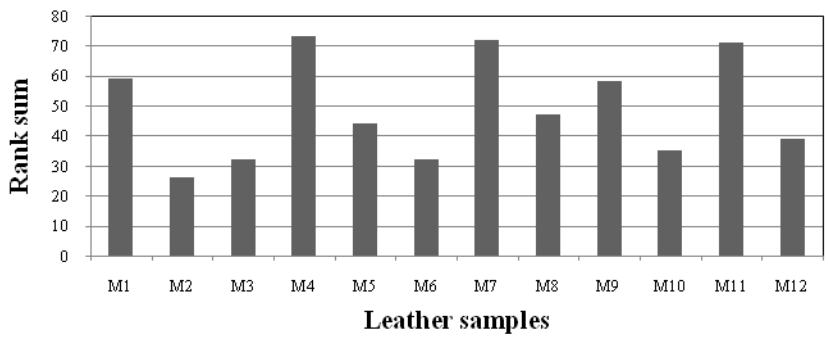

Figure 1. Rank sum scores for the twelve samples of apple leathers. The lower rank sums indicate higher preference and higher rank sum scores indicate lower preference.

Descriptive quantitative analysis. The trained panel evaluated the attributes apple flavor, sweetness, chewiness, harshness and acidity; the results are shown in Figure 2. Was significant difference between samples (ρ <0.005) for attributes of apple flavor, sweetness, hardness and acidity. However, the analysis of the values concerning the chewiness between samples did not show a statistically significant difference (ρ = 0.074).

Focus group. The apple leathers with higher tendency to acceptability were M2 (Red delicious, sugar, 60 ° C) and the M6 (*Golden delicious*, chili, 60 ° C) as shown in Figure 2. The 13 consumers gave their opinions on the attributes of the leathers they considered important for election were the apple flavor and sweetness which highlighted the leathers containing sugar and the acidity; the chewiness and acidity were more acceptable to the leathers with chili; for moreover, the hardness of the leathers dried at 70 ° C were decisive for its low acceptability.

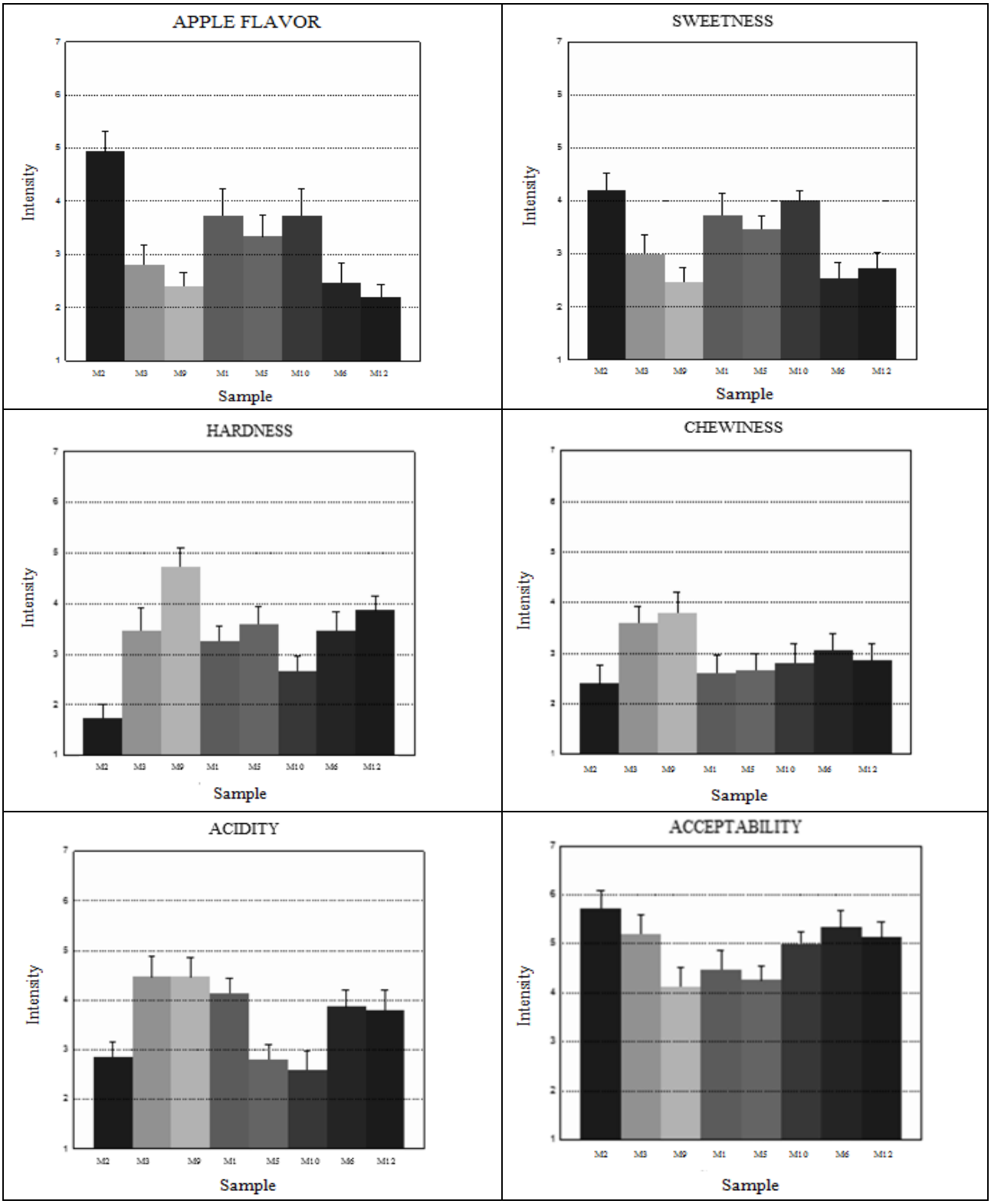

Figure 2. Results of the focus group and the descriptive quantitative analysis.

Conclusions

The drying temperature and the formulation of the leathers had a significant effect in the acceptability of the product. The leathers with higher acceptability were dried at a temperature of 60°C with the two apple varieties employed and with the formulations of sugar and chili respectively. Although the results show eight leathers scores above the average of 4, whereby a good tendency is observed in general acceptability.

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Exploration of Aroma Quality of Mezcal by Sensory Analysis and Chromatographic Analysis

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Introduction

Mezcal is a Mexican alcoholic beverage, obtained by the artesian fermentation and distillation from agave syrup. The sensory properties of alcoholic beverages such as mezcal, wine, whiskey, rum and others are determined by the composition and combination of volatiles such as alcohols, esthers, terpenes and some other compounds (1, 2, 3). The aroma quality is one of the most determining criteria for food acceptance (3, 4). Therefore, to have a complete understanding it is important to get information of both volatile compounds and sensory properties (4). Methods based on gas chromatography are extensively used for instrumental analysis of flavor compounds. On the other hand, sensory evaluation is oriented to identify and quantify the impact of the compounds on the senses, the product perception and their impact on the level of acceptance and liking (5, 3). Quantitative descriptive analysis is one of the most comprehensive and informative tools used in sensory evaluation. An important aspect on flavor studies is the relationship between sensory and instrumental data (5). Multivariate analysis has been used for flavor studies; especially Partial Least Squares (PLS) that has been applied to correlate sensory and instrumental results (3, 4, 5).

Keywords: Descriptive Analysis, Mezcal, Volatile Compunds, Correlation.

Materials and methods

The samples consisted of two mezcals, of different batches, that were produced by different makers of Oaxaca State, and one mezcal of the region of San Luis Potosí (Table 1).

Table 1. Information the mezcals

Mezcal	Batch	Fermentation type	Region
Mezcal 1 (M1)	2008	Juice and bagasse, in wooden tubs, spontaneous, high population of bacteria	Matatlan, Oaxaca
Mezcal 2 (M2)	2008 (batch 1)	Juice and bagasse, in wooden tubs, fermentation spontaneous	San Pedro Totolopan, Oaxaca
Mezcal 3 (M3)	2009	Juice and bagasse ,in wooden tubs, fermentation induced	Matatlan, Oaxaca
Mezcal 4 (M4)	2008 (batch 2)	Juice and bagasse, induced fermentation with ammonium sulfate	San Pedro Totolopan, Oaxaca
Mezcal 5 (M5)	2007	Juice no bagasse, in wooden tubes, fermentation with ammonium sulfate	San Luis Potosí

Odoractive compounds were extracted and concentrated by solid phase microextraction (SPME), and identified by gas chromatography-mass spectrometry (GC-MS), according to De León Rodriguez (2). Ten representative odor compounds were selected to construct calibration curves for quantification by gas chromatography-flame ionization detection; there were included substances of each chemical family (all chemicals used as standards with purity up to 99.0% GC grade were purchased from Sigma-Aldrich).

Sensory measurements were made with a group of 12 trained assessors according to Official French Standard (AFNOR V09-003). The homogeneity between batches from the same manufacturer was verified using triangular tests (AFNOR V09-13). Odor descriptors were generated and agreed upon using objective references for developing the quantitative odor profiles of each mezcal. A ranking test (ISO; 8587) was used to establish low, medium and high reference of odor intensity. The intensity of each descriptor was evaluated using a semi structured scale of 16 cm. (ISO: 11035).

Results

Ninety-seven volatile compounds were identified by SPME-GC-MS in all the samples; they belonged to different chemical families (table 2).

Table 2 Number of volatile compounds identified by chemical family

Chemical Family	Identified compounds
Acetals	2
Acids	7
Alcohols	12
Ketones	2
Aldehydes	3
Esthers	22
Phenols	8
Furans	5
Terpenes	24
Hydrocarbons	12

Among the volatile compounds present in the mezcal, there was a selection made to choose compounds representing different chemical families which have already been reported present in mezcal (Table 3).

Table 3 Concentration of identified compounds in gas phase

Ret. Time (min)	Compound	M1		M2		M3		M4		M5	
		Con. (PPM)	Std Dev	Con. (PPM)	Std dev	Con. (PPM)	Std dev	Con. (PPM)	Std dev	Con. (PPM)	Std Dev
3,06	Ethyl acetate	0	0	110,631	1,436	287,228	0,917	169,399	2,046	310,878	7,612
8,94	3-methyl-1-butanol	4,517	0,184	1,143	0,096	1,639	0,246	1,756	0,047	0,88	0,004
10,04	D-limonene	Ns		Ns		Ns		3,235	0,264	4,013	0,513
20,59	Ethyl Octanoate	Ns		Ns		Ns		Ns		4,37	0,212
21,55	Furfural	22,575	1,88	0,677	0,028	9,286	1,573	6,267	0,432	10,347	0,516
21,57	Acetic acid	3,443	0,661	0,351	0,244	0,351	0,244	2,886	0,288	2,427	0,235
23,77	Linalool	2,654	0,452	4,296	0,339	1,941	0,045	3,093	0,119	5,268	0,004
33,25	Propionic acid	Ns		10,817	3,163	Ns		6,795	0,57	14,281	0,449
35,4	Ethyl decanoate	3,706	0,472	0,677	0,028	1,079	0,485	3,086	0,068	1,703	0,095
39,74	Eugenol	Ns		0,136	0,04	1,191	0,017	0,475	0,046	Ns	

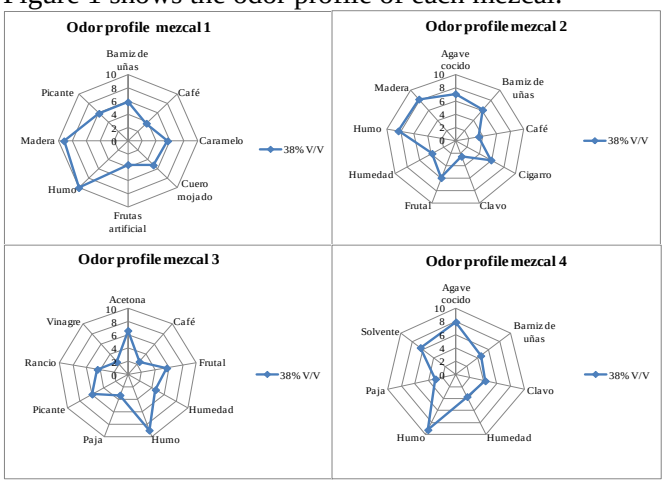
The concentrations of the quantified compounds were variable even among mezcals of the same manufacturer. Some of them were present in low concentrations as furfural or 3-methyl-1-butanol, however the contribution of these compounds in odor characteristics could be significant. It might suppose that even those compounds found in low concentrations and in combination with other compounds could determine the characteristic odor of each mezcal. The results of triangle test are showing in table 4. The objectives of this test were: to familiarize judges with sensory evaluation protocols, to familiarize judges with particular characteristics of the mezcals, and verify the homogeneity between different batches of the same manufacturer.

Table 4 Results of triangle test in mezcals

Alcoholic concentration	Mezcals	Observations with differences	Observations with no differences	Probability according to one tail table (p=1/3), O´Mahony (1986)
38% v/v	Mezcal 1 vs Mezcal 3	14	6	0.001
	Mezcal 2 vs Mezcal 4	17	3	≤0.001
20% v/v	Mezcal 1 vs Mezcal 3	18	2	≤0.001
	Mezcal 2 vs Mezcal 4	18	2	≤0.001

Triangle test showed differences between batches of mezcal from the same manufacturer, resulting in a heterogeneous aromatic quality of mezcal within each manufacturer. Confirming that there are real differences in the manufacturing process, which probably have some influence on the volatile compounds profile (6). On the other hand, the differences between batches were accentuated in mezcals evaluated at 20% V/V of alcohol.

Figure 1 shows the odor profile of each mezcal.



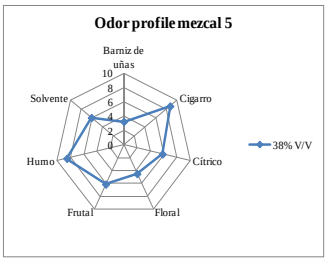


Figure 1. Quantitative descriptive profile odor for the mezcals

Each mezcal presented a unique profile with a common descriptor (humo). Mezcals 2 and 4 from the same manufacturer presented the "agave cocido" descriptor, which is highly desired among manufactures of mezcal. After obtaining the data of sensory and instrumental measurements, a correlation between them was explored through Partial Least Squares (PLS). According to the literature, it is a widely used tool for the correlation between instrumental and sensory measurements. To observe any correlation, the predictive matrix (X) was the quantified concentrations of compounds in headspace, against the intensity data of the differentiating sensory descriptors for batch and producer as response matrix (Y). Table 5 shows the results of PLS analysis.

Table 5. Results of Partial Least Squares analysis for the descriptors and quantified compounds

Descriptor	Regression R^2	Number of components	P value	Mezcals
Cuero mojado	0,9959	9	<0.0000001	M1
Fruta artificial	0,9946	3	0,000356531	M1
Caramelo	0,9945	9	<0.0000001	M1
Floral	0,9931	5	0,000545923	M5
Clavo	0,9854	6	0,000158371	M2, M4
Rancio	0,9281	4	0,00763082	M3
Acetona	0,9053	6	0,000201707	M3
Vinagre	0,8877	6	0,00130951	M3
Picante	0,7274	4	0,00109998	M1, M3
Cítrico	0,723	5	0,00470372	M5
Agave cocido	0,4911	7	0,000871824	M2, M4

The regression coefficient indicates the reliability of the model to predict the relationship between compounds and descriptors. This coefficient was obtained from the observed and predicted results. The method of partial least squares regression generates functions or components that predict each descriptor (Y). The number of components generates to predict Y is obtained by cross validation method and refers to the number of functions that achieve an accurate prediction, the P value is the significance level of the regression model and the last column indicates in which mezcal these descriptors were present.

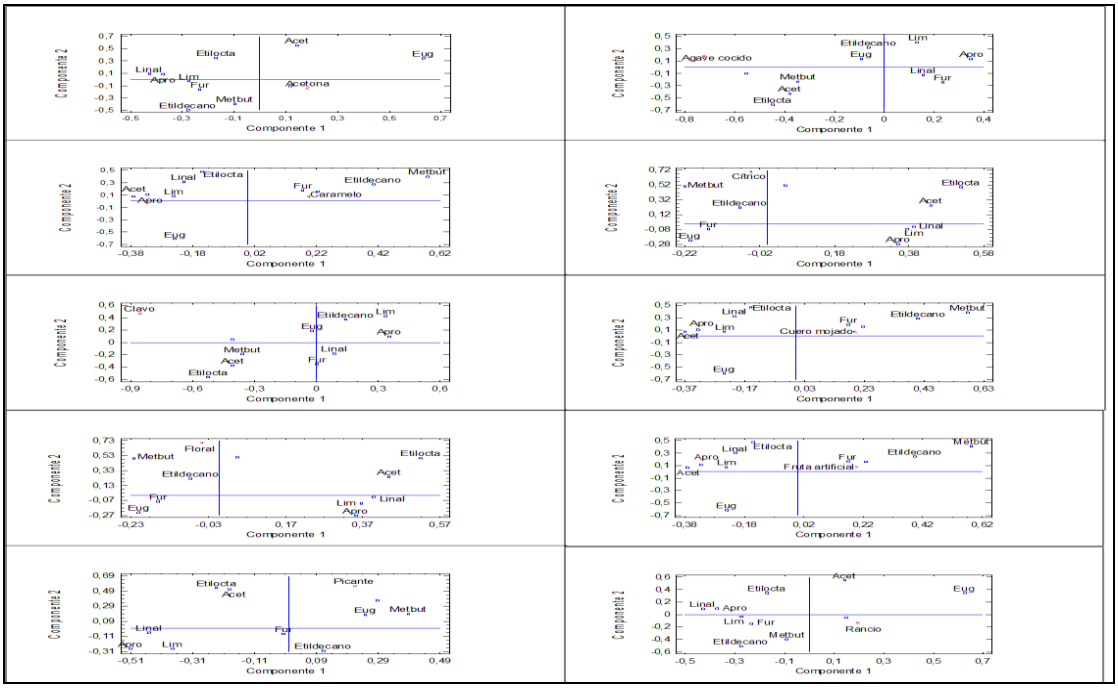


Figure 2. Loadings of PLS-components for each descriptor

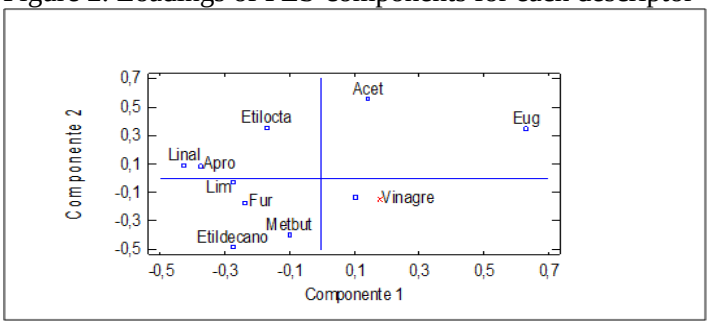


Figure 3 Loading of PLS-components for “vinagre” descriptor

It is noted that descriptors have a high correlation with the volatile compounds (cuero mojado, fruta artificial, caramel, floral, clavo, rancio, acetone and vinagre), only in the case of “agave cocido” and “picante” descriptors showed that the regression coefficients were relatively low with respect to the others, probably because of different patterns of association between sensory and instrumental variables (3). According to the analysis were some kind of associations between descriptors differentiators and quantified compounds. They had different patterns of both positive and negative correlations that explain each descriptor. The correlations between descriptors and formation of volatile compounds may indicate that the generation of a descriptor is due both to the presence of a few components which together form the descriptor and the presence of other compounds affecting their generation.

As final conclusions, the triangle test showed differences between the mezcals of the same manufacturer. Some sensory discriminative descriptors were identified and their relationship with volatile composition was modeled by partial least squares using the quantities of 10 representative compounds as predictors.

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Sensory Evaluation Of Handcrafted Corn Malt Beverages: Sendechó And Corn Beer

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Introduction

The consumption of alcoholic drinks is not just a current phenomenon in Mexico; ancient civilizations also made fermented beverages from a variety of products (6). From them, corn (*Zea mays L.)* has been an important cereal widely used as raw material for the production of typical fermented products in Mexico.

Sendechó is a malted corn beverage with low alcoholic graduation created by Otomíes and Mazahuas in Estado de Mexico, its method of production and sensory properties are similar to beer. This beverage is prepared with malted corn and chili, two typical ingredients in Mexican food and it used pulque as inoculum for fermentation (2)

Nowadays, corn is used for manufacturing starch and as an adjunct by breweries. Whereas malting of barley and wheat has advanced, corn has not received similar attention, so far (6).In order to recover our traditions and to offer the consumers and innovative product, samples ofhandcrafted corn beer were prepared using malted corn and a controlled fermentation, the same as using in the production of barely beers.

The aim of the present study was the sensory evaluation of handcrafted beverages, Sendechó and Corn beer, based on Quantitative Descriptive Analysis method (QDA) as a tool for measuring sensory proprieties and to obtain avocabulary of attributes for appearance, flavor, odor, and taste (1), (4). They will become quality indicators for the characterization of the sensory properties of both Sendechó and the new Corn beer.

Materials and methods

Samples

Two varieties of corn, red and blue, were used to elaborate the samples of both beverages, Sendechó and Corn Beer.

Sendechó was prepared with combinations of malted corn, pulque as inoculum of fermentation, and chili, as indicate in Table 1. The samples of beer were prepared with the same technic of malting corn, a controlled fermentation, and combinations of corn, chili and hop as indicate in Table 1.

Tabla3. Composition of Sendechó and Corn beer samples.

Sendechó	Red corn with chili	Blue corn with chili	
Cornbeer	Red corn with chili (RCCH)	Blue corn with hop (BCH)	Red corn with chili and hop (RCCHH) Blue corn with chili and hop (BCCHH)

Preparation of Sendechó and Corn Beer Samples

The method used in the preparation of Sendechó and Corn samples is indicated in Figure1.

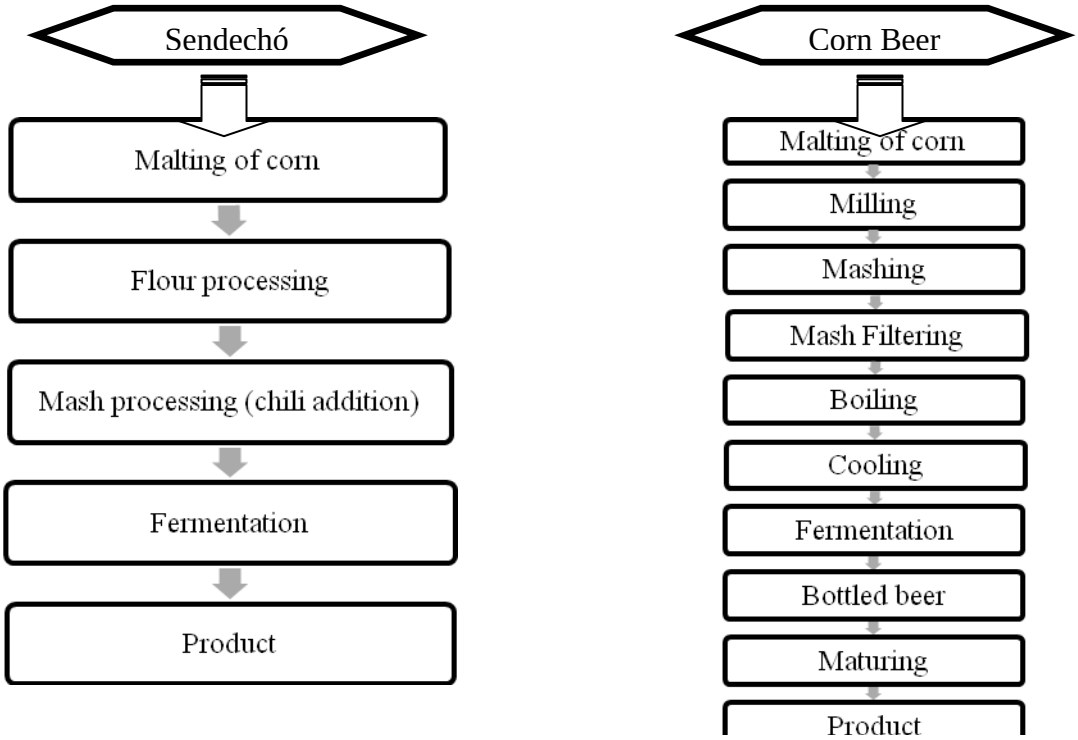

Figure 1. Preparation of Sendechó and Corn Beer Samples

Sensory Evaluation

Two fermented beverages were characterized by trained panel at Sensory Laboratory of Universidad AutónomaMetropolitana-Iztapalapa. The panel comprised twelve assessors. Tasting sessions were carried out one time a week. In each session, from three to four samples of beer and Sendechó were randomly presented to the panelists. Beer was cooled down to 5±1 °Cand served in clean glasses (40ml).

Attributes of appearance, flavor, odor and taste were evaluated for each beverage by judges. Quantitative Descriptive Analysis (QDA) was used as a sensory method to describe all sensory attributes of the samples of Sendechó and Corn Beer. An unstructured line scale was used for each attribute. Marks were converted to a score of 15 for data analysis purposes (1), (4).

Statistical Analysis

Sensory profiles for every product were obtained with the average for each descriptor represented in radial plots. Results of Corn Beer were analyzed using Principal Components Analysis (PCA), a multivariate method that can be applied to QDA data to reduce the set of dependent variables (attributes) to a smaller set of uncorrelated variables (factors) based on patterns of correlation among the original variables (3).

Results

Table 2. Attributes of Sendechó and Corn Beer

Attribute			
Sendechó		**Corn Beer**	
Corn		Floral	
Nixtamal		Banana	Sweet
Toasted	Corn odor	Fruity	Salty
Yeasty	Nixtamal odor	Green Apple	Bitter
Cooked	Toasted odor	Butter	Acid
Vegetables	Yeasty odor	Yeasty	Citric Fruit-odor
Pepper	Cooked vegetables odor	Sweet Corn	Green Apple-odor
Chili	Pepper odor	Cooked Corn	Cooked corn-odor
Peanut	Chili-odor	Cooked Vegetables	Hoppy
Pulque	Peanut-odor	Molasses	Cooked vegetable-odor
Tamarind	Pulque-odor	Nixtamal	Spicy
Lactic	Tamarint-odor	Fermented Fruit	Metallic
Fermentation	Lactic fermentation-odor	Chili	Astringent
Fermented Fruit	Fermented fruit-odor	Alcoholic	Carbonation
Mature Cheese	Mature cheese-odor	Solvent-like	Body
Acid		Tortilla	
Sweet			

To create a visual profile of product attributes, radial plots were created by plotting average intensity values on each scale, and then joining the points (5). Figure 2a and 2b show attributes of Sendechó and Corn Beer respectively. These plots illustrate that "cornflavor" "nixtamal flavor" and "chili odor" were characteristics found in both beverages.

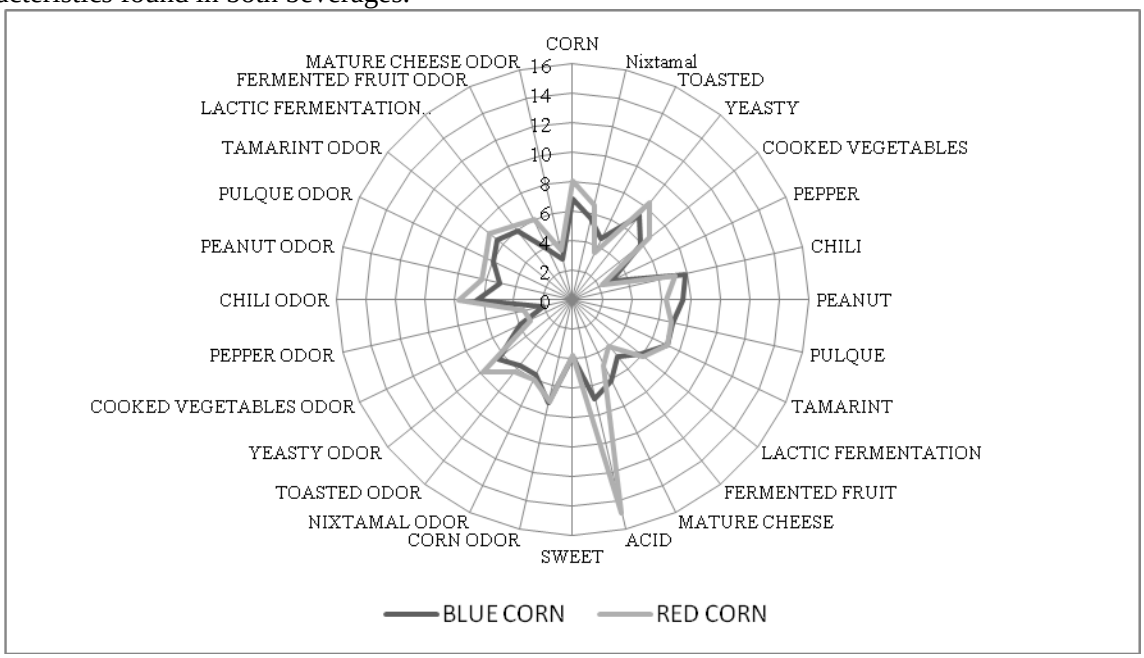

Figure 2. (a)Plot obtained through QDA of Sendechó

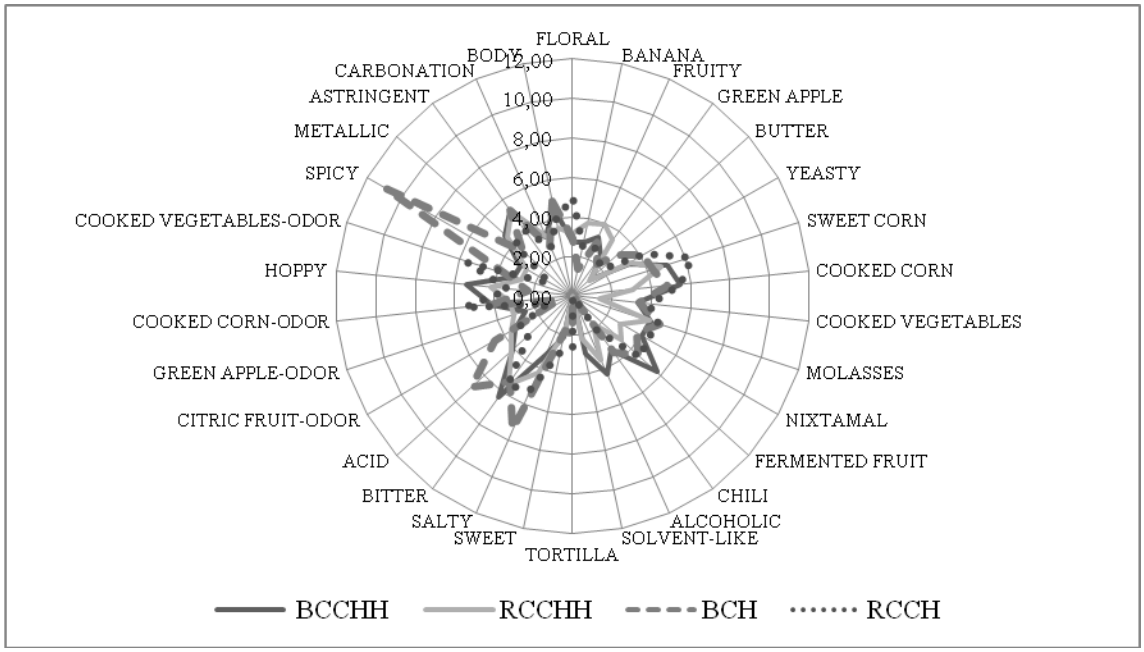

Figure 2. (b)Plot obtained through QDA of Corn Beer

PCA was applied to the results of corn beerfor the attributes listed in Table 2 and the four products (Table 1). PCA specify the location of each product along of the principal components and enabled the generation of biplots or maps that represented the variability of samples, the same as the more important variables (sensory attributes) related to sample differentiation. Products that are similar to one another are positioned close to one another in the map, and products that are different, are far apart (2).

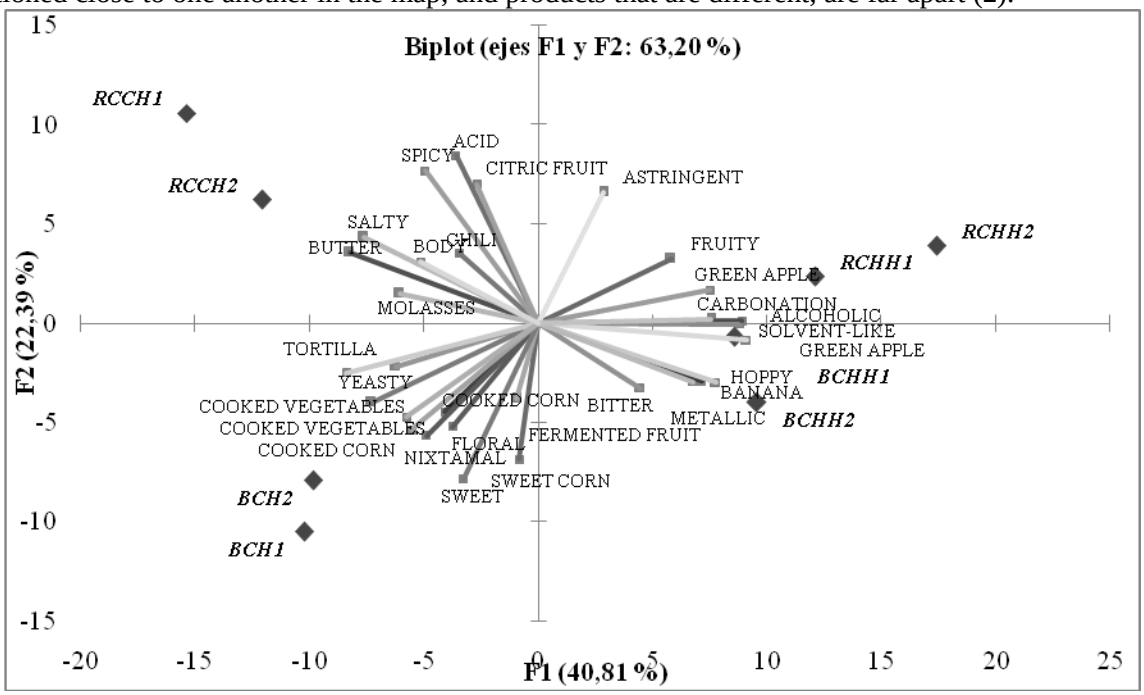

Figure 3.Results of PCA of Corn Beer.

PCA scores and loadingsplot for the first two principal components are shown in Fig. 3. The first two components account for 40.81 and 22.39% of total variance correspondingly. The first component differentiates between mixed beers (i.e. combination with chili and hop BCHH and RCHH) from the unmixed beers (i.e. made whit chili or hop). The first component is related to attributes as spicy, acid,

citric fruit, astringent, sweet corn, sweet and nixtamal. The second component differentiates between beers made with red corn from beers made with blue corn. The attributes that contributing to the second principal component are alcoholic, carbonation, solvent-like, green apple, tortilla, butter and salty.

Conclusions

The attributes that best represented these two beverages were corn flavor, chiliodor andnixtamal flavor. These descriptors complement some others commonly used for the flavor evaluation of the conventional beer made of malted barley.

Acknowledgements.

ICyTDF (Project 250/2010) for research financial support and Scholarship of Nicté Cruz and CONACYT for Angélica Romero's scholarship.

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Sensory Evaluation Of Marinated Beef With Chía-Base Marinator

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Introduction

Sensory evaluation is concerned of the measurement and quantification of the characteristics of a product or ingredient, which are perceived by the human senses (1) and is probably the most important step in the process of developing new products, because acceptance of the final product depends basically on the appreciation of consumers. In the development of new products is common to use ingredients that provide added value to them, and these may modify the aroma and flavor profiles of the final product. In the meat industry it is usual to use marinators in order to provide color and flavor while allowing the preservation of the meat for longer, and remains soft and juicy; also provide increased water retention and thus higher yields, which means higher profits; also allows the use of cuts of low commercial value. The marinade is founded on water-protein interactions, this is due to the attraction of the positive and negative charges in the molecules, but these may be affected by the conditions of the system, such as the final pH of the meat (2). The way of action of marinators is to change the pH in muscle myofibrillar proteins so they get free and let them interlaced, which allow the phosphates to link with protein. (3). Another feature that may be involved in the performance is the high content of fiber, because of its high water holding capacity (4). The fiber in chia (*Salvia hispanica*) holds 14 times more water than wheat bran and 16 times more than flaxseed (5), previous studies by our team observed that chia has an impact on water retention in meat products helping to improve performance; it may be related with health benefits to consumers. Since chia seed contains nutrients essential to human health such as omega-3 and 6 (6). The aim of this study is to evaluate the sensory perception of meat marinated in two forms with different amounts of flour chia seeds (*Salvia hispanica*).

Material y methods

Samples of Chia seeds (*Salvia hispanica*), type beef flank steak, lemon-pepper seasoning, and a commercial marinator which used in this study were obtained from a local supermarket. The reagents used were corn starch from Arancia industries, sodium triphosphate DEQ lot: 12321jiv, 2,6-di-tert-butyl-batch 4methylphenol DEQ: 0001439850, Lactic acid 85% DEQ Lot: 110219-01 AL.

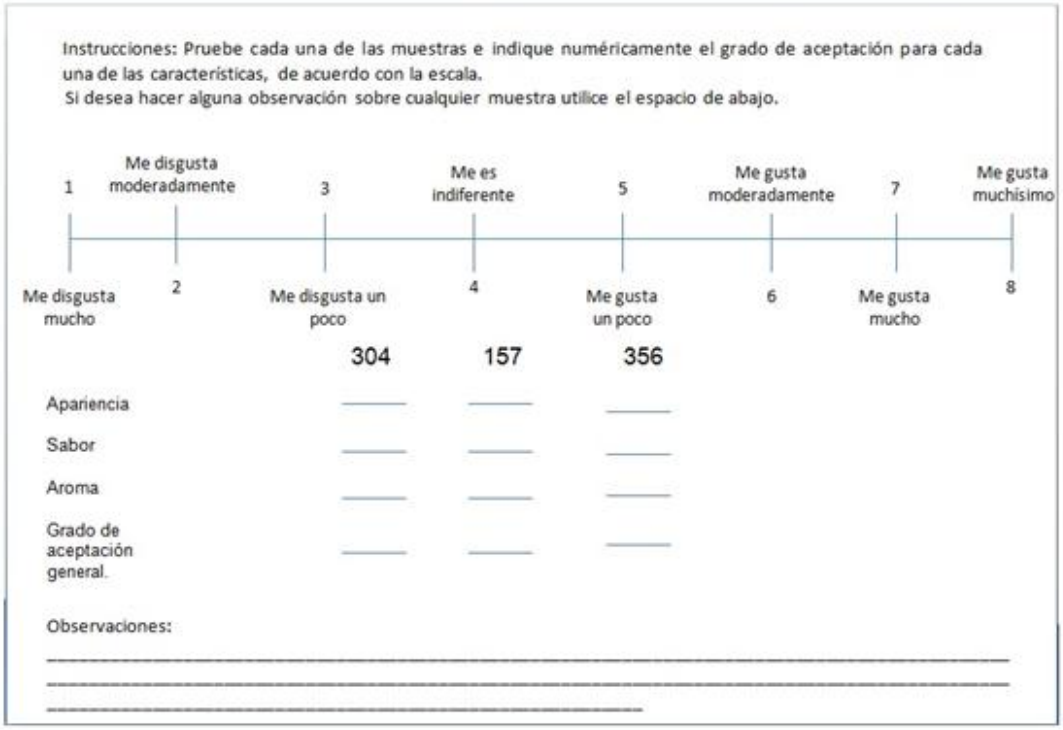
Each tested formulas marinator is shown in Table 1. And as a control we used a commercial marinator of wich the exact formulation is unknown.

Table 1. Formulations per 100ml of water of each marinator tested in this study.

		M1	M2
Corn Starch	g	5	5
Chia seed flour	g	5	5
BHT	g	0,05	0,05
sodium triphosphate	g	10	10
Lemon-Pepper	g	5	5
Lactic acid	ml	0	5

We prepared a dilution in water and used 20% of the weight of the meat for marination, leaving them in a vaccum tumbler, marinator meat mixer at a pressure of 15mm of mercury for 15 minutes, with 5.81 RPM.

Fluid was removed, and all treatments underwent to similar thermal processing, up to 137 ° C in the thermal center.
Was then applied a sensory evaluation. Performed by 22 non trained judges using a hedonic scale of 1 to 8, with 1 being "I dislike it" and 8 "I really like it." Evaluation includes appearance, flavor, aroma and general acceptance. An example of the evaluation sheet is shown in the Image 1. Finally, the data were analyzed using an ANOVA with the program Minitab version 14. (7)


Instrucciones: Pruebe cada una de las muestras e indique numéricamente el grado de aceptación para cada una de las características, de acuerdo con la escala.
Si desea hacer alguna observación sobre cualquier muestra utilice el espacio de abajo.

1 Me disgusta mucho
2 Me disgusta moderadamente
3 Me disgusta un poco
4 Me es indiferente
5 Me gusta un poco
6 Me gusta moderadamente
7 Me gusta mucho
8 Me gusta muchísimo

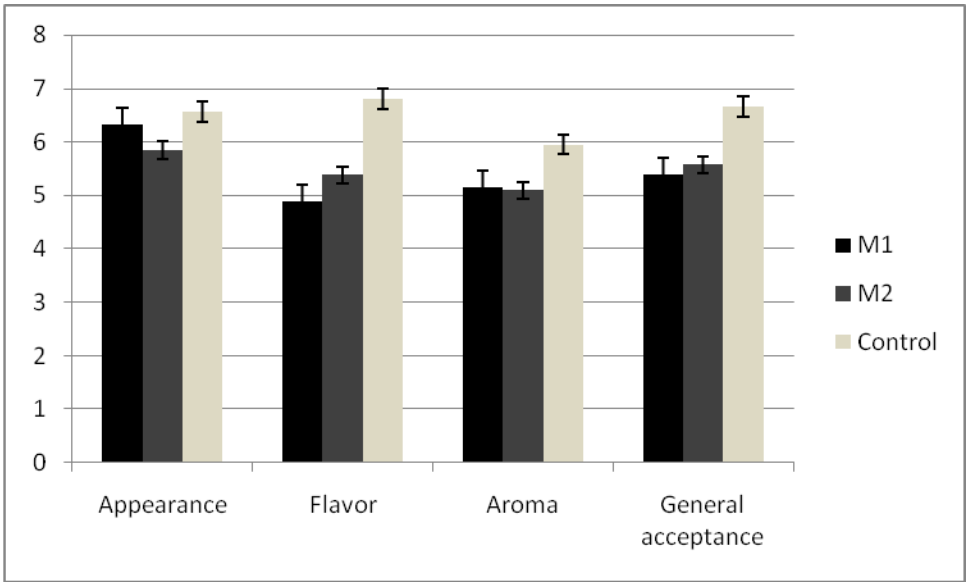
	304	157	356
Apariencia			
Sabor			
Aroma			
Grado de aceptación general.			

Observaciones:

Image 1. Format of evaluation of sensory analysis test.

Results and discussion

As shows in the Graphic 1 none of the evaluated parameters shows significant differences between the three marinators tested.



Graphic 1. Results of the sensory evaluation of beef treated with three different marinators

Despite the limiting factor in the shelf life of fresh meat is microbial total count, the consumer selects by the aspect of the product, in particular by color or appearance. The two chia marinators (M1, M2) and control marinaror did show significant difference ($p > 0.05$). M1 and M2 showed a slight sour taste, the acid taste in certain foods can be a sign of deterioration, but this flavor was not noticeable by all judges. The presence of this flavor may be due in part to the addition of lactic acid and the acids of chia itself.
The aroma is one of the main factors determining the acceptance or rejection of a product. In this case, there are no significant differences between marinators. According to the literature the aroma is the most difficult to determine. Some of the factors influencing the results are: adaptation or fatigue of the judge, the cleanse of the air, or the inability to quantify odorant reaching the mucosa (8). That's why there was no difference between the three marinadores. In conclusion chia seeds flour can be used even in a proportion of 20% of the marinator without affecting the sensorial characteristics of meat.

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Effect Of The Storage Time On The Sensory and Physicochemical Profile Of Tomato *Roma* And *Globe* Variety

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Abstract

In Mexico, tomato has an annual production of more than 2 billion tons, with per capita consumption of 15.3 kg. Tomatoes are included in the diet because it reduces or control prostate cancer and cardiovascular problems, and counteracts vision´s problems especially in diabetic persons. In order to fulfill consumer demands, tomatoes are growing in greenhouses to be harvest throughout the year. Therefore, producers have been concerned to offer a high quality fruit (considering flavor, aroma, texture and aesthetics). The objective of this study was to evaluate how the sensory and physicochemical characteristics of tomatoes changed as the storage time increased. The material used was *Roma* (*Lycopersicon esculentum*) and *Globe* (*Lycopersicon esculentum* variety *Gironde*), were grow at a greenhouse from Santiago Maravatío, Guanajuato, Mexico. They were harvested at ¾ of mature stage, in January-June 2010, August-December 2010 and January-June 2011; and were stored for 28 days at 8°C and 60% RH. A descriptive sensory evaluation was performed using trained panelists, the parameters considered were appearance, aroma, flavor and texture; and also a physicochemical analysis was carried on. The data was collected using a randomized block design. ANOVA was employed, and in those cases where the null hypothesis was rejected, a mean comparison was achieved by the Bonferroni method. For *Roma* tomatoes, as storage time increased color; while fresh aroma, acid flavor and hardness intensity decreased; the overall aroma and sweetness intensity did not changed; the pH, °Brix, carotenes and lycopene concentration increased. In the other hand, for *Globe* variety color intensity and hardness increased; overall aroma and acid flavor did not changed; the titratable acidity, soluble solids by dry weight, carotene and lycopene concentration increased; pH was the only one decreasing. Concluding that, the effect of storage on tomatoes is reflected in the sensory attributes, mainly in color, and therefore the increased concentration of carotenes and lycopene in physicochemical tests; tomatoes had the best sensory and physicochemical properties between 21 day of storage.

(Keywords: Tomato, *Roma*, *Globe*, Storage, Sensory)

Introduction

The geographical origin of the *Lycopersicon* is located at the Andean region, ranging from southern Colombia to northern Chile. However, Mexico has had a more profitable domestication. The term "tomato" has been used since 1695, first by botanical travelers, who took the word from the Aztecs that called this plant "xitomate" or "xitotomate" [1,2,3].

It is a low calorie food; its main ingredients are water and carbohydrates [4]. Tomatoes are rich in vitamin C (ascorbic acid) and A (carotenoids), vitamins B and K, plus minerals, especially iron, phosphorus, calcium, manganese, magnesium, copper, potassium, zinc and sodium [5]. Sugars and organic acids are responsible for its characteristic flavor [4]. Lycopene is another of his attributes; this substance accountable for its color and it has antioxidant properties, because it protects against many cancers and prevents atherosclerosis [5].

Globally, Mexico ranks tenth in terms of annual production of tomato, with China being the largest producer with an output of approximately 33 billion tons, followed by the United States. The main producers of tomatoes in Mexico are Sinaloa, followed by Michoacán; with an estimated national annual production of 2.2 million tons [6,7].

Sensory Analysis

Sensory analysis is a tool that allows objective techniques to evaluate the sensory properties of food products and determine its acceptance by the consumer. A panel of trained tasters is a fundamental tool that evaluates all the attributes of a product, providing valuable information to [8]: quality control of raw materials or of the finished product, reformulation of products, process optimization, and product comparison. Sensory evaluation panels are classified into three types: highly trained or expert panels, laboratory panels (trained judges) and consumer panels (use a large number of untrained judges.) The first two are used in quality control in the development of new products or to measure changes in the composition of the product. Consumer panels are mainly utilized to determine buyers' reaction to the product [9]. In the other hand, human being tends to perceive the attributes generated by food in the following order: appearance, smell/aroma/fragrance, consistency and texture [10].

The aim of this study was to evaluate how the sensory and physicochemical profiles of two tomato varieties were affected by storage and harvested times.

Methodology

Roma and *Globe* tomatoes were purchased having ¾ green color appearance, from greenhouses located in Santiago Maravatío, Guanajuato, Mexico. They were washed and stored at 8C and 60% of relative humidity. The tests were performed at time 0 (day of harvest), at 7, 14, 21 and 28 days after being harvested. The fruit was collected from January-June 2010, August-December 2010 and January-June 2011.

Sensory Analysis

Twelve panelists were selected and trained, following the descriptive method. This method uses an intensity scale of 15 points (1=just detectable, 7=moderate intensity and 15=extreme intensity). The parameters evaluated included appearance, aroma, flavor, texture by touch and by mouth.

Physicochemical Analysis

The analysis carried out were pH, titratable acidity, soluble solids per °Brix and dry weight, carotenes, chlorophyll and lycopene; according to the AOAC.

Statistical Analysis

The sensory data was collected following a complete randomized block design. Both sensory and physicochemical data were analyzed by ANOVA, and in those cases when the nule hypothesis was rejected a mean comparison was achieved using the Bonferroni method; both analysis were at α=0.05 of significance.

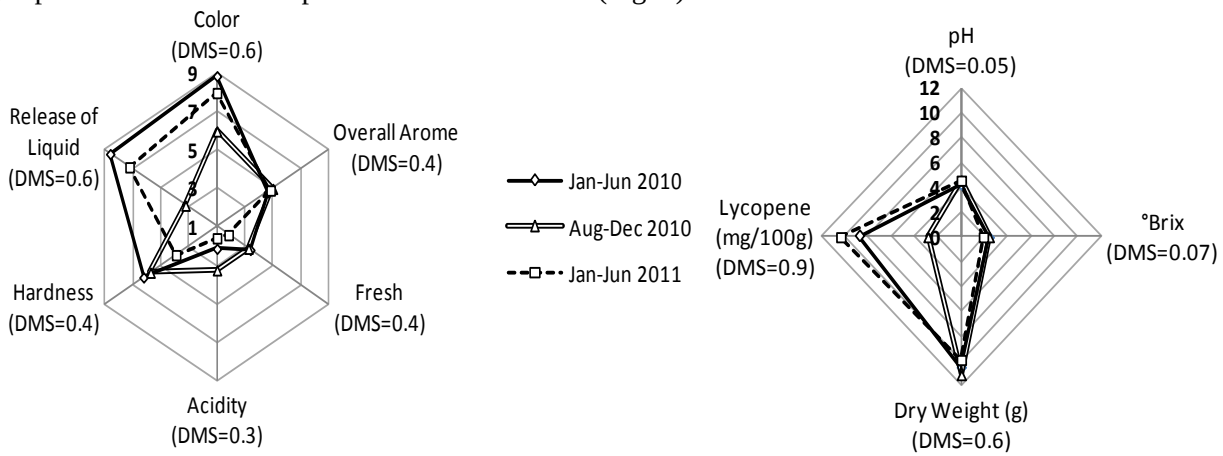
Results

In this paper only the data obtained at the 21st day of storage are shown. For the reason that on this date the fruit had the best sensory properties to be consumed as fresh.

Rome

In regards to the harvest time, the Jan-Jun 2010 had the greater color intensity, while the Aug-Dec 2010 presented the lowest. There were not significant differences in the overall aroma intensity among harvested times. The Jan-Jun 2011 harvest had an intensity almost imperceptible in fresh aroma, also was the lowest acidity and hardness. As for the release liquid parameter, Jan-Jun 2010 scored the highest value compared to the other harvest times (Fig. 1).

In the physicochemical tests, the three harvest times there were not significances different on the pH, °Brix and dry weight (approx. at 4.5, 2.2 and 10.5, respectively). Jan-Jun 2011 harvest scored the highest lycopne concentration compared to the others times (Fig. 1).



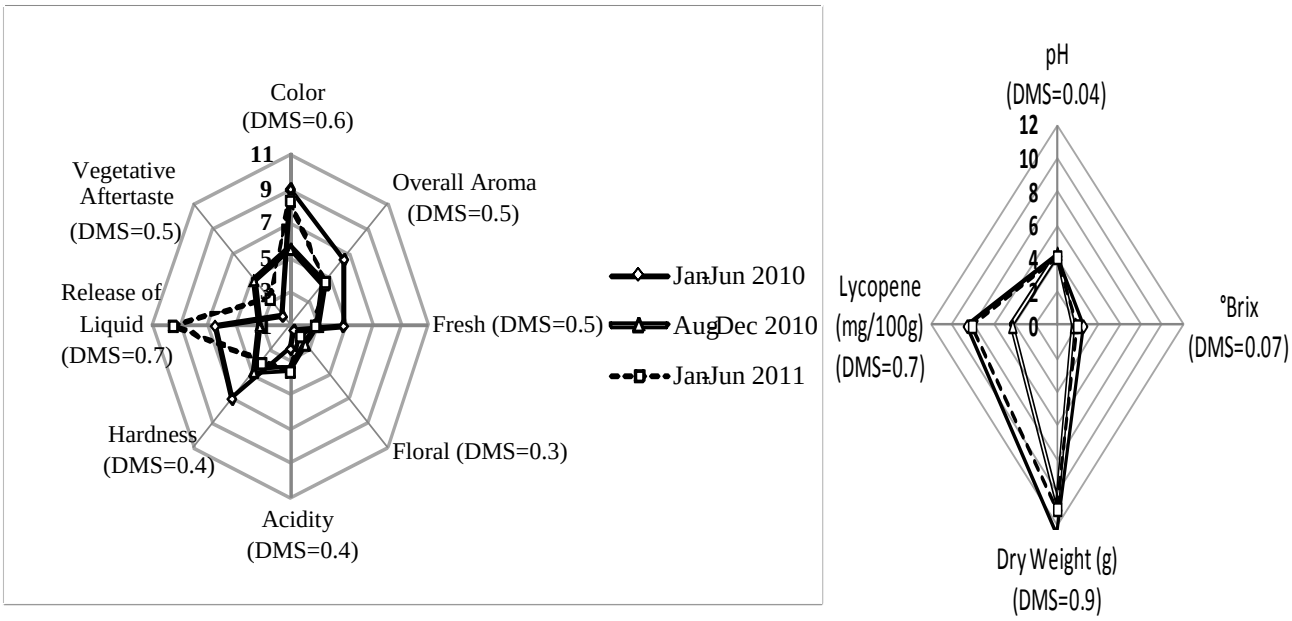
a) **b)**

Fig. 1. Effect of the harvest time evaluated at 21 days on roma tomatoes: a) Sensory profile, b) Physicochemical parameters.

Globe

The color intensity was higher in Jan-Jun 2010 and 2011 harvest compared to Aug-Dec 2010. The Jan-Jun 2010 harvest presented more overall aroma, fresh and hardness intensity compared to the other harvests. The 3 harvested times had a barely detectable floral aroma (close to 2). Aug-Dec 2010 and Jan-June 2011 harvest were the most acidic and vegetative aftertaste. Jan-Jun 2011 had higher to release liquid intensity compared to the other harvest times (Fig. 2).

In physicochemical parameters, there were not significant differences among the harvest time in the pH (close to 4.2). The Jan-Jun 2010 harvest scored higher concentration of solids per ° Brix and dry weight. While Jan-June 2010 and 2011 harvest had higher lycopene content (Fig. 2).



a) **b)**

Fig. 2. Effect of the harvest time evaluated at 21 days on globe tomatoes: a) Sensory profile, b) Physicochemical parameters.

Pearson´s Correlation

Table 1 shows the main correlations between sensory and physicochemical parameters for *Roma* and *Globe* tomatoes. The vegetative aftertaste had a negative correlation with lycopene content, meaning that the vegetative aftertaste disappears when the pigment precursors are metabolized [11,12]. In the cohesiveness, that measures the degree to which the tomato is deformed, was correlated to the amount of soluble solids per dry weight, when the sample had higher solids concentration it is deformed up to fracture [13.14]. The concentration of titratable acids is correlated to color and release of liquid [15]. Hardness is correlated to ^{o}Brix and to carotenes content.

Table 1. Sensory and Physicochemical Parameters with High Correlation.

	Titratable Acidity (meq/Kg)	°Brix	Dry Weight (g)	Carotenes	Chlorophyll (mg/g)	Lycopene (mg/100g)
Color	0.61					
Aroma a Fresh			0.51			
Acidity						-0.50
Hardness		0.72		0.56		
Release of Liquid	0.65				-0.53	0.57
Cohesiveness		0.52	0.66			
Number of Bites		0.58	0.55			
Residual Geometric					0.55	
Vegetative Aftertaste						-0.62

Conclusion

The effect of storage time on tomatoes is reflected directly in the sensory parameters of color, acid taste, hardness and release of fluids by texture; while for the physicochemical determinations the concentration of carotenoids and lycopene increased. On the 21st day of storage the best sensory and physicochemical parameters were found, which indicate that it is the best time to be consumed as fresh considering any of the three-harvested times and for either *Roma* or *Globe* tomatoes.

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Sensory And Physicochemical Characterization Of Commercial Beverages

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Abstract

In Mexico, at home 7% of the expenses are destined to acquire various drinks; their marketing is not constrained by their availability. An advantage of this type of food is that due to their composition and process, their shelf life is longer, so they can be easily found in the market. The drinks are products made in the same way like nectars, but with less fruit content, more sugar or other sweeteners and water, and some additives are included such as vitamin C, dyes and artificial flavors. In the last 10 years, drinks were sought to hydrate the body and provide consumer satisfaction. Nowadays it have been included other two requirements, drinks have to be healthy and natural, and to be liked by consumers. Therefore the objective of this work was to determine the sensory and physicochemical profiles of commercial healthy beverages. The study materials were four commercial beverages, a sensory analysis was carried out by trained panelists following the methodology Spectrum ®. The sensory parameters evaluated were appearance, aroma, and flavor. The physicochemical tests included pH, titratable acidity, and soluble solids per °Brix and dry weight. The data was collected using a randomized block design. ANOVA was applied, and in those cases where the null hypothesis was rejected, a mean comparison was achieved by the Bonferroni method. On the appearance parameter, color was evaluated, which intensity ranged between 8 and 12.5. For aroma, were evaluated aroma overall, natural and artificial, obtaining intensity between 4 and 6 in overall aroma, predominating the artificial aroma. The specific aromas detected were floral, caramel and fruit such as cherry, raspberry, blueberry, apple, among others. In relation to the basic tastes tested, sweetness and acid had the higher intensities, predominating artificial flavor over the natural flavor, such as grape, apple, cherry, pomegranate, blueberry, among others. In conclusion it was observed the importance of acidity, measured by sensory and physicochemical tests, the four drinks presented high values, reflecting the influence that the acidity has on the sensory properties of the final product therefore it is of great interest the realization of these types of analyzes.

(Key words: Beverages, sensory analysis, physicochemical analysis, color, flavor).

Introduction

Fruits are an excellent source of minerals, vitamins, phytochemicals and enzymes. They are usually consumed after being processed and preserved in different forms and types, such as jams, juices or drinks [1].

Beverages are products made in the same way as nectars, but fruit content is even lower, to which are added sugar and other sweeteners, water and additives such as vitamin C, artificial colors and flavors [2].

In their preparation, there are factors that affect quality, such as color, sugar, or natural acids, which give sweetness and acidity, is necessary to balance them, so that the beverages have higher acceptability [1, 3]. Given the importance of these in the market, it is thereby necessary to use new techniques such as sensory evaluation to measure the quality of a product and to determinate the specific attributes that lead to the choice of the quality, in appearance, aroma and flavor [4].
Furthermore, the need to obtain valid, precise and reproducible information is what has impulsed the birth of what is now known as sensory analysis, in order to obtain a descriptive sensory profile [5]. This sensory profile is used in research, development and manufacture of a product to define sensory properties in the development of new products, define characteristics/specifications for better quality control, in addition to track product's sensory changes over time with respect to their shelf life [3].

Methodology

Four commercial beverages were evaluated (Table 1), obtained from commercial centers of the city of Celaya in the state of Guanajuato. Mexico. They eere stored at 20 °C, for their posterior sensory and physicochemical evaluation.

Table 1. Commercial beverages.

Beverages	Fruit ingredients
1	Pomegranate
2	Carrot, apple, cherry, strawberry, raspberry, blackberry
3	Blueberry
4	Blueberry (different mark from above)

Sensory analysis

Fifteen panelists were selected and trained, following the methodology established by Spectrum™. An intensity scale of 15 points was used (1= just detectable intensity, 7= moderate intensity and 15= extreme intensity) Parameters evaluated included appearance, aroma and flavor.

Physicochemical analysis

The analysis carried out were pH using a potentiometer, titratable acidity using NaOH 0.1 N, soluble solids per °Brix and dry weight; according to the AOAC.

Statistical Analysis

The data was collected using a complete randomized block design. ANOVA was employed for both analysis, and in those cases where the null hypothesis was rejected, a mean comparison was achieved by the Bonferroni method. Both at α=0.05 of significance.

Results and discussion

Sensory analysis

According to the mean comparison by the Bonferroni method, only the parameters most representatives are reported here. To the color intensity (Table 2), beverage 2, 3 and 4 presented the highest intensity, while the beverage 3 presented the lowest.

Table 2. Appearance intensity of each beverage

Beverage	Appearance color
1	11.57
2	12.51
3	8.36
4	12.59
DMS	1.13

With regards to aroma (Figure 1), the beverage 2 and 4 had the highest overall aroma intensity while the beverages 1 and 3 had lower intensities. In aroma prevailed the artificial over the natural notes, being the beverage 2 the one with higher intensity.

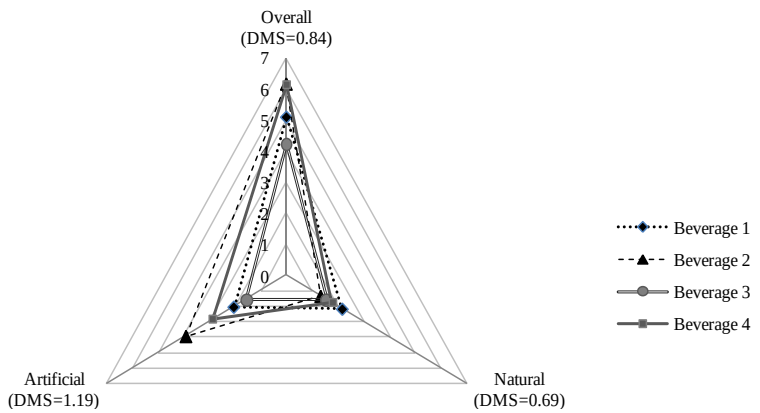

Figure 1. Overall, artificial and natural aroma intensities of each beverage

The aromas predominating are shown in table 3, being more intense the aroma a caramel, floral and fruit such as grape, cherry and blueberry in the four beverages.

Table 3. Descriptive aroma intensity of each beverage

	Beverage 1	Beverage 2	Beverage 3	Beverage 4	DMS
Caramel	3.06	3.94	2.23	4.19	0.8
Apple	2.76	ND	1.7	3.04	1.0
Grape	2.97	2.86	2.36	3.63	1.3
Floral	3.03	3.35	2	3.18	1.1
Raspberry	ND	3.66	ND	6	2.8
Cherry	2	5.13	3	3.12	4.5
Blueberry	4	4	3.62	5.5	4.5

ND=Not detected

The flavor notes that dominate were sweet, acid, artificial and natural (Figure 2). For the sweet, the beverage had intensities between 4 and 6. In acidic taste, the beverage showed low values with intensities between 2 and 3. The artificial flavor was higher in the beverages 2, 3 and 4, while in the beverage 1 natural and artificial flavor were very similar.

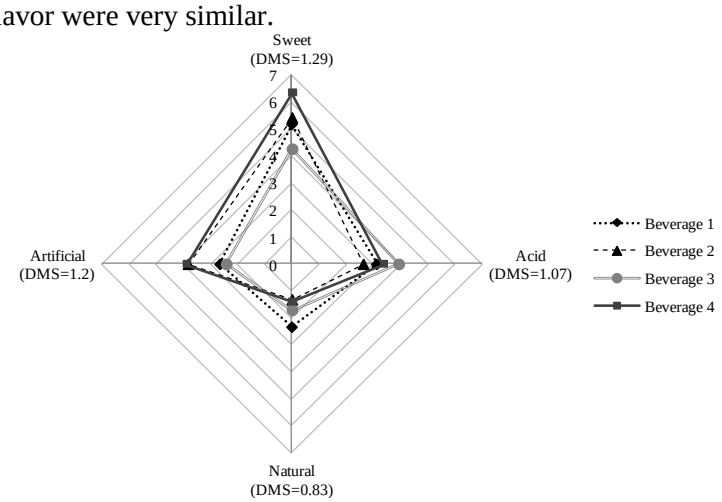

Figure 2. Flavor intensity for each beverage.

Some of the representative flavors are described in table 4, being more intense the caramel and fruit such as cherry, blueberry and pomegranate in the four beverages.

Table 4. Descriptive flavor intensity of each beverage

	Beverage 1	Beverage 2	Beverage 3	Beverage 4	DMS

Caramel	3.38	3.42	2.83	3.95	0.89
Apple	2.46	2.50	2.43	3.05	2.01
Grape	3.60	2.42	2.70	3.04	1.25
Cherry	5.00	4.75	3.16	4.05	4.21
Blueberry	7.50	3.00	4.50	5.50	4.15
Pomegranate	4.00	ND	3.37	5.75	1.92
Strawberry	1.50	2.25	ND	3.50	4.93

ND=Not detected

In the part of aftertaste (Figure 3), sweet and acid were the highest in all the beverages, while artificial also predominate over the natural.

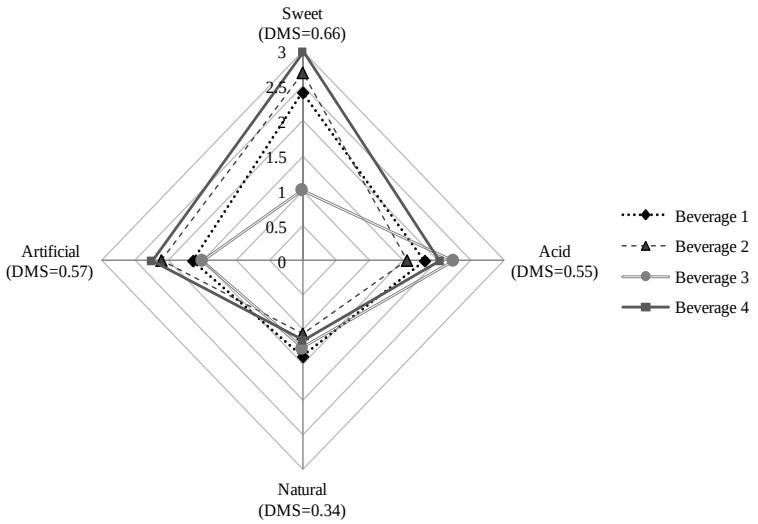

Figure 3. Aftertaste intensity of sweetness, acid, artificial and natural flavor for each beverage

Physicochemical analysis

Furthermore, the physicochemical analysis (Table 5), where the pH and soluble solids were very similar in the four beverages, in the case of °Brix the difference between them was low. In the case of acidity, the beverage 2 was lowest than the rest of them.

Table 5. Values for physicochemical analysis of each beverage

	Beverage 1	**Beverage 2**	**Beverage 3**	**Beverage 4**	**DMS**
pH	3.24	3.52	3.11	3.25	2.83
°Brix	14.1	11.75	12.75	12.6	2.83
Titratable acidity (meq/Kg)	81.5	53.5	84	84	2.83
Soluble solids (g)	8.73	8.24	8.67	8.63	2.83

The Pearson correlation coefficient was also calculated (Table 6). Where it was found that the taste of jamaica is directly related to the axes a* and b* of the CIELAB space, this means that while the values of a * and b * increased the flavor of Jamaica, in a constant proportion. Furthermore, the flavor of jamaica was relates inversely to titratable acidity, soluble solids and color axis L*.

Table 6. Pearson correlation coefficients

	pH	°Brix	Titratable acidity (meq/Kg)	Soluble solids (g)	L*	a*	b*
Appearance color	0.51						
Aroma artificial	0.71	-0.52	-0.69		-0.67	0.70	0.65
Bitter taste	-0.62				0.61	-0.66	
Natural flavor		0.55					

Artificial flavor	0.60	-0.52			-0.65	0.65	
Artificial aftertaste					-0.52		
Aroma caramel						0.50	
Apple aroma	0.88	0.73		0.61	-0.73	0.59	0.68
Citrus aroma	0.81		-0.66		-0.75	0.82	0.72
Aroma floral							0.51
Aroma raspberry	0.50	0.50	-0.50	0.50	0.51		
Aroma jamaica	0.64				-0.67	0.90	
Aroma cherry	0.71	-0.62	-0.65			0.71	0.68
Jamaica flavor			-1.00	-1.00	-1.00	1.00	1.00
Cherry flavor	0.81	-0.58	-0.62		-0.75	0.85	0.70

Conclusions

The Pearson correlations shows the linear relationship between two quantitative random variables, making it a useful tool in cases where a group of trained panelists is not available, because there are physicochemical parameters that are directly related to the sensory parameters, therefore physicochemical analysis may relate directly to sensory parameters. Moreover, the most important attributes of the beverages were described, presenting acceptable characteristics of appearance, flavor and aroma.

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Sensory Comparison Between Tortillas Made From Sorghum [Sorghum bicolor (*L.) Moench*] White And From Corn (*Zea mays* L.)

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Abstract

One of the principal foods in the Mexican diet is corn tortillas, and today due to the continuing drought, many crops have been lost, increasing the price of tortillas. This is why it is sought to find an alternative to made tortillas. Therefore, the objective of this work was to made tortillas using sorghum with good sensory quality similar to corn. The material used in this study were sorghum tortillas made from white sorghum [Sorghum bicolor (L.) Moench] variety RB Paloma and Fortuna, and corn (*Zean mays*) tortillas made form fresh masa, and from nixtamalized corn flour (Maseca®). The tortillas were analyzed at day 0 and 7th after being made, by trained panelists following the Spectrum® methodology; the parameters tested were appearance, aroma, flavor, texture, and aftertaste. The data was collected using a randomized block design; a factorial design was utilized to determine the level of lime and cooking time. ANOVA was employed, and in those cases where the null hypothesis was rejected, a mean comparison was achieved by the Bonferroni method. The results show that there were no significant difference in many sensory parameters from both sorghum and corn tortillas. All tortillas presented a nejayote, nixtamal, corn, and masa aromas with intensities between 2 and 4. Concerning flavor and aftertaste, tortillas had no significant differences among them; predominantly lime, flour and corn flavor, holding a low intensities. In assessing texture by touch, was observed a significant increase on the 7th day of storage in sorghum tortilla compared to corn tortillas on the parameters of curvature, rolling, elasticity and flexibility; while at day 0 there were no significant differences. The hardness at first bite and moisture absorption in sorghum tortillas with both increased significantly at day 7th, while the corn tortilla showed an increase but with lower intensity than those from sorghum. Concluding that it is feasible the sorghum tortillas production using either RB Paloma or Fortuna variety, because they have similar characteristics and behaviors on days 0 and 7th compared to the corn tortillas.
(Key words: White sorghum, Sorghum bicolor (L.) Moench, RB Paloma, Fortuna, Sensory analysis)

Introduction

In Mexico and Central America the tortilla is a basic food [1]. It is consumed with most foods and is used as a substitute for bread, snacks and eating utensils [2].
Sorghum constitutes a typical species of warm climate areas, is a cereal that its composition is very similar to corn, constituting an excellent source of energy (Table 1) [3]. Sorghum belongs to the division of the phanerogams, of the family of the Gramineas [4].

Table 1. Chemical composition of sorghum grain (5).

Component	Sorghum	Corn
Protein (%)	7-14	10
Lipids (%)	2.4- 6.5	4.5
Carbohydrates (%)	70-90	71
Fiber (%)	1.2-3.5	2.0
Calcium ($mg \cdot 100g^{-1}$)	11-58.6	12
Phosphorus ($mg \cdot 100g^{-1}$)	167-751	340
Iron ($mg \cdot 100g^{-1}$)	0.9-20	2.5
Thiamine ($mg \cdot 100g^{-1}$)	0.2-6.4	2
Riboflavin ($mg \cdot 100g^{-1}$)	0.1-0.2	0.13

Sorghum has the morphological and physiological capacity of adaptation being superior to other species, attracting the interest of researchers who conduct studies to find better yields in this crop and production techniques, pest and disease resistance, also economically accessible to all people, especially for the low-income [6].

Sensory analysis

Sensory evaluation of food is today a cornerstone in the design and development of new food products and therefore anticipates the acceptability that it may have [7]. The main applications of the sensory techniques are: quality control, research and product development [8].

There are three types of analysis: discriminative, consumer level and descriptive. The first is based on product differentiation, while the second refers to the degree of preference and acceptability, neither of the two analyses does not require an extensive training [9]. The third is based on the detection and description of the qualitative and quantitative sensory aspects, demand for 6 to 8 training sessions [10].

Methodology

The sorghum variety RB-Paloma and Fortuna were provided by the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP, Campo experimental Bajío) of the 2011 harvest. It was stored at a temperature of 8 C in order to avoid the proliferation of pest; sorghum was subsequently cleaned and selected for processing.

Sorghum underwent an alkaline cooking, at a temperature of 92 C. The nixtamalized grain was allowed to stand for 10 to 12 hours for subsequent grinding, resulting in a mass of malleable consistency, this was used for the elaboration of tortillas. Sorghum tortillas were analyzed with corn tortillas at day 0 and day 7th after being processed.

Table 2. Tortilla samples evaluated.

Samples	Meaning
F0, F7	Tortillas of sorghum variety Fortuna, evaluated at the day 0 and 7.
P0, P7	Tortillas of sorghum variety RB-Paloma, evaluated at the day 0 and 7.
H0, H7	Corn tortillas from nixtamalized flour, evaluated at the day 0 and 7.
M0, M7	Corn tortillas from fresh mass, evaluated at the day 0 and 7.

Sensory analysis

Fifteen panelists were selected and trained using the descriptive sensory methodology. This methodology uses an intensity scale of 15 points (1 = barely detectable, 7 = intermediate, 15 = extreme). The parameters evaluated were: appearance, aroma, flavor, texture and aftertaste.

Statics analysis

The data was collected using a randomized block design and a factorial design was utilized to determine the level of lime and cooking time, which proved to be 0.8% of calcium hydroxide and 50 minutes of cook time; in this study the other nixtamalization conditions are not presented. Sensory data were analyzed by ANOVA, and in those cases that the nule hypothesis was rejected a mean comparison was achieved using the Bonferroni method; both at α=0.05 of significance.

Results and discussion

Sensory analysis

Within the all parameters evaluated in appearance, aroma, flavor, texture and aftertaste, the most representative ones, turned out to be: overall flavor, nixtamal notes, fresh masa notes, lime flavor, corn flavor, corn aftertaste, color, rugosity, elasticity, flexibility, hardness, adhesiveness, mass cohesiveness and particle size.

According to the behavior of the aroma, taste and aftertaste, at day 0 and 7th showed not significant differences intensities with respect to corn tortillas. For the overall aroma, tortillas with nixtamalized flour and fresh mass showed intensities of 6 and 5, respectively; while it was an intensity of 4 for sorghum tortillas variety Fortuna and RB-Paloma. Within the nixtamal and fresh masa notes, the samples showed intensities of 2 and 3, respectively. For lime and corn flavors, corn and sorghum tortillas had an intensity of barely detectable (2). Finally the aftertaste, demonstrated low intensities with a tendency to 2 (Fig. 1 and Fig. 2).

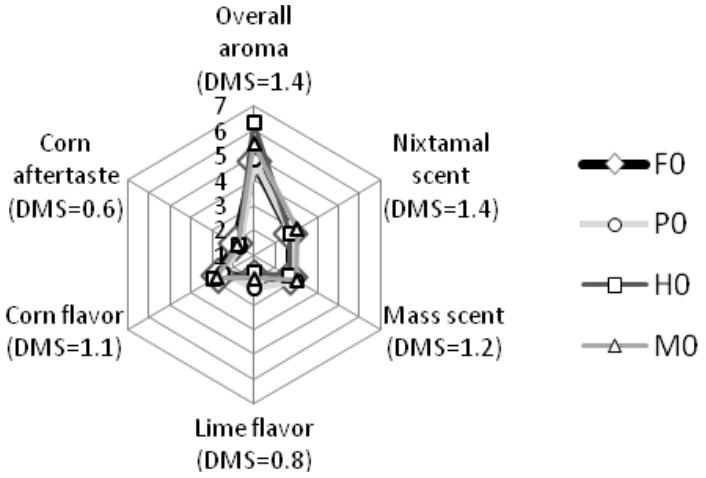

Fig. 1. Intensities of aroma, taste and aftertaste at day 0 for sorghum and corn tortillas.

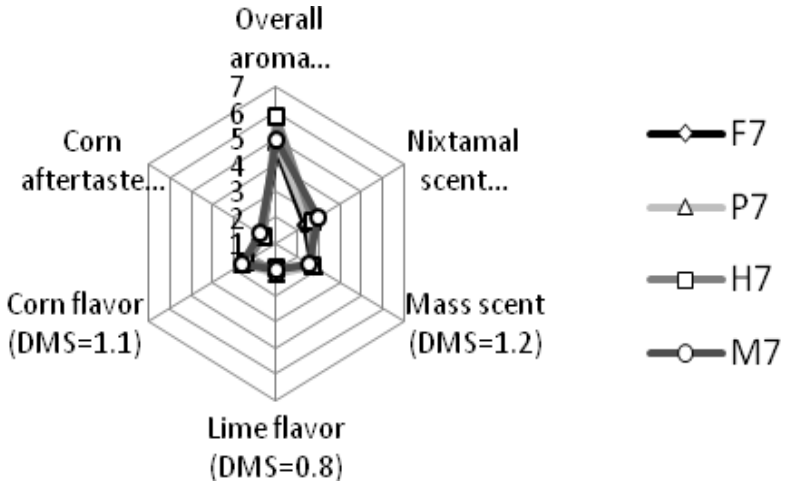

Fig. 2. Intensities of aroma, taste and aftertaste at day 7 th for sorghum and corn tortillas.

In appearance, the color intensity for corn tortillas was between 3 and 5, at the day 0 and 7th. While for the sorghum tortillas presented an intensities of 12 and 13 (Fig 3 and Fig. 4).

In the part of texture several parameters did not presented significant differences throughout the storage period, for the corn and sorghum tortillas, which were: rugosity with values from 3 to 5, hardness from 4 to 6, adhesiveness from 4 to 5, and the particle size from 6 to 7. Referring to elasticity, at day 0 showed values from 4 to 6, however for the day 7 th presented a decrease with values from 2 to 3; same behavior had the mass cohesiveness, at day 0 showed values from 6 to 7 and for the day 7th was from 5 to 6. On the other hand, the moisture absorption increased their values at the seventh day, initially showed values between 6 and 7 passing to values between 8 and 9 (Fig 3 and Fig. 4).

Finally, the intensities obtained for flexibility were significant different between corn and sorghum tortillas, the latter being lower. For the day 0, the Fortuna sorghum tortilla presented an intensity of 6, while the Paloma sorhum was 9, for nixtamalized corn flour tortilla was 12 and for fresh masa corn tortilla was 13. While for the day 7th, samples Fortuna sorghum and Paloma sorghum showed a value of 4, nixtamalized corn flour was 5, and fresh masa corn tortilla was 8 (Fig 3 and Fig. 4).

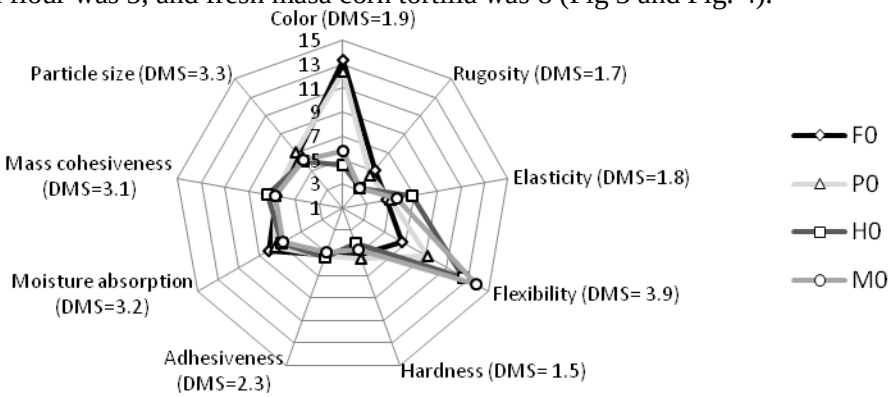

Fig. 3. Intensities of appearance and texture at day 0 for sorghum and corn tortillas.

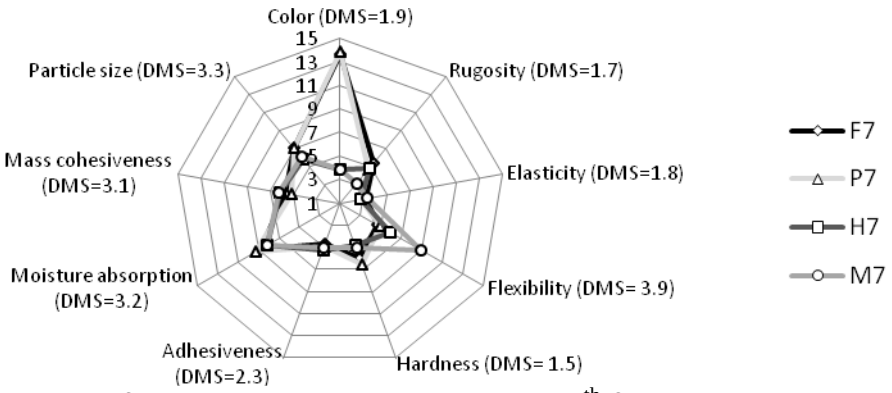

Fig.4. Intensities of appearance and texture at day 7 th for sorghum and corn tortillas.

Conclusions

Sorghum tortillas of both varieties showed similar behavior with the corn tortillas in terms of aroma, taste and aftertaste; however in texture and appearance, the parameters of: color, flexibility and moisture absorption presented higher variability, at day 0 and 7. It can be said that both varieties of sorghum, Fortuna and RB-Paloma, are suitable for making tortillas. They presented the best characteristics at the day 0.

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Effect Of The Addition Of Different Types Of Carrageenans In The Textural Properties And Stability Of Cooked Preferred Type Turkey Ham

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Abstract
The use of carrageenans in meat products, improves sensory attributes, maintaining more stable products during storage, increasing yields and reducing costs. The objective of this study was to evaluate the effect of adding 1% of two types of carrageenan, semi-refined (standardized with dextrose) and carrageenan refined (with potassium ions) in preferred type turkey hams. 2 batches were produced, of 6 hams each one, under the same process conditions, and formulation; with the only difference of added carrageenan type. In these meat products texture profile analysis (TPA) at three points (hardness, stickiness, cohesiveness, elasticity and gumminess) were evaluated; slice-ability and syneresis during 42 days of storage at 4 °C as indicators of stability at days 7, 14, 21, 28, 35 and 42 were also evaluated Syneresis was measured by weight difference at removal of exuding liquid. Slice-ability was determined with a Torrey FS12 slicer, by means of a subjective scale which considers that a product is stable when it allows to obtain 10 slices of uniform thickness (0.1cm) without breaks of each one of them at 21 ° C. The results were evaluated by two-way ANOVA. The batch of hams made with refined carrageenan, showed greater stability, less loss of exudates and the system remained stable enough to obtain homogeneous slices for a longer storage time. There was no effect on textural attributes of each batch tested associated with the use of each type of carrageenan in each formulation determined by ANOVA two-way ($\alpha = 0.05$); however in all cases and all attributes, the storage time significantly affected the results. Data showed that at 28 day of storage, lots of ham had a peak indicating that the product starts the end of their shelf life. They also presented the greatest syneresis losses although very homogeneous slices at 42 days of storage were obtained.
Keywords: turkey preferred Ham, Carrageenan, syneresis, TPA, slice-ability.

Introduction
The addition of carrageenan in the formulation of meat products such as ham, responds to the need of improve their sensory attributes, avoid negative effects on the product during storage, lower costs and reduce inputs (2).

The carrageenans not only give the product a smooth texture, but serve as binders between meat proteins and carrageenan and impart stability to the product by controlling fluid loss, which benefits both the yields and quality of the final product (4). In a study by Ayadi, 2009, presented the results of evaluating the influence of carrageenan using textural attributes as well as the water retention ability in formulated products and turkey cooked meat.

Surmacka, 2002 could determine acceptance parameters of the tested products from a texture profile analysis, defined by the attributes of cohesiveness and hardness, and thus establish a better formulation. Moreover, O'Neill et al. (2003), demonstrated the correlation of the slice-ability as an indicator of the quality of hams prepared from soft and exudative meat Pale (PSE) during refrigerated storage at 4 ° C.

Methods and materials
2 batches of preferred type cooked turkey ham were prepared, under the same process conditions, made with two types of carrageenan. The formulations were named: Refined carrageenan formulation and Semi-refined carrageenan formulation. A spreadsheet which allows to obtain balanced formulation (3) was used

to develop meat formulas. According to the different degrees of quality of food products on the market, products must meet the following specifications (6 and 7). Adding carrageenan meets the specifications set by NOM-158-SCFI-2003, preferred type for cooked ham with maximum level of addition of carrageenan permitted of 1%.

Table 1. Ham Technical Specifications

Trade ranking	% PFF* minimum	% Fat minimum	% Humidity Máximo	% Protein added	% Carrageenan máximum	% Starch máximum
Preferred	14	8	76	2	1.5	5

Source: NOM-158-SCFI-2003. *Total Protein Fat Free, including the added protein.

In this case, the regulation considered to add a maximum value of 1.5% of carrageenan in the formulation. In this paper we used 1% of each type, refined carrageenan (with potassium ions) and semi-refined (standardized with dextrose). Assessments established in this project were: syneresis, slice-ability and Texture Profile Analysis (TPA). In order to sustain and quantify the stability and textural attributes provided by the addition of the two types of carrageenans in turkey ham formulation for 42 days at 4 °C storage, assessments were scheduled at days 7, 14, 21, 28 , 35 and 42. The syneresis was quantified by weight difference between ham and fluid exudate during storage (Restrepo and Carrera, 2010). The syneresis was quantified using the formula

$$Syneresis: \frac{(P_{1,0} - P_2)}{P_{1,0}} X100 \ldots\ldots\ldots (11)$$

The slice-ability was determined by obtaining 10 ham slices of 0.1 cm thickness with a Torrey FS12Slicer. As slices were obtained, it was monitored how many of them kept uniform shear. Slices were scored using a subjective scale which was established from 1 to 4 where: 1 was poor, 2 regular, 3 good, 4 very good. The results were expressed as a percentage (9).

Figure 1. Evaluation of the slices obtained from each batch

A texturometer TXT2 TA-cylinder device with a ¼ inch in diameter and a compression force of 30% for the Texture Profile Analysis (TPA) was used, in slices of 1 cm thick at 3 points of the slice (Figure 2). The textural attributes studied were, adhesiveness, cohesiveness, hardness, elasticity and gumminess.

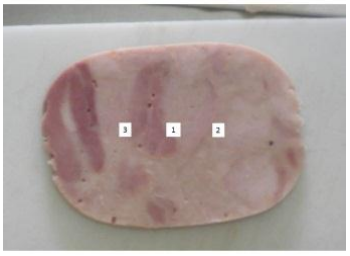

Figure 2. Slice evaluated

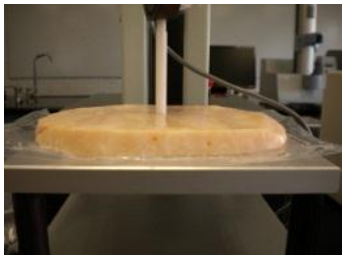

Figure 3. Texture Profile Analysis

Results and discussion

A two-way analysis of variance (ANOVA) with an α significance level of ±0.05 was used to evaluate results, making possible to corroborate the influence of each carrageenan tested in the textural and stability attributes as are loss of fluids and slice-ability during storage at 4 ° C for 42 days on turkey ham preferred type formulation. Results of each of the textural attributes in both formulations (refined or semi-refined carrageenan), showed no significant differences (α = ±0.05) in product stability in terms of fluid loss during storage, although batch of ham added with semi-refined carrageenan remained stable during a shorter storage time because, as can be seen in Figure 4, the major loss of exuded liquid was at day 28, which in addition would enhance the development of microorganisms and is a defect in appearance to the consumer.

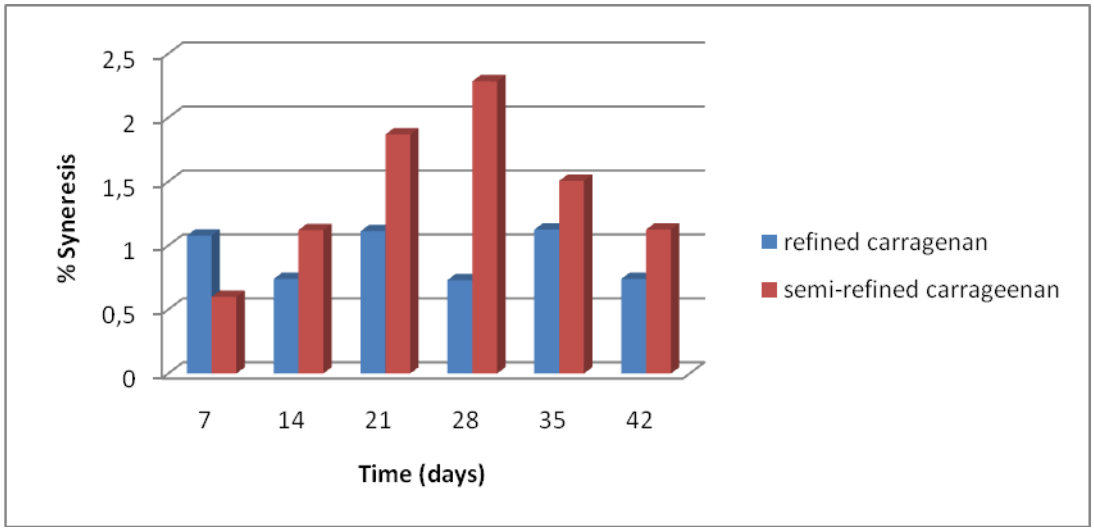

Figure 4. Syneresis over time storage at 4°C

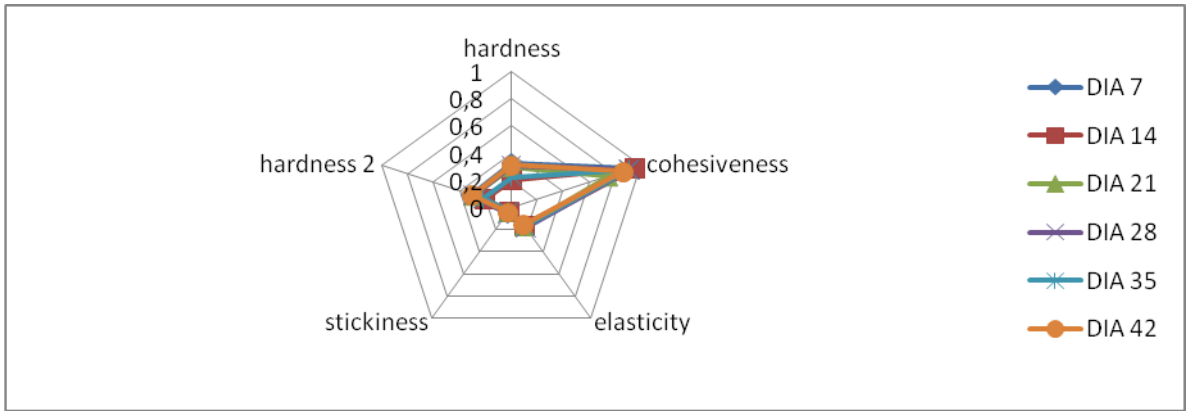

Figure 5. Parameters in the TPA of turkey ham added with refined carrageenan

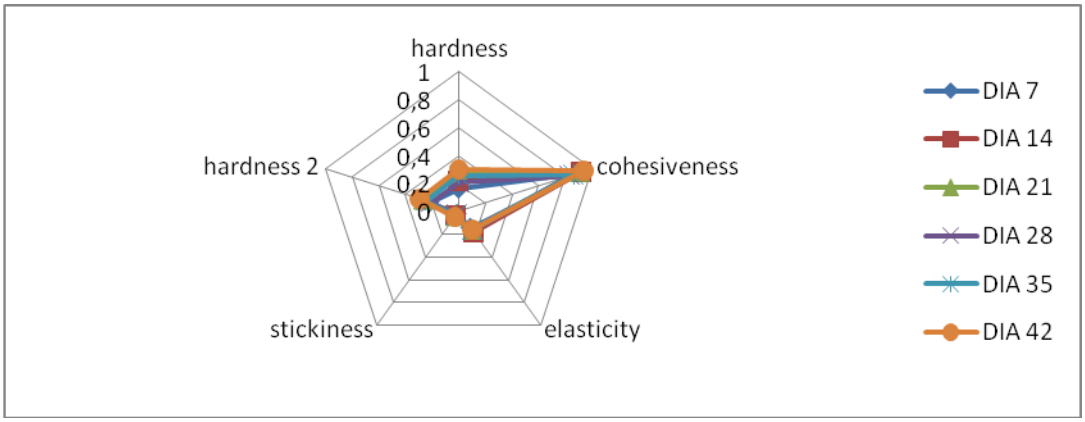

Figure 6. Parameters in the TPA of turkey ham added with semi-refined carrageenan

Figures 5 and 6 show influence of added carrageenan (refined or semi-refined), during storage on product's cohesiveness, which allowed the product to stand firm and increased product's hardness as it lost fluids. The two-way ANOVA ($\alpha = \pm 0.05$) calculated for each textural attribute in batches made with refined carrageenan formulation, did not significant influenced the product during storage.

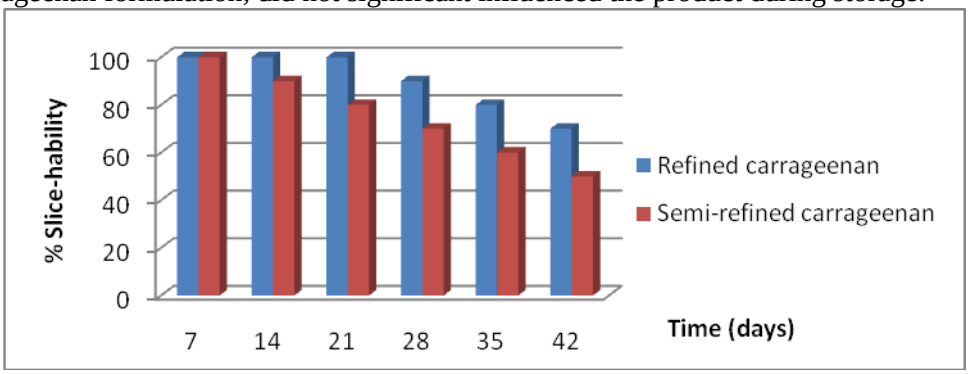

Figure 7. Slice-ability of ham storage at 4°C

Based upon results of two-way ANOVA ($\alpha = \pm 0.05$) of product's slice-ability it can be said that carrageenan type used, had an influence in this parameter during storage. It can be seen in Figure 7 that, after 28 days of storage, the lots of ham not only were adversely affected by fluid losses, but also by changes in sensory attributes and in obtaining inhomogeneous slices. Considering the slice-ability as a quality parameter, this assessment predicts that the product's shelf life is reaching its limit. The addition of semi-refined and refined carrageenan used in preparing turkey hams, for this project, were instrumental in contributing to better textural attributes (cohesiveness, hardness, elasticity, chewiness); however, no significant differences between the use of refined or the semi-refined carrageenan were found in reducing losses achieved by syneresis during refrigerated storage at 4 ° C, although the use of refined carrageenan produced best results on slice-ability.

Concluding Marks

Ham batches formulated with refined carrageenan, had lower percentage of exudate fluid during storage. The type of carrageenan did not significantly influence the textural attributes, even if slice-ability significantly influenced the product. Assessments of lots of cooked preferred type turkey ham, can predict the shelf life of the product.

Aknowledgements

DGAPA-PAPIME PE 202010, DGAPA-PAPIIT IT 202312, PACIVE 2010, 2012.

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Evaluation Of Stabilizers For The Acceptance Of Goat Milk Ice Cream

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Introduction

Milk contains five major nutrients: fat, protein, lactose, vitamins and minerals, from the first three is where you get the flavors, aromas and main characteristics of dairy products (1). In Mexico, goat milk is mainly consumed as fluid milk, cheese and caramel (6), in eastern countries as local produce is consumed mainly acidified (3). Ice cream, sweetened food products are obtained from an emulsion of fat and protein or a mixture of water with the addition of other ingredients which are frozen with or without incorporation of air it. These products are stored, distributed and are sold partially or completely frozen (5).

The ice cream goat milk shake is a product formed by air bubbles bounded by thick film of a uniform mixture of proteins, sugars, salts and other components as well as emulsified fat into small ice crystals (2). The market demand current is ice cream the excellent characteristics in texture and body, which together with the taste, largely determine the acceptability of these. In this industry is marked tendency towards the use of stabilizers which are integrated potentiate synergism between components, obtaining better quality final products (4). Gums in the manufacture of ice cream, increase the viscosity of the aqueous phase, thus improving the stability of the emulsion; avoid flaws in the texture, smoothness control during storage, prevents ice crystals from forming large give body and creaminess to the finished product. Moreover, the gums improve the properties of ice cream (5).

It has reported various types of gums for ice cream, including gums karaya, locust bean, guar and xanthan, carrageenan, sodium alginate and carboxymethylcellulose. Has also been reported that gum arabic, karaya and ghatti, provide good texture, body and creaminess to ice cream made with buffalo milk. We found that guar gum and carob, the carrageenans and carboxymethylcellulose, used at concentrations between 0.2 - 0.4%, give ice cream, excellent sensory properties (4).

Materials and methods

The development of formulations and sensory evaluation thereof was conducted in the Department of Science and Food Technology Oriented Health (CyTaOS) School of Biological Sciences at the UAC. Three formulations were developed in strawberry and chocolate for each formulation, evaluating four different gums added in different percentages. Determining the formulation and taste more pleased by a not trained panel of 30 judges who like to eat ice cream using a five-point hedonic scale.

Table 1. Percentages of added stabilizers in each of the formulations

Formulations	1	2	3
Pectin	0.3%	-	0.3%
Guar Gum	0.1%	0.3%	-
Xanthan	0.1%	0.25%	-
Carrageenan	0.02%	-	0.02%

Results and discussion

Results are interpreted by the allocation of points, whereby was obtained a better acceptance of a formulation containing the mixture of gums Carrageenan, Xanthan, Guar and Pectin flavored chocolate. The graph 1 shows that the formulation of a higher score was the one with the highest score in the assignment I really like. The graph 2 shows that the chocolate flavor was better accepted by the judges.

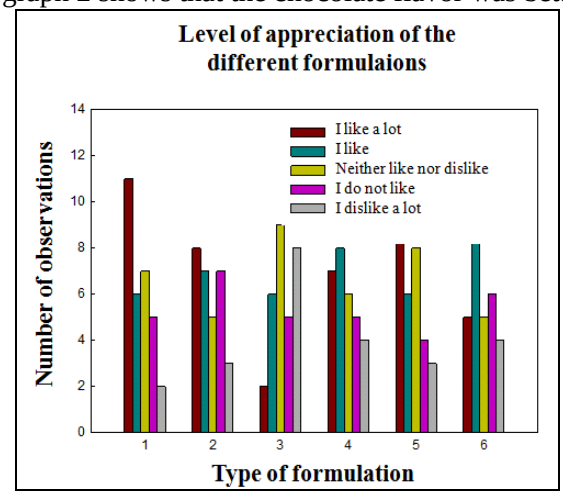

Figure 1. Observations obtained for each formulation.

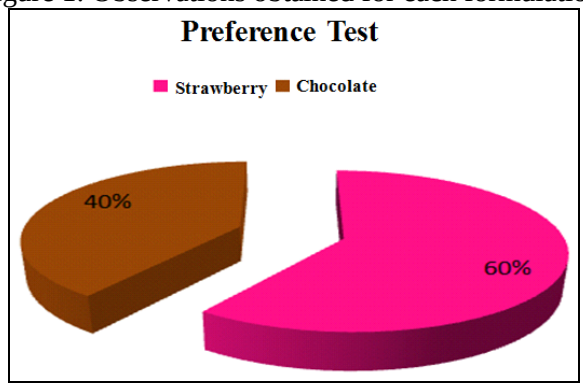

Figure 2. Percentage of acceptance for each flavor

There have been several studies aimed to evaluate different mixtures of gums, one of them is made by Martinez in 2002 who developed a mixture of gums and compare it to a commercial mixture in ice cream making, can determine that both mixtures can be used, replacing each other, for their similarity in the behavior characteristics of both the mixture and the final product. Posada Furthermore evaluated in 2009 a mixture of carob gum, guar against a commercial mixture also containing carrageenan, sodium alginate, dextrose in ice cream, better results with the mixture of guar gum and carob that this mixture favored maturation times of ice cream, addition to being added in a lower percentage the commercial mixture decreasing costs.

Conclusions

The mixture of gums such as carrageenan, guar, xanthan, pectin, have synergy between generating the elastic gels, cohesive and reducing syneresis due to hydrogen bonds that form through the liquid, conferring quality characteristics, texture, flavor, color increase the consumer acceptability of icecream goat milk. The development of gums mixtures decreases production costs compared to commercial mixtures. The production of ice cream from goat's milk, increases diversity the products that goat can be developed and thereby increase their income.

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