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PHYSICAL, MECHANICAL, BARRIER PROPERTIES AND MICROSCOPIC STRUCTURE OF CACTUS MUCILAGE-BASED EDIBLE FILMS MIXED WITH GELATIN AND BEESWAX

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Abstract: The purpose of this study was to develop edible films from cactus mucilage, gelatin and beeswax in two or three-component combination and in variable concentrations. Physical, mechanical and barrier properties as well as microscopic structure were evaluated in the films through transparency, water vapor permeability and tensile and puncture strength assays, as well as scanning electron microscopy. An increase in the concentration of the mucilage and beeswax addition had caused a decrease in transparency of the films. Likewise, the addition of beeswax contributed to the decrease in water vapor permeability. Films with 0.5% (w/v) showed the least water vapor permeability (6.547 g*mm/). Two-component edible films based on 1.5 (w/v) gelatin with 0.5, 1 and 1.5% (w/v) of mucilage showed the highest values of tensile and puncture strength, being of 8.01-9.05 and 0.25-0.36 MPa, respectively. The cactus mucilage has an important potential to develop edible films.

Keywords: Edible films, *Opuntia ficus-indica*, mucilage, gelatin, beeswax.

INTRODUCTION

Lately, an increasing interest has arisen to develop edible films (EF) due to the need of increasing life shelf of perishable foods such as fruit products. Moreover, there has been a strong effort to decrease the use of non-degradable packaging materials as they greatly contribute to environmental pollution (Sharma *et al.,* 2009).

An EF is a structure formed by some thin and continued material coats that can be eaten by human beings without any adverse effects on their health. They are applied on the surface of the food products where they act as barriers against water, oxygen, carbon dioxide, some aromatic and lipid compounds transfer. They create a means of transport for some additives (antimicrobial, antioxidants, flavor, etc.) as well as they improve the entire structure and handling of food products (Falguera *et al.*, 2011).

The EF components can be classified into three main categories: hydrocolloids (protein and polysaccharides), lipids and hydrocolloids and lipids mixtures (Baldwin y Hagenmaier, 2012). Many polysaccharides are capable to form gels and they occur naturally in plants. Most of them, like pectin, carrageen, alginates, and xanthan have been widely researched for their use in edible films and coatings. Polysaccharides from cactus mucilage have been recently used for edible films and coatings (Espino-Díaz *et al.*, 2010; Del-Valle *et al.*, 2005).

The cactus, belonging to the genus *Opuntia*, is well known for its capacity to produce and storage mucilage (Peña-Valdivia *et al.*, 2012), which can be extracted from cactus pads (cladodes), as well as fruit peel, with variable efficiency (Sepúlveda *et al.,* 2007). The mucilage is a complex polysaccharide with high molecular weight ($2.3\ X10^4$-$4.3X10^6$ g/mol). It is formed mainly by varied proportions of the following monomers: L-arabinose, D-galactose, L-rhamnose and D-xylose as well as D-galacturonic acid. The monomer proportion depends on variety, age, and weather conditions (Sáenz *et al.*, 2004). This mucilage is a hydrocolloid which can form molecular bonds capable to retain a great amount of water (Sepúlveda *et al.*, 2007).

In Mexico, there are about 30,200 Ha of cultivated cactus. From them, 17,747 Ha are used for fodder and 12,799 Ha are planted for human consumption (SIAP, 2021). The main variety planted is 'Milpa Alta', whose name comes from the place it is planted: Milpa Alta, Mexico City. Therefore, the use of cactus mucilage to develop edible films will represent an alternative process for cactus with the correspondent extra economical income for the producers. On the other hand, gelatin has been used as thickening and texturizing agent for the food industry. Due to its good gelling properties, it has a great capacity to form EF, which show in general terms, acceptable mechanical properties, although with great water vapor permeability (Pérez-Gago, 2012). Finally, beeswax is capable to decrease water vapor permeability (WVP) in polysaccharide and/or protein-based EF (Navarro-Tarazaga *et al.,* 2011). Although there is previous research on the use of cactus mucilage in the production of EF (Espino-Díaz *et al.*, 2010) or edible coatings (EC) (Adetunui *et al.*, 2012), its capability to form EC in combination with other components as well as the subsequent modification of their functional properties have been scarcely studied. Therefore, the aim of this research was to evaluate the physical, mechanical and barrier properties of cactus mucilage-based EF in combination with gelatin and beeswax.

MATERIALS AND METHODS

Biological material for obtaining cactus mucilage. Cactus cladodes from `Milpa Alta' variety weighing from 210 and 280 g and 20-25 cm long were used. All of them were obtain in Milpa Alta, D.F., Mexico.

Mucilage extraction. The extraction of the mucilage was carried out following the method described by Contreras-Padilla *et al.* (2012) with some modifications.

Edible film preparation. The following mixtures were prepared using mucilage (M) obtained from cactus variety 'Milpa Alta', gelatin (G) and beeswax (B): (M_1G_1), (M_2G_1), (M_3G_1), (M_1G_2), (M_2G_2), (M_3G_2), (M_1G_3), (M_2G_3), (M_3G_3), ($M_1G_1B_1$), ($M_3G_1B_1$) and ($M_1G_1B_2$). Sub-indexes 1, 2 and 3 indicate the concentrations 0.5, 1 and 1.5 % (w/v), respectively. The mucilage was dispersed in distilled water at 25 °C with constant stirring for 24 h. Once it reached 35 °C, G and/or B was added in the correspondent quantities. Glycerol was added as plasticizer and Tween as emulsifier at concentrations of 0.5 and 0.9% (w/v), respectively. Finally, the mixture was heated at 90 °C using an Ultraturrax (Mod.T25 basic; IKA-Werke, Gmbh & Co. KG, Staufen, Alemania) for 1 min at 12,000 rpm plus 4 min at 22,000 rpm (Navarro-Tarazaga *et al.*, 2011). Aliquots of 30 mL were taken from the forming solution and later were poured into flat-bottomed containers of 15 cm diameter. They were placed on a flat and leveled surface at room temperature (25 °C and 50% RH approximately). They were dried for 24 hours until EF were formed. Finally, the films were pre-conditioned in a desiccator at 23±2 °C y 50±5 H.R. for 48 hours (ASTM D618-00., 2003) for its further characterization.

CHARACTERIZATION OF EDIBLE FILMS

Transparency. 0.5 x 0.4 cm rectangular samples were placed in spectrophotometer cells (GENESYS 10 UV-Visible) in perpendicular position to the light path to assess the absorbance at a wavelength of 550 nm. Transparency was calculated according to the following equation: $\mathbf{T=A_{550}/X}$, being $\mathbf{A_{550}}$ the absorbance value and **X** film thickness measured in six random positions for each film using a digital micrometer (Mitutoyo, Modelo Quickmike Series 293-IP-54; Mitutoyo Corp., Kanawava, Japon). The result was expressed as transparency of the six samples mean (Al-Hassan and Norziah, 2012).

Water vapor permeability (WVP). It was calculated using the method described by Espino-Díaz *et al.* (2010) with some modifications. The previously conditioned films were cut into discs and placed in 8.4 cm-diameter testing cells with anhydrous calcium chloride as desiccator agent (0% H.R). Once their original weight was measured, they were introduced into a chamber with 100% de RH at 25±2 °C. The loss of weight was assessed in 90-minutes periods for 24 h and the water vapor transfer rate (WVTR) was calculated through the slope to the lineal regression analysis. The WVP was calculated using equation (1).

$$WVP = WVTR * x/p_3 - p_2 \quad (1)$$

$$p_2 = P - (P - p_1)\exp(R * T * \Delta Z * WVTR/P * D) \quad (2)$$

Where *WVP* is the water vapor permeability (g/m*s*Pa), *WVTR* is the water vapor transmission rate, x is the film thickness (m), p_3 is the water vapor pressure inside the chamber (kPa), p_2 is the corrected water vapor pressure (kPa), P is the total atmosphere pressure (kPa), p_1 is the partial water pressure at the surface of the desiccant agent in the cell (p_1=0), T is the absolute temperature during the assay (K), R is the ideal gas constant (m^3*kPa/g*K); D is the water vapor diffusion coefficient in the air (m^2/day) and ΔZ is the space between the solution and the film (m^2).

Mechanical properties. Puncture strength (PS) and tensile strength (TS) were calculated by the method described by Gontard *et al.* (1993) with light modifications. A mechanical test machine was used (SINTECH 1/S, MTS, USA) with a 100N load cell. For PS, the films were cut in 8 cm-diameter circles and fixed to a metallic holder where they were penetrated uniaxially with a 1.3 cm – diameter- cylindrical puncture at a speed of 6mm/min. For the TS, the films were cut in 1cm x8 cm. They were held by clamps with 5 cm initial separation. Traction speed was of 60 mm/min. The puncture and tensile strength were registered in 15 samples per formulation.

Microscopic structure. A scanning electron microscopy (JEOL 35 CF Tokio, Japon) was used to obtain the microscopic structure. Profile and superficial images of each conditioned film were taken (Jiménez *et al.*, 2010).

Statistics. Data obtained were analyzed by Analysis of Variance (ANOVA), followed by means comparison by the Duncan test, establishing a significance of (α) =0.05. IBM, SPSS Statistics (versión 20.0) software was used.

RESULTS AND DISCUSSION

TRANSPARENCY

Transparency of edible films depends on the concentration of its components and the interaction between them, related mainly to the development of hydrogen bonds (Gorgieva y Kokol, 2011), as well as its morphology and its thickness (Fakhoury *et al.*, 2012). Moreover, it is a characteristic that enables us to judge the component compatibility of the EF from its high or low light dispersion (Ma *et al.*, 2012). EF with less concentration of mucilage and gelatin presented higher transparency values (Figure 1). This can be explained by the fact that gelatin forms the continuous phase of the gel formed by the mixture of the two components, and it is also possible that the retained water in the protein network dissolved, in a certain degree, the mucilaginous phase. At the same time, gelatin chemical structure did not crystalize, maintaining an amorphous state, allowing that more light passed through the EF (Fakhoury *et al.*, 2012). Likewise, higher concentration and more components had a significant effect ($p \leq 0.05$) on the EF transparency. With increasing concentration of mucilage and gelatin, the thickness values of the EF were higher, provoking lower light passing, thus, lower transparency.

On the other hand, a reduction in transparency was shown when beeswax was added ($p \leq 0.05$) increasing thus the opacity values in them, even though an emulsifier was used to favor the lipid miscibility. The decrease in transparency may indicate a hydrophilic and lipophilic phase separation. This generates a reduction in components compatibility. Moreover, the presence of lipid clusters on the surface provokes a higher light dispersion (Monedero *et al.,* 2009).

Transparency values showed for two- and three-component films were similar to those reported by Limpisophon *et al.* (2010) and Pires *et al.* (2013).

WATER VAPOR PERMEABILITY

Water vapor permeability is a very important film characteristic when films are selected to be applied in fruit, because this property indicates their capacity to protect fruit from weight loss and/or dehydration (Navarro-Tarazaga *et a*l., 2011).

Addition of beeswax had a significant effect ($p \leq 0.05$) on WVP of the EF. Edible films added with beeswax showed less permeability (8.173 g*mm/m^{2}*d*kPa) in comparison to those made only with hydrocolloids (12.50 g*mm/m^{2}*d*kPa), being those elaborated with 0.5% mucilage, gelatin, and beeswax the ones which showed the lowest value (6.547 g*mm/m^{2}*d*kPa) (Figure 2). These results confirm that the addition of beeswax reduce the hydrophilic-lipophilic balance of the EF, increasing its water vapor barrier, meaningfully. Therefore, EF added with beeswax could have a better potential to be applied in fruit and vegetables products.

On the other hand, Pérez-Gago and Krochta (2005) reported that with an increase of lipidic content the water vapor barrier is improved, only if the lipid is dispersed homogenously in the hydrocolloid matrix. This causes an increase of the length of migration of the water molecules and immobilizes the hydrocolloid in the interphase. As a result, a better-arranged structure and stronger reticulation are obtained. Nevertheless, in the present study, the films elaborated with higher concentration of beeswax showed higher WVP values ($p \leq 0.05$) in comparison to those elaborated with lower concentration. This indicates that even though higher concentration of lipids was used, the distribution in the matrix was not totally homogeneous. Moreover, Pérez-Gago and Krochta (2005) reported that there is a critical lipid content from which WVP is not improved even if it is increased several times. This is due to the saturation of the lipids in the formulation or due to their bad distribution in the hydrocolloid matrix. Therefore, in the present study, increasing beeswax resulted in a poor distribution of the hydrocolloid matrix, causing coalescence of the lipid particles during the drying of the EF, resulting in lipid cluster presence on their surface.

WVP values (12.50 g*mm/m^{2}*d*kPa) for two-component EF is lower than those reported by Navarro-Tarazaga *et al.* (2011) and Espino-Díaz *et al.* (2010). However, they are very similar to those reported by Fakhreddin-Hosseini *et al.,* (2013) and Muñoz *et al.,* (2012). Finally, WVP values for EF containing beeswax (8.173 g*mm/m^{2}*d*kPa) are closed to the ones reported by Pires *et al.* (2013) and Pires *et al.* (2011).

MECHANICAL PROPERTIES

Tensile strength in the EF elaborated with two components increased with higher gelatin composition (Figure 3a). EF based on 1.5% of gelatin with 0.5, 1 and 1.5% of mucilage showed 85.5% more of TS (8.1-9.5 MPa) ($p \leq 0.05$) than those developed with 0.5% gelatin with 0.5, 1 and 1.5% of mucilage, which needed a lower TS (1.21-1.35 MPa). Therefore, increasing gelatin concentrations had a significant effect ($p \leq 0.05$) on the increasing TS. This is because the protein increases the number of superficial chains, which are responsible of improving the intermolecular interactions within the polymeric matrix, generating, thus, a higher tension resistance (Fakhoury *et al.*, 2012).

Regarding to puncture strength, films based on two components showed an increase mainly because of the percentage of gelatin used and the increase of mucilage concentration (Figure 3b). Films with 1.5%

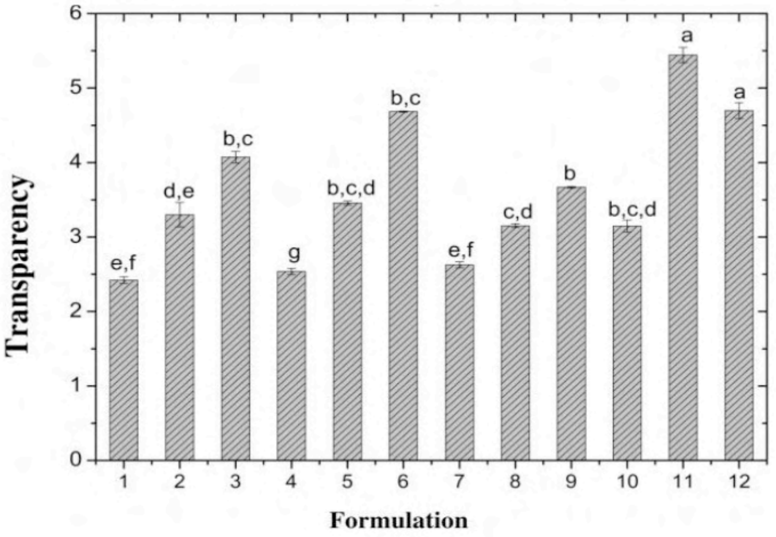

Figure 1. Transparency of edible films based on mucilage, gelatin and beeswax. The numbers of the abscissa represent the formulation used for each edible film; 1 (M_1G_1), 2 (M_2G_1), 3 (M_3G_1), 4 (M_1G_2), 5 (M_2G_2), 6 (M_3G_2), 7 (M_1G_3), 8 (M_2G_3), 9 (M_3G_3), 10 ($M_1G_1B_1$), 11 ($M_3G_1B_1$), 12 ($M_1G_1B_2$). M=mucilage, G=gelatin, B=beeswax and 1=0.5%, 2=1%, 3=1.5 %. Verticals bars represent the mean of films evaluated ± SD. Different letters indicate significant difference (P≤0.05).

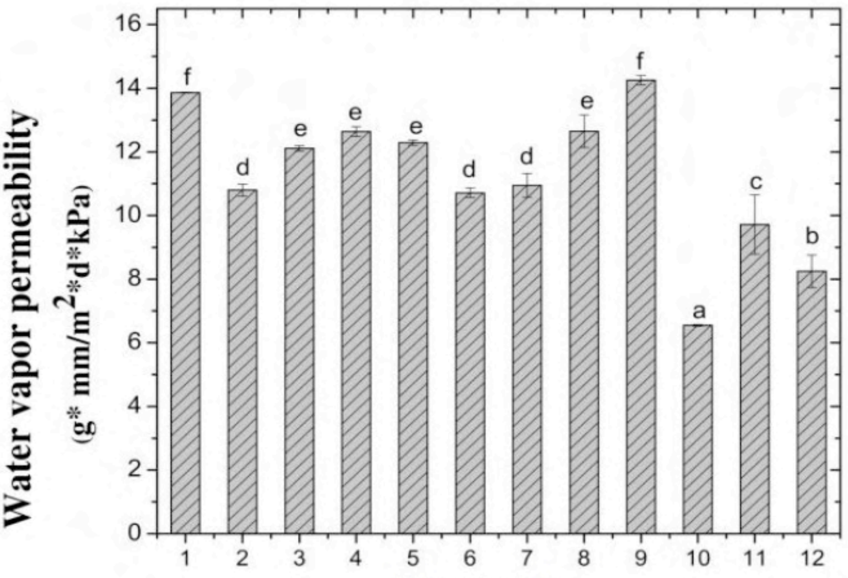

Figure 2. Water vapor permeability of edible films based on mucilage, gelatin and beeswax. The numbers of the abscissa represent the formulation used for each edible film; 1 (M_1G_1), 2 (M_2G_1), 3 (M_3G_1), 4 (M_1G_2), 5 (M_2G_2), 6 (M_3G_2), 7 (M_1G_3), 8 (M_2G_3), 9 (M_3G_3), 10 ($M_1G_1B_1$), 11 ($M_3G_1B_1$), 12 ($M_1G_1B_2$). M=mucilage, G=gelatin, B=beeswax and 1=0.5%, 2=1%, 3=1.5 %. Verticals bars represent the mean of films evaluated ± SD. Different letters indicate significant difference (P≤0.05).

of gelatin and combined with 0.5, 1 and 1.5% of mucilage needed, in average, 84% more PS (0.250-0.357 MPa) ($p \leq 0.05$) than those elaborated with 0.5% of gelatin with 0.5, 1 and 1.5% of mucilage, which needed the lowest PS (0.029-0.064 MPa). Therefore, an increase in gelatin and mucilage concentration had a significant effect ($p \leq 0.05$) on an increase of puncture strength. This is due to the fact that higher concentration of hydrocolloids helps to increase their interaction and to form a more resistant structural network. Moreover, the polysaccharides and proteins can provide additional interactions due to hydrogen bonds between polymeric chains, resulting in a more resistant film (Wang *et al.,* 2011).

The results agree with those mentioned by Coughlan *et al.* (2004) which stated that EF based on mixtures of proteins and polysaccharides showed better mechanical properties than those elaborated only with one of the components.

On one hand, a decrease in the tensile strength was shown in the films with three components due mainly to the addition of beeswax ($p \leq 0.05$). These films shown, on average, 84.6% less TS (0.78 MPa) than those elaborated with only hydrocolloids (5.06 MPa). From the EF elaborated with beeswax, those with 0.5% of mucilage, 1% of beeswax and 0.5% of gelatin showed lower TS (0.38 MPa), than those elaborated with 1.5% of mucilage, 0.5% beeswax and gelatin, which showed a higher TS (1.43 MPa).

On the other hand, adding and increasing concentration of beeswax had a significant effect ($p \leq 0.05$) on the decrease of the PS in three-component EF causing fewer resistant films. Films with beeswax showed 86.2% less in PS (0.020 MPa), than those elaborated only with hydrocolloids (0.143 MPa). Those elaborated with 0.5% of mucilage with 1 and 0.5% beeswax and gelatin required lower PS (0.010 MPa) in comparison to the ones elaborated with 1.5% mucilage with 0.5% beeswax and gelatin, which showed the highest force (0.037 MPa).

It has been broadly reported that TS and PS in films are affected by the type and concentration of plasticizers (Fakhoury *et al.*, 2012), temperature emulsion (Guo *et al.*, 2012) and drying conditions (Fernández-Pan *et al.*, 2010). However, in this study these conditions were kept constant. On one hand, there is evidence that shows that these properties are strongly affected by the type and concentration of the polymeric ingredients in the formulation (Silva-Weiss *et al.,* 2013). The results indicate that the addition and increase of beeswax content provoke less resistant EF. This agrees with the finding of Navarro-Tarazaga *et al.* (2008), who reported that adding lipids in the hydrocolloid-based films produces a heterogeneous structure. Since these molecules are dispersed in the hydrocolloid matrix, the continuity of the phase is interrupted, producing fracture points that reduce the tensile and puncture resistance and a modification of their mechanical properties in general. Furthermore, these discontinuities, low structural cohesion, and a high lipid fracturability, can increase fragility and a lower tensile and puncture strength in the EF.

Besides, Maftoonazad *et al.* (2007) reported that lipids as beeswax, show higher rigidity and deformation resistance due to their high melting point (61-65 °C). The drops that form the disperse phase in the polymeric matrix are solids, causing that this phase becomes very rigid, provoking that it cannot be deformed easily, thus reducing their capacity to tension. This causes that beeswax addition in hydrocolloid-based EF produces poor malleable, very rigid, and less adaptable films for their application on food products. This is mainly due its low structural cohesion, so they are easily broken.

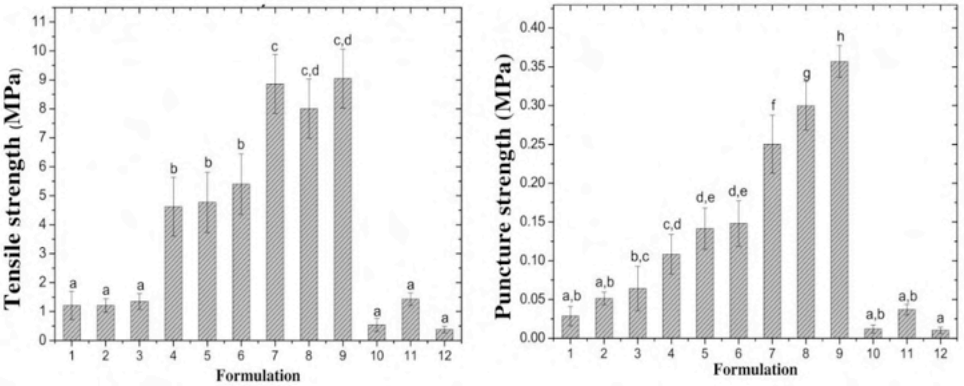

Figure 3. Tensile strength (a) and Puncture strength (b) of edible films based on mucilage, gelatin and beeswax. The numbers of the abscissa represent the formulation used for each edible film; 1 (M_1G_1), 2 (M_2G_1), 3 (M_3G_1), 4 (M_1G_2), 5 (M_2G_2), 6 (M_3G_2), 7 (M_1G_3), 8 (M_2G_3), 9 (M_3G_3), 10 ($M_1G_1B_1$), 11 ($M_3G_1B_1$), 12 ($M_1G_1B_2$). M=mucilage, G=gelatin, B=beeswax and 1=0.5%, 2=1%, 3=1.5 %. Verticals bars represent the mean of films evaluated ± SD. Different letters indicate significant difference (P≤0.05).

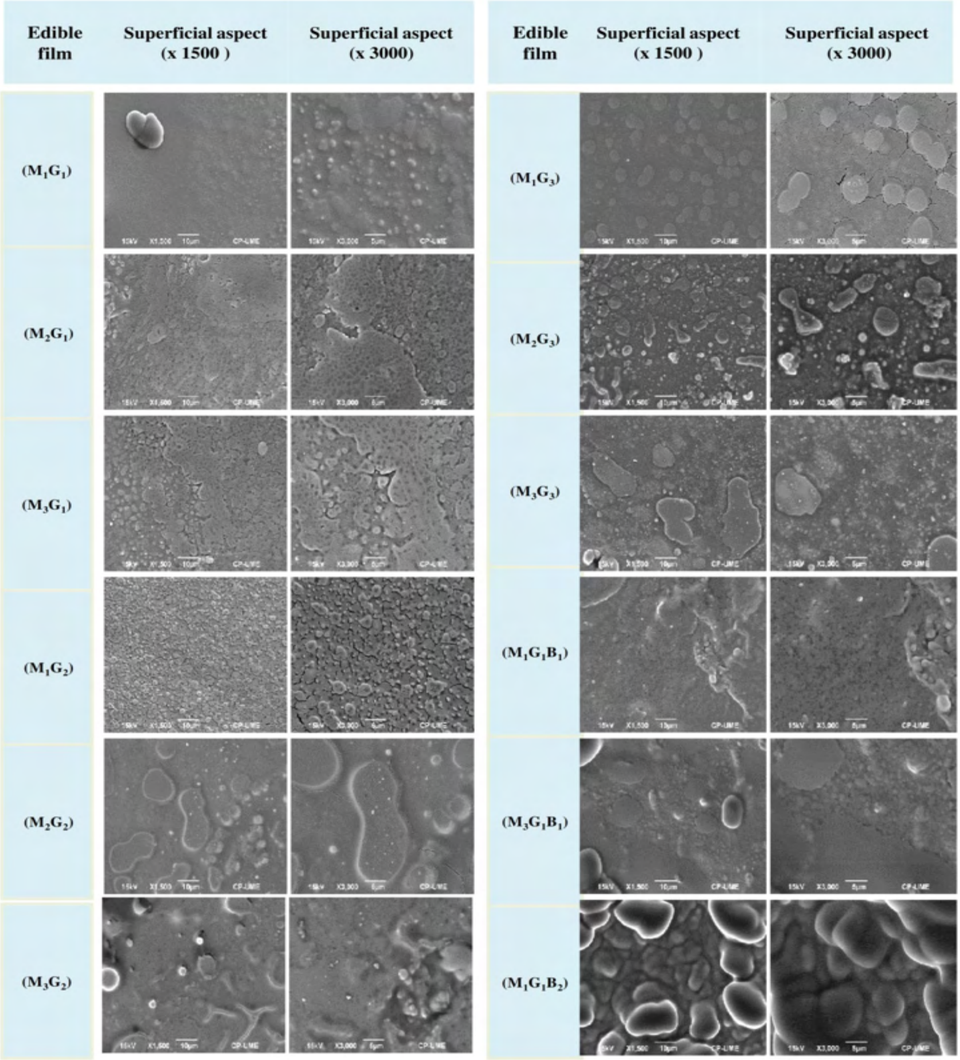

Figure 4. Images from scanning electron microscopy of different films based on mucilage, gelatin and beeswax. The numbers represent the formulation used for each edible film; (M_1G_1), (M_2G_1), (M_3G_1), (M_1G_2), (M_2G_2), (M_3G_2), (M_1G_3), (M_2G_3), (M_3G_3), ($M_1G_1B_1$), ($M_3G_1B_1$), ($M_1G_1B_2$). M=mucilage, G=gelatin, B=beeswax and 1=0.5%, 2=1%, 3=1.5 %.

Likewise, it has been observed that in emulsified films there is less water content and lower values in their mechanical properties than those based only in hydrocolloids. This can be the result of the lipid addition, apart from creating discontinuities in the continuous phase, lipids also increase hydrophobicity in the edible films, reducing their water adsorption capacity and their plasticizer role in the formulation (Navarro-Tarazaga *et al.,* 2008). The high resistance of the beeswax to water is due to its high hydrophobicity (high content of fatty alcohols and long chain alkanes) and its high molecular organization. Water flow is hindered, as waxes show a strong compact orthorhombic crystalline arrange which goes perpendicular to the water flow (Fabra *et al*., 2008).

On the other hand, Espino-Díaz *et al.* (2010) mentioned that EF mechanical properties depend on other factors such as the molecular weight of their components. Lazaridou *et al.* (2003) reported that EF mechanical properties were affected by molecular weight, showing that the tensile strength is increased as the molecular weight is increased, as well. Therefore, the differences that beeswax, which is a low molecular weight compound, shows regarding the rest of polysaccharides and proteins, which are macromolecules with high molecular weight, could represent another factor which affects the mechanical properties in EF. The results obtained in this study regarding tensile strength agree with those reported by Fakhreddin-Hosseini *et al.* (2013) and Navarro-Tarazaga *et al.* (2011). Likewise, the values obtained for puncture strength (0.250-0.357 MPa) are similar to those reported by Soazo *et al.* (2011) for whey protein with beeswax.

MICROSCOPIC STRUCTURE

The scanning electron microscopy (SEM) of the films showed differences in the surface and structure morphology of their internal structure. The microphotographs obtained showed the effect of more than one component and beeswax addition on the EF structure (Figure 4).

Mucilage-gelatin based EF showed a softer and more homogenous structure. However, mucilage-gelatin-beeswax based films had a tougher appearance in the surface, probably due to coalescence and creaming phenomena during drying. This produced the migration of lipidic drop to the surface increasing its rugosity (Zúñiga *et al.,* 2012). In the same way, Fabra *et al.* (2009) reported that sodium caseinate-based EF rugosity is increased with higher contents of beeswax due to larger lipid drop, which increases destabilization phenomena during drying, and the accumulation of lipid clusters in the surface. Even though three-component EF were the roughest, their structure was the most closed and with less cavities and cracks, being the ones elaborated with less concentration of beeswax the ones that showed the less water vapor permeability. This confirms the tight relationship kept between WVP and EF microstructure.

Also, in two-component edible films there were some granular particles on the surface, due to the mucilage clusters that were not well-dissolved during EF preparation method and that were deposited on the surface during drying (Muñoz et al., 2012). Microscopic structure of mucilage and gelatin-based films were relatively comparable with that reported by Muñoz *et al.* (2012), Al-Hassan and Norziah (2012) and Limpisophon *et al.* (2010). Besides, the structure of three-component edible films was relatively comparable to those obtained by Zúñiga *et al.* (2012).

CONCLUSIONS

Mucilage-gelatin-beeswax edible films properties showed a significant difference on the transparency, water vapor permeability, tensile and puncture strength, and microscopic structure. Gelatin did not have an effect on transparency and film thickness in two-component edible films. However, it contributed to the development of softer and more homogeneous structure. It also improved the mechanical properties by increasing the tensile and puncture strength. In three-component edible films, beeswax addition contributed to the development of a rougher appearance, although with a more closed and compact structure. This study demonstrated that the cactus mucilage combined with other components has important potential to develop edible films, besides that this type of films has a great potential to be used in the post-harvest handling of fruits and vegetables.

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