

Biopolymeric films based on cactus (*Opuntia ficus-indica*) mucilage incorporated with gelatin and beeswax

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ABSTRACT

Mucilage from stems of cactus (*Opuntia ficus-indica*) 'Milpa Alta' (Mm) and 'Toluca' (Mt), in a single ingredient condition and in mixture with gelatin and beeswax, was used to prepare biopolymeric films, which were assessed in terms of microscopic morphology, thickness, transparency, tensile strength, and permeability to water vapor, O₂, and CO₂. Films based on Mm were thinner, with higher transparency, and higher tensile strength than those of Mt, but they had similar barrier properties to water vapor, O₂, and CO₂. The addition of gelatin did not affect thickness and transparency of such films, but caused modification of the microscopic morphology, the mechanical strength, and the barrier properties to gases and water vapor. Furthermore, the addition of beeswax promoted the formation of aggregates in the microscopic morphology of films and these acquired smaller thickness, higher tensile strength, and higher barrier properties to O₂, CO₂, and water vapor, which may give them better potential to be used in the postharvest conservation area of horticultural products, although they were less transparent.

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

INTRODUCTION

The cactus plant (*Opuntia ficus-indica*) can be processed to obtain products with application in foods and beverages, livestock feed, pharmaceuticals, cosmetic, and natural additives (Saenz, 2013). Through many studies, the importance of the industrial processing of such species to

promote the economic development of rural communities in arid regions has been well established (Bhatt and Nagar, 2013). The genus *Opuntia*, similarly to other cacti, is known by the capacity to produce and store mucilage (Peña-Valdivia *et al.*, 2012), a complex carbohydrate formed by arabinose, galactose, rhamnose, xylose, and uronic acids (Matsuhiro *et al.*, 2006), which can imbibe large quantities of water (Saenz *et al.*, 2004), and this is an important factor in the adaptation of the plant to warm and dry environments (Cardenas *et al.*, 1997). The mucilage has found application in effluent treatments (Miller *et al.*, 2008), in water purification for human consumption (Pichler *et al.*, 2012), and in pharmaceutical to favor suspending, thickening, binding, emulsifying, and stabilizing of suspensions (Malviya *et al.*, 2011). Further, the characterization of mucilage of cactus stems has been carried out to evaluate its use as a nourishing additive, either as a viscosity-increasing agent or together with other gums to obtain a synergistic effect with such polymers (Yahia, 2012). According to Espino-Diaz *et al.* (2010), mucilage from cactus could represent a good option for the development of edible films in countries, such as Mexico, where this species is highly produced at low cost, constituting a processing alternative, and a source of economic resources for low income communities. In fact, the mucilage obtained from *Opuntia* spp. has already been included as ingredient in edible films and these have been applied on fruits as strawberry (Del-Valle *et al.*, 2005) and mango (Adetunui *et al.*, 2012), with favorable results in prolonging postharvest shelf-life.

Edible films are thin layers of biopolymeric materials that are applied on horticultural commodities. Among their functions is the protection of the product from mechanical damage, physical, chemical, and microbiological activities (Falguera *et al.*, 2011). Also, edible films are applied on many materials to control moisture transfer, gas exchange or oxidation processes (Rojas-Graü *et al.*, 2009b). These biopolymeric films are formulated with carbohydrates, proteins, and lipids. According to Baldwin and Hagenmaier (2012), moderate barriers to O₂ and CO₂ are developed with films made with the first two, and this may allow their use to modify internal atmosphere of products, delaying their senescence (Falguera *et al.*, 2011), although with poor barriers to water vapor, which may cause dehydration and loss of quality. Baldwin and Hagenmaier (2012) pointed out that, in contrast, films based on lipids are more permeable to gases, but their water vapor permeability is low. Therefore, it is common to observe the use of mixtures of hydrophilic and lipophilic ingredients in the polymeric films formulation to favor the modification of the atmosphere at tissue level, preventing at the same time from dehydration (Bourtoom and Chinnan, 2009; Falguera *et al.*, 2011). In addition, plasticizers compounds as glycerol are frequently added in formulations, due to they can modify the appearance and the mechanical and permeation properties of films through reducing intermolecular forces between polymeric chains (Rodriguez *et al.*, 2006).

Although edible films based on cactus mucilage have shown potential to be used in the postharvest horticultural commodities preservation area, it is necessary to assess if their functional properties can be improved. In particular, the permeabilities to gases like O₂ and CO₂ are unknown and, if a conservation strategy based on mucilage films is required to preserve horticultural products, it is necessary to know the effect on the internal atmosphere (Falguera *et al.*, 2011). Espino-Díaz *et al.* (2010) conducted the unique published work about the assessment

of cactus mucilage films, and observed, for instance, that these had low tensile strength and intermediate water vapor permeability. Thus, it is necessary to evaluate whether the water barrier property and the mechanical resistance can be improved in order to increase protection of products against dehydration and damage derived from postharvest handling. In this regard, composite films have demonstrated to have better functional properties (Falguera *et al.*, 2011) and, in that sense, the incorporation of compounds like gelatin, that exhibits good gelling properties, could contribute to increase the tensile strength (Farris *et al.*, 2011). Besides, when films formulations have included a lipid component like beeswax, their water vapor permeability has been reduced (Fabra *et al.*, 2008; Velickova *et al.*, 2013). Thus, the objective of this study was to evaluate the microscopic morphology, the barrier properties to gases and water vapor, the tensile strength, and the optical properties of biopolymeric films made with cactus mucilage as single ingredient and incorporated with gelatin and beeswax.

MATERIALS AND METHODS

Materials

Young cladodes of cactus (*Opuntia ficus-indica*) 'Milpa Alta' and 'Toluca', with length between 20 and 25 cm, grown in Texcoco, Mexico, Mexico (19° 23' 40" N, 98° 39' 28" W, 2250 masl) were used. Powder of food grade pigskin gelatin and beeswax were provided by Ferandelh S.A. de C.V. (Mexico). Besides, glycerol and Tween 80 were provided by Hycel de Mexico S.A. de C.V., Mexico, and ethanol and NaOH by Sigma Aldrich Inc., USA.

Mucilage extraction

The method of Peña-Valdivia *et al.* (2012) was applied, with washed and boneless cladodes, cut into sections of 2 cm × 2 cm, which were mixed with water at 90 °C for 15 min in proportion 1:2 (w/v). The liquid was decanted, centrifuged at 4500 × g for 10 min, and the supernatant was mixed with ethanol in 1:1 ratio (v/v) and allowed to stand overnight at 4 °C. The mucilage was separated by decantation and dialyzed in membrane of 5-6 kDa (Spectra/Por@Dialysis Membrane, Spectrum Laboratories, Inc.) with deionized water for 72 h. The membrane content was frozen at -35 (± 3) °C, and lyophilized (Labconco, Freeze Dry System I Lyph Lock@4.5, Labconco Co., Kansas City, Missouri, USA). Thereafter, 0.5 g of mucilage were solubilized in 99.5 mL of deionized water and submitted to evaluation of pH with a portable potentiometer (Hanna Instruments, Romania), acidity by titration with NaOH 0.01 N (AOAC, 1999), and viscosity with a LVT Brookfield viscometer (Brookfield Engineering Laboratories, Inc., USA). The mucilage from cactus 'Milpa Alta' had pH of 7.23 (± 0.13), acidity of 0.033% (± 0.004%), and viscosity of 18.92×10⁻³ (± 1.07×10⁻³) Pa s, while values for mucilage obtained from cactus 'Toluca' were 7.44 (± 0.12), 0.042% (± 0.007%), and 17.67×10⁻³ (± 0.82×10⁻³) Pa s, respectively.

Biopolymeric films development

The following mixtures were prepared using mucilage extracted from cactus 'Milpa Alta' (Mm), mucilage from cactus 'Toluca' (Mt), gelatin (Ge), and beeswax (W): (Mm₅₀-Ge₂₅), (Mt₅₀-Ge₂₅), (Mm₅₀-Ge₂₅-W₂₅), (Mt₅₀-Ge₂₅-W₂₅), (Mm₅₀-Ge₅₀), (Mt₅₀-Ge₅₀), (Mm₅₀-Ge₅₀-W₅₀), and (Mt₅₀-Ge₅₀-W₅₀). The subscripts 25 and 50 indicate concentrations of 0.25 and 0.50%, respectively. Also, films of Mt, Mm, and Ge, in a single ingredient condition, were developed. During preparation, 0.5 g of rehydrated mucilage was mixed with 99.5 mL of distilled water, and heating was applied at 30 °C for 10 min with constant stirring at 220 rpm. Glycerol was added as plasticizer and Tween 80 as emulsifier, at concentrations of 0.6 and 0.4%, respectively. Finally, Ge and W were added at the required amounts, with constant stirring at 220 rpm and heating at 60 °C, for an additional interval of 20 min. The process of film formation was carried out as described by Espino-Díaz *et al.* (2010) with modifications. Briefly, aliquots of 30 mL were taken from each emulsion and were poured into flat-bottomed containers of 15 cm diameter, which were placed on a leveled horizontal surface at 24 °C and 50% relative humidity, for a period of one to three days to allow films formation.

Microscopic morphology

Samples of films were crashed and fixed with 2.5% glutaraldehyde for 24 h. Three washes with a phosphates buffer were applied, and materials were after sequentially suspended in ethanol solutions (50, 60, 70, 80, 90, and 100% v/v) and subsequently dried. A fine layer of gold was applied using a sputter coater (JFC-1100 Fine Coat; JEOL LTD, Japan). Finally, a scanning electron microscope (JSM-6390; JEOL Ltd., Japan) was used to obtain images of the frontal surface of films. A categorical scale was defined to qualify films with the following levels: (1) smooth, (2) slightly roughness, (3) intermediate roughness, (4) intense roughness, (5) slightly lumpiness, (6) intermediate lumpiness, and (7) intense lumpiness. Three persons participated in the observation and qualification of films.

Transparency and thickness

Rectangular pieces of films of 0.5 cm × 4.0 cm were prepared and placed in cells of a spectrophotometer (Genesys 5, Milton Roy, USA), in perpendicular position to the light path to evaluate transmittance (T_{550}) at 550 nm. An empty cell was used as control. Transparency (Tr) was determined as $[Tr = \log(T_{550}/x)]$ (Chana-Thaworn *et al.*, 2011), where (x) was the film thickness (mm), measured at six points with a digital micrometer (Starret, model 216, USA), with resolution of 1 μ m.

Tensile strength

Rectangular pieces of films of 10 cm × 2 cm were prepared and subjected to stretching in a texture analyzer (TA-TX2i, Stable Micro Systems, UK), using a routine where samples were

deformed up to rupture at a velocity of 1 mm/s. The resulting force was divided by transversal area of the film and results of tensile strength were expressed in MPa (Espino-Díaz *et al.*, 2010).

Permeability to water vapor (WVP)

The method of McHugh *et al.* (1993) was applied, with containers of 9.6 cm diameter and 1.4 cm deep, with a lateral port of 1 cm in diameter, which was sealed with a rubber stopper, through which 35 mL of a saturated aqueous solution of K_2SO_4 were injected to generate a relative humidity (RH) of 97% inside the container. The vessel was covered with the film to be evaluated and the assembly was placed in the headspace of a sealed chamber containing a saturated solution of NaCl at 20 (± 2) °C to develop an internal environment with 75% RH. The water vapor flow (r_v ; kg/s) through the film was evaluated registering the weight loss of the K_2SO_4 solution during a 24-h interval. Partial vapor pressures (Pa) were evaluated inside (p_{vi}) and outside (p_{vo}) of the cell with the expression ($p_v = RH p_{vs}$), where p_{vs} is the saturation vapor pressure (Pa) evaluated from a steam table. Partial vapor pressures were corrected as proposed by Gennadios *et al.* (1994), considering the effect of the gas diffusion through the static air layer on both sides of the film. The water vapor permeability (WVP; mol m / s m² Pa) was calculated as $[WVP = (r_v x) / (A (p_{vi} - p_{vo}))]$, where A is the film water vapor transmission area (m²).

Permeability to gases

Containers of 9.6 cm diameter and 1.4 cm deep, with two lateral monitoring ports of 1 cm were used. The film to be evaluated was placed covering the open mouth of one container, and a flow of N_2 was applied during 30 min. Upon completion, 1 mL of high purity (99.8%) CO_2 was injected, and a monitoring process of the CO_2 and O_2 concentrations in container was carried out during 2 h, with 100- μ L aliquots taken from cell at 15-min intervals. Samples were injected into a chromatograph (Varian, model 3400CX, USA), equipped with a Chrompack Poraplot-Q capillary column, a thermal conductivity detector, and a flame ionization detector. The operating conditions in this equipment were 80, 150, and 170 °C in the column, injector, and detectors, respectively, and the column operated at 158.5 kPa (gauge pressure). Concentration data (weight fraction) were treated with non-linear regressions to fit models of the form $[y_{Ox}^t = y_{Ox}^0 + k_2 (1 - \exp(-k_1 t))]$ and $[y_{CDOx}^t = y_{CDOx}^0 + k_2 (1 - \exp(-k_1 t))]$, where (y_{Ox}^0 , y_{CDOx}^0) and (y_{Ox}^t , y_{CDOx}^t) are O_2 or CO_2 concentrations at zero time ($t=0$) or elapsed time t (s), and k_1 (s⁻¹) and k_2 (weight fraction) are regression constants. Permeabilities (mol m / s m² Pa) to oxygen (O_2P) and carbon dioxide (CO_2P) were obtained with the calculation $[O_2P / CO_2P = (k_1 x V_c) / (R (T+273.15) A)]$, where x , V_c , R , T , and A , are film thickness, volume of the test cell (m³), ideal gas constant (8.134 J/mol K), temperature (°C), and gas transfer area of the film (m²), respectively (Valle-Guadarrama *et al.*, 2008).

Data analysis

The work was based on a completely randomized design with a 2 × 4 factorial arrangement. One variation factor was the mucilage source (S), with two levels (Milpa Alta and Toluca cactus

varieties), and the other was determined by the ingredient type in films (I), with levels given by mucilage (M) as single ingredient, gelatin (Ge) as single ingredient, mixtures of mucilage and gelatin (M-Ge), and mixtures of mucilage, gelatin, and beeswax (M-Ge-W). Analysis of variance and means comparison routines (Tukey, 0.05) were applied to analyze data (SAS Institute, Inc., 1989). Besides, in order to complement the analysis of the factorial arrangement, the formulations (Mm), (Mt), (Mm₅₀-Ge₂₅), (Mt₅₀-Ge₂₅), (Mm₅₀-Ge₂₅-W₂₅), (Mt₅₀-Ge₂₅-W₂₅), (Mm₅₀-Ge₅₀), (Mt₅₀-Ge₅₀), (Mm₅₀-Ge₅₀-W₅₀), and (Mt₅₀-Ge₅₀-W₅₀) were considered treatments and data were submitted to an additional analysis of variance and means comparison routines (Tukey, 0.05).

RESULTS AND DISCUSSION

Microscopic morphology

Films formulated with cactus mucilage as single ingredient were very lumpy, regardless of the variety used, with granules that seemed to be independent one from another, thus the structure had many discontinuities. In contrast, films made with gelatin, also as single ingredient, had a very smooth and homogeneous appearance (Figure 1), suggesting a distinct chemical structure. Polysaccharides present in cactus stems include pectin with gelling properties in presence of Ca²⁺, and mucilage without gelling properties (Goycoolea and Cárdenas, 2003). The present work used the mucilaginous fraction as polymerizing material, which, according to McGarvie and Parolis (1981), has a highly branched structure, and these features allow explaining the irregular appearance showed in Figure 1. On other hand, the gelatin has the capacity to form gels, and intra- and inter-molecular cross-links among protein chains are formed in a continuous network (Dangaran *et al.*, 2009), producing the mentioned homogeneous appearance, and coinciding with the report of Andreuccetti *et al.* (2009), who observed in films of gelatin that the internal structure was compact and with scarce discontinuities.

Gelatin was incorporated to mucilage films and an interaction between both biopolymers occurred producing microscopic morphology with values between 3 and 5 when materials dried (Figure 1), which corresponded to intermediate and intense roughness, respectively, and these resulted from the high lumpiness given by the mucilage and the smoothness provided by the gelatin. All films in the present work were plasticized with glycerol and emulsified with Tween 80. In this regard, Rodríguez *et al.* (2006), working with starch films, found that the glycerol molecules can interfere with the polymerizing material, decreasing the intermolecular attraction and increasing polymer mobility, thus films become more flexible. However, authors found that with the additional incorporation of Tween the superficial tension is reduced and a synergistic effect with the glycerol is developed, decreasing in a more extent the intermolecular attraction.

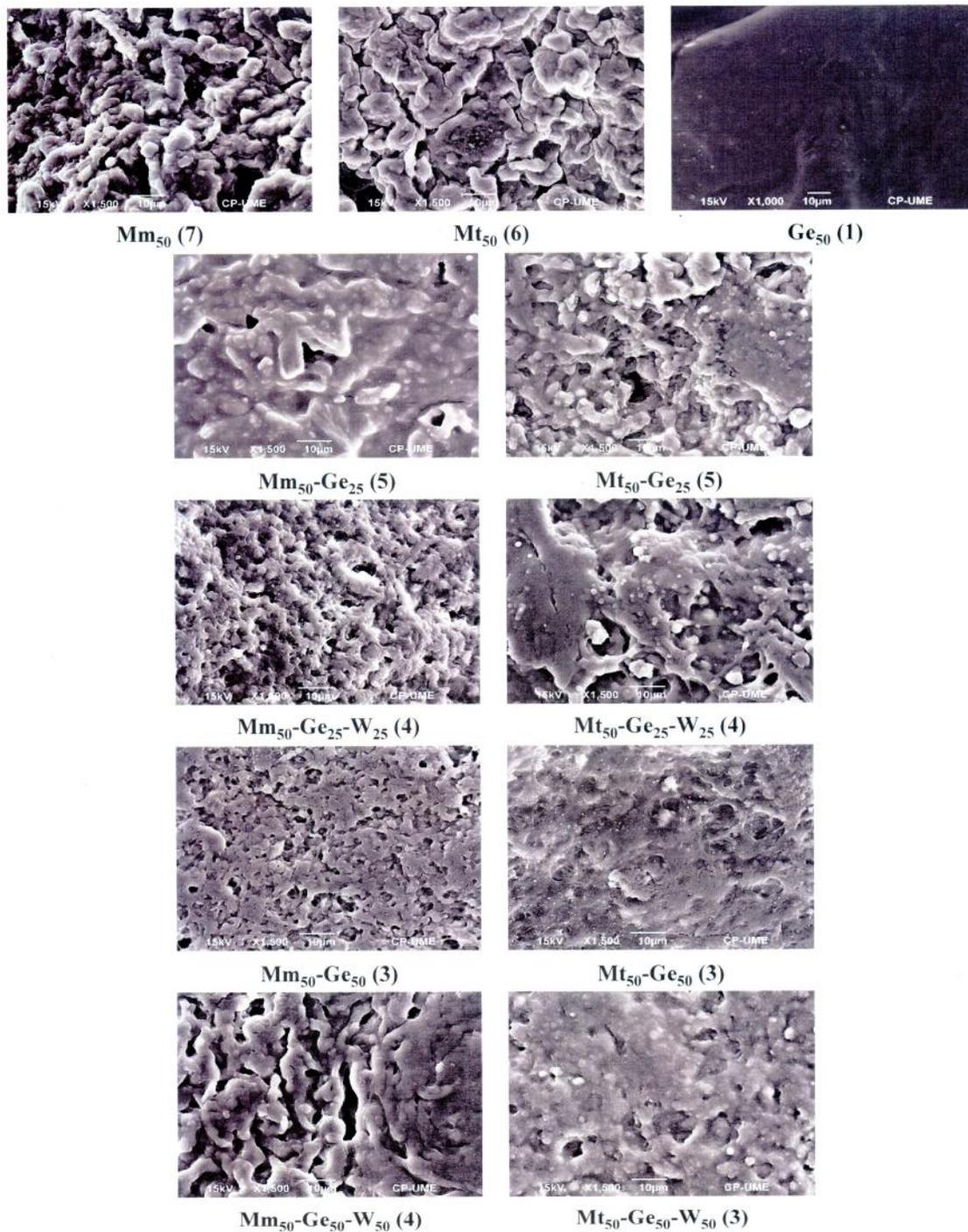


Figure 1. Images from scanning electron microscopy of films prepared with 'Milpa Alta' (Mm) and 'Toluca' (Mt) cactus mucilage, gelatin (Ge), mixtures of them, and mixtures with beeswax (W). Subscripts 25 and 50 indicate concentrations of 0.25 and 0.50%, respectively. Numbers in parenthesis correspond to the following scale: (1) smooth, (2) slightly roughness, (3) intermediate roughness, (4) intense roughness, (5) slightly lumpiness, (6) intermediate lumpiness, and (7) intense lumpiness.

If such phenomenon also occurred in mucilage-gelatin films, the morphology of these could result from a combined network of both structures, giving place to the formation of a filled-gel type structure, which is a gel matrix with particulate inclusions (Banerjee and Bhattacharya, 2012), wherein the gelatin partially filled the spaces left by the mucilaginous network and became the continuous phase (Figure 1).

The addition of beeswax tended to favor lumpiness, which was also reported by Arcan and Yemenicio lu (2013), who observed, in general, that the addition of waxes, including beeswax, into zein films, caused dramatic changes in films morphology and formed some amorphous aggregates within the polymeric matrix. According to these authors, the melting degree of waxes at the moment they are incorporated to formulations is important to avoid formation of aggregates, although in the present work such phenomenon could have occurred during the cooling phase of mixtures and the subsequent drying process. Microphotographs included in Figure 1 show that the addition of beeswax produced again a grainy consistency, but with smaller granules than those observed in films of mucilage without gelatin and wax, and apparently the filled-gel type structure was partially lost, giving place to another structure, where the hydrophobicity of the wax could cause phase separation and release of water from the gelling network structure, allowing evaporation and turning the film into a kind of xerogel, which is a solid formed from a gel by drying with unhindered shrinkage, that usually retain high porosity, enormous surface area, and very small pore size (Banerjee and Bhattacharya, 2012), although this phenomenon was clearer in films that used mucilage from cactus 'Milpa Alta' than in materials based on the gum that came from cactus 'Toluca'.

Thickness

The mucilage source (S) and the ingredient type (I) caused differences in thickness of films, without significant interaction between these two variation factors (Table 1). The use of mucilage from cactus 'Toluca' (Mt) generated thicker films (0.033 mm) than mucilage from cactus 'Milpa Alta' (Mm; 0.030 mm; $P > 0.05$), this small difference, probably, may have no practical relevance. On the other hand, the analysis of the ingredient type factor (I) showed that the highest thickness was exhibited by films of mucilage (M) and gelatin (Ge) in condition of single ingredient, and also in films made with mixtures of these compound (M-Ge) (0.040, 0.038, and 0.035 mm, respectively), which contrasted ($P < 0.05$) with the cross section size of films based on formulations of mucilage-gelatin-beeswax (M-Ge-W; 0.021 mm). Particularly, in the absence of beeswax (W), the presence of Ge did not significantly affect thickness ($P > 0.05$), which strengthens the argument of the combined network of M and Ge, where the mucilaginous fraction resulted immersed in the gelatin. In contrast, when the wax component was incorporated with a concentration of 0.25% the thickness diminished significantly ($P < 0.05$), but no additional effect was observed with further increment in concentration of that component (Figure 2A). In this regard, the addition of lipid substances has been associated with a lower water absorption capacity, and with an increase of hydrophobicity in coatings (Ghasemlou *et al.*, 2011), which may explain the effect of the beeswax in reducing the thickness of films in this work.

Table 1. Fisher (F) (Tukey, 0.05) values and means comparison corresponding to the analysis of variance of the properties of edible films formulated with six replications, with mucilage (cactus 'Milpa Alta' and 'Toluca'), gelatin, beeswax, and the mixtures of them, affected by the mucilage source (S) and the ingredient type (I).

Variation source	DF	Thi	Tra	MM	TS	WVP	O ₂ P	CO ₂ P
		(mm)			MPa	10 ⁻¹² mol m / s m ² Pa		
Mucilage (S) ¹	1	6.7 *	44.1 *	0.1 ns	30.0 *	0.3 ns	1.6 ns	0.6 ns
Mm ²		0.030 b ³	2.94 a	4.0 a	2.61 a	73.4 a	10.3 a	7.4 a
		(0.002)	(0.05)	(0.8)	(0.26)	(12.57)	(1.28)	(0.86)
Mt ²		0.033 a	2.82 b	3.8 a	1.44 b	63.2 a	9.4 a	6.8 a
		(0.002)	(0.05)	(0.7)	(0.19)	(16.96)	(1.38)	(0.93)
HSD		0.002	0.036	1.6	0.42	38.0	1.4	1.6
Ingredients (I) ¹	3	52.5 *	309.0 *	10.1 *	27.7 *	6.8 *	81.2 *	20.6 *
M ²		0.040 a	2.96 b	6.5 a	0.49 c	87.2 ab	14.4 a	8.9 a
		(0.001)	(0.03)	(0.5)	(0.05)	(16.21)	(0.62)	(0.12)
Ge ²		0.038 a	3.37 a	1.0 b	3.38 a	63.4 ab	16.6 a	10.5 a
		(0.002)	(0.02)	(0.0)	(0.12)	(3.46)	(1.12)	(0.44)
M-Ge ²		0.035 a	2.93 b	4.0 ab	1.48 b	116.3 a	11.0 b	8.7 a
		(0.001)	(0.02)	(0.6)	(0.18)	(26.68)	(0.63)	(0.89)
M-Ge-W ²		0.021 b	2.56 c	4.0 ab	2.67 a	13.2 b	3.1 c	2.8 b
		(0.010)	(0.02)	(0.5)	(0.36)	(1.78)	(0.46)	(1.11)
HSD		0.005	0.072	3.50	0.84	75.0	2.7	3.2
Interaction SxI ¹	3	0.6 ns	3.9 *	0.1 ns	5.3 *	0.5 ns	1.1 ns	0.2 ns
CV (%)		15.84	2.67	25.53	29.47	35.44	20.12	32.92

¹Values correspond to Fisher test (Tukey, 0.05). ²Quantities correspond to means values; units on column head apply to these values. ³Same letters mean no significant difference (Tukey, 0.05). Other meanings: DF: Degrees of freedom; Thi: thickness; Tra: transparency; MM: microscopic morphology; TS: tensile strength; WVP: Water vapor permeability; O₂P: oxygen permeability; CO₂P: carbon dioxide permeability; Mm: mucilage from cactus 'Milpa Alta'; Mt: mucilage from cactus 'Toluca'; M: films made with mucilage as single ingredient; Ge: films made with gelatin as single ingredient; M-Ge: films made with mucilage and gelatin; M-Ge-W: films made with mucilage, gelatin, and beeswax; CV: Coefficient of variation; *: significant (P < 0.05); ns: non-significant (P > 0.05); HSD: honest significant difference (Tukey, 0.05). Values in parenthesis are standard errors.

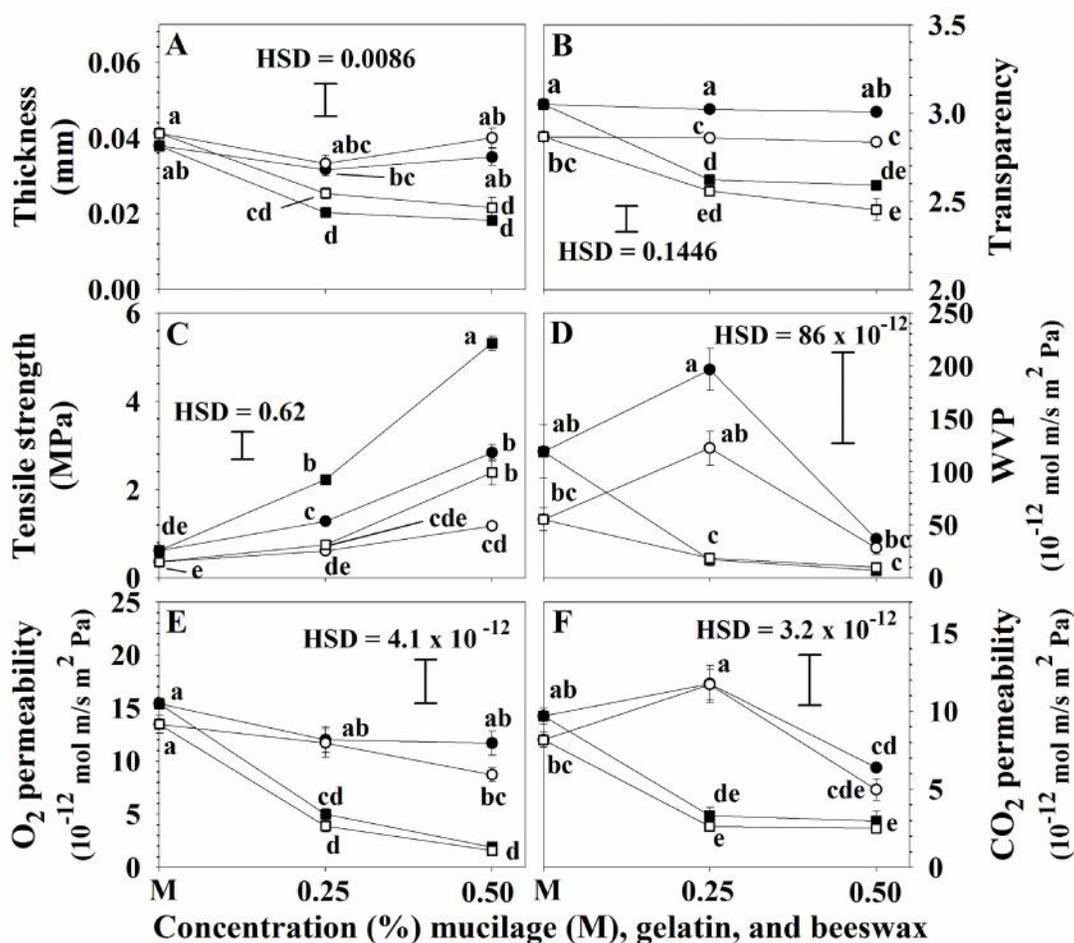


Figure 2. Behavior of thickness (A), transparency (B), tensile strength (C), water vapor permeability (D), permeability to O₂ (E), and permeability to CO₂ (F) of biopolymeric edible films formulated with mucilage extracted from cactus ‘Milpa Alta’ (Mm, ●) and ‘Toluca’ (Mt, ○) at 0.50%, and incorporated with gelatin (Ge, 0.25%, 0.50%), and beeswax (0.25%, 0.50%). The annotation M in concentration axis points out films made with mucilage as single ingredient. Circles (○, ●) correspond to films with mucilage plus Ge. Squares (□, ■) correspond to films with mucilage plus beeswax, and plus Ge. Error bars correspond to standard error. HSD is honest significant difference. Different letters inside each box indicate significant difference (Tukey, 0.05).

Transparency

The light transmission capacity was affected by both the mucilage source (S) and the ingredient type (I) ($P < 0.05$). In the first case, when mucilage (M) from cactus ‘Milpa Alta’ was used in films

their transparency was higher (2.94) than when mucilage from cactus 'Toluca' was used (2.82). In the second case, the highest transparency ($P < 0.05$) was observed in films of Ge as single ingredient (3.37), followed by materials made with M (2.96) and with the combination M-Ge (2.93), which contrasted with films that resulted from the combination M-Ge-W (2.56) (Table 1). Fakhoury *et al.* (2012) pointed out that transparency is affected by films morphology, and in the case of Ge they reported that the chemical structure of this compound does not permit to crystallize, and an amorphous state is maintained, allowing light to go through film. However, in the case of mucilage the high transparency may be explained by the fact that films had a structure with large granules and a large amount of space between them, suggesting low tortuosity to light transmission. In this variable, the interaction SxI was statistically significant (Table 1), because when the gelatin (Ge) was incorporated the transparency was not affected, while when the beeswax was added, as third component (M-Ge-W), the light transmission capacity was reduced significantly as the concentration of such lipid ingredient increased (Figure 2B). It is reported that the transparency of a polymeric film depends on the ingredients used, and the interaction between them, which is based on development of hydrogen bonds (Gorgieva and Kokol, 2011).

In the present work, when films were obtained from the mixture of mucilage and gelatin a filled gel was obtained, and the resulting structure tended to be similar to that of gelatin, due to the protein component became the continuous phase, and also because it is possible that the water trapped in the protein network dissolved in some degree the mucilaginous fraction, allowing similar light transmission to that of gelatin. On other hand, the reduction of transparency with the addition of waxes is a behavior that has been reported in several works (Fabra *et al.*, 2009; Ma *et al.*, 2012; Rodrigues *et al.*, 2014), and particularly Velickova *et al.* (2013) observed a reduction in films transparency with the addition of beeswax to a chitosan solution. Although in the present work an emulsifier was used to favor miscibility of beeswax with mucilage and gelatin, the reduction in transparency suggests a separation of the hydrophilic and lipophilic phases and, as it was stated by Ma *et al.* (2012) and Rhim *et al.* (2007), the transmittance through films might have decreased due to a reduction in compatibility of components, and also due to light dispersion or reflection at the two-phase interface. In this regard, based on the proposal of a xerogel-type structure with very small granules, and high porosity and surface area, the passage of light may have been hindered by the presence of many reflection points.

Tensile strength

Films formulated with mucilage from cactus 'Milpa Alta' in combination with other ingredients showed higher tensile strength (TS; 2.61 MPa in average) than when mucilage from cactus 'Toluca' was used (1.44 MPa in average), the difference was significant ($P < 0.05$; Table 1), although in the single ingredient condition there was no effect ($P > 0.05$) caused by one or another type of mucilage, which suggested that the interaction between components in formulations occurs in different form depending on the mucilage used (Figure 2C). On the other hand, TS was also affected by the ingredient type, and, in this case, those films prepared only with mucilage had the smallest mechanical strength (0.49 MPa). This situation coincided with

values obtained by Espino-Díaz *et al.* (2010), but such magnitude was significantly smaller than the tensile strength obtained in films of gelatin (3.38 MPa) and those based on mixtures M-Ge (1.48 MPa) and M-Ge-W (2.67 MPa) (HSD = 0.84 MPa; Table 1). Tensile strength is the maximum stress that a film can withstand against an applied tensile stress before the film tears (Espino-Díaz *et al.*, 2010). There is abundant evidence to show that this property is strongly affected by the concentration and type of ingredients in formulations (Prakash Maran *et al.*, 2013; Silva-Weiss *et al.*, 2013), and by the microscopic morphology developed by the interaction between them (Arcan and Yemenicio lu, 2013), this being given by the ability of the mixture to form hydrogen bonds (Wu *et al.*, 2012). In this regard, Fakhoury *et al.* (2012) and Wang *et al.* (2011), working with starch/gelatin and carrot puree/gelatin films, respectively, showed that the increment in the protein component produced higher TS values, this coinciding with results of the present work. Besides, the low mechanical strength exhibited by materials formulated only with mucilage could be a result of the lumpy structure that films acquired with drying, since many separated granules were formed (Figure 1), thus a weak structure was obtained.

Rodriguez *et al.* (2006), working with starch films, observed that the combination of glycerol with Tween can reduce the tensile strength, which could have also occurred in mucilage films of the present work. In the case of gelatin films, the structure corresponded to a polymeric gel where peptide chains may result in a high aggregation of protein with high number of cross-linking zones, thus giving a stronger structure to the film (Jridi *et al.*, 2013). The statistical analysis reported that the interaction between variation factors (S and I) was significant ($P < 0.05$; Table 1). In fact, as the gelatin concentration increased in the mixture, the tensile strength was also increased ($P < 0.05$), but that phenomenon was more pronounced when the mucilage came from cactus 'Milpa Alta', where values of 1.28 and 2.83 MPa were observed in films of treatments $Mm_{50}\text{-}Ge_{25}$ and $Mm_{50}\text{-}Ge_{50}$, respectively. This behavior contrasted with the values of 0.61 and 1.18 MPa from the mixtures $Mt_{50}\text{-}Ge_{25}$ and $Mt_{50}\text{-}Ge_{50}$, respectively (HSD equal to 0.62 MPa; Figure 2C), such disparity may be caused by a different arrangement of the mucilage granules when films dried, since in films of mucilage from cactus 'Toluca' the space between granules was lesser than in films based on mucilage from cactus 'Milpa Alta'. The formed 'Toluca' mucilage films had to occupy more volume, which derived in thicker materials, but in each particular portion of them a fewer quantity of gel remained, thus the tensile strength was lesser than in films based on mucilage from 'Milpa Alta'. On the other hand, the incorporation of beeswax caused additional increment ($P < 0.05$) in the mechanical strength and values as high as 5.32 and 2.39 MPa were observed in films of the mixtures $Mm_{50}\text{-}Ge_{50}\text{-}W_{50}$ and $Mt_{50}\text{-}Ge_{50}\text{-}W_{50}$, respectively. Such increment in tensile strength with beeswax was in contrast with the effect that has been commonly observed in films when this lipid compound has been added, in the sense that it induces reduction in the mechanical resistance, derived from a relaxation of the polymeric matrix (Fabra *et al.* 2008; Navarro-Tarazaga *et al.*, 2011).

In the present work, the morphology obtained with the incorporation of wax could correspond to a xerogel structure, where the evaporation of water could increase rigidity in films. Besides, the lesser space between granules and the resultant requirement of higher volume to the filled gel in Toluca mucilage films caused lesser tensile strength than in Milpa Alta mucilage.

Water vapor permeability

The source of mucilage did not cause difference ($P > 0.05$) in the water vapor permeability, but did the ingredient type ($P = 0.05$) (Table 1). Average values of 116.3×10^{-12} , 87.2×10^{-12} , 63.4×10^{-12} , and 13.2×10^{-12} mol m / s m² Pa were found in films made with formulations of M-Ge mixtures, with mucilage as single ingredient, with gelatin as single ingredient, and with M-Ge-W mixtures, respectively, the difference was significant between the latter and the three former (HSD equal to 75×10^{-12} mol m / s m² Pa), and the interaction between mucilage source and ingredient type factors was not significant (Table 1). On the other hand, mucilage films of the present work resulted with similar water vapor barrier properties to cactus mucilage films obtained by Espino-Díaz *et al.* (2010), since they observed WVP between 98.47 and 146.81 g mm/d m² kPa (between 63.3×10^{-12} and 94.3×10^{-12} mol m / s m² Pa). The films of this work had similar WVP values as the prepared with quince seeds mucilage (4.23×10^{-12} mol m / s m² Pa; Jouki *et al.*, 2014), methylcellulose (5.2×10^{-12} mol m / s m² Pa), zein (6.5×10^{-12} mol m / s m² Pa), chitosan (27.2×10^{-12} mol m / s m² Pa), wheat gluten (5.1×10^{-12} mol m / s m² Pa), whey protein (61.0×10^{-12} mol m / s m² Pa), and soy protein (19.3×10^{-12} mol m / s m² Pa) (Lin and Zhao, 2007).

In M-Ge formulations a tendency to increase WVP was observed when the protein component was added at 0.25%, but this trend was reversed when the compound was used at concentration of 0.50% (Figure 2D). The exchange of water through a film occurs with a permeation phenomenon, where the moving compound is first dissolved at the high concentration side, and then it flows throughout the film by a diffusion mechanism to be subsequently evaporated at the low concentration side (Kester and Fennema, 1989). According to Farris *et al.* (2011), gelatin films possess high swelling index and their molecules assemble into aggregates containing short segments (200 to 400 nm in length and about 6 nm in height) linked by an intermolecular triple helix formation among which the water is trapped, and it is probable that with the increment in gelatin concentration the cross-linking also increases, making difficult the liberation of water to the environment, reducing WVP.

The addition of beeswax (W) caused a decrease ($P = 0.05$) in WVP values, between 6.9×10^{-12} and 18.7×10^{-12} mol m / s m² Pa (Figure 2D), and such effect was also reported in chitosan-base films by Velickova *et al.* (2013), suggesting that the incorporation of this lipid reduced the hydrophilic-lipophilic balance, increasing the water vapor barrier. Transpiration is a deterioration factor that produces important losses during the postharvest handling of horticultural products, thus a biopolymeric film could be used as part of a conservation strategy to provide protection against dehydration. Thus, water vapor permeability (WVP) is an important property to be considered in a polymeric material selection (Navarro-Tarazaga *et al.*, 2011).

Permeability to gases

The permeabilities to O₂ and CO₂ were not affected ($P > 0.05$) by the mucilage source; however, the type of ingredient affected ($P = 0.05$) these parameters (Table 1). In the case of O₂, values of 16.6×10^{-12} , 14.4×10^{-12} , 11.0×10^{-12} , and 3.1×10^{-12} mol m / s m² Pa were found for films of Ge, M,

M-Ge, and M-Ge-W, respectively, where the difference was significant between the first two and the last two (HSD equal to 2.7×10^{-12} mol m / s m² Pa; Table 1). In the case of CO₂, values of 10.5×10^{-12} , 8.9×10^{-12} , 8.7×10^{-12} , and 2.8×10^{-12} mol m / s m² Pa were found for films of Ge, M, M-Ge, and M-Ge-W, respectively, and in this case there was significant difference only between the first three and the last (HSD equal to 3.2×10^{-12} mol m / s m² Pa).

The interaction between variation factors (S and I) was not significant ($P > 0.05$; Table 1) and, although there were some variations in O₂ and CO₂ permeabilities when the gelatin was incorporated to the mucilage, the differences had no practical relevance. However, when the beeswax was added, the permeability decreased ($P = 0.05$) to values lesser than 1.9×10^{-12} and 2.9×10^{-12} mol m / s m² Pa in the case of O₂ and CO₂, respectively (Figures 2E and 2F). Coatings based on polysaccharides and proteins are hydrophilic materials and develop moderate barriers to gas exchange, but they are poor barriers to water vapor. In contrast, films based on lipids are more permeable to gases, but they generate better barriers to water vapor (Baldwin and Hagenmaier, 2012). The results from the present work showed that the addition of beeswax to films of mucilage and gelatin caused a decrease in the permeation gas exchange rates for water vapor, O₂ and CO₂.

According to Lin and Zhao (2007), the films of this study represented a greater barrier to O₂ and CO₂ than those made with methylcellulose (1.6×10^{-4} mol m / s m² Pa and 2.8×10^{-5} mol m / s m² Pa) and hydroxypropyl cellulose (1.3×10^{-4} mol m / s m² Pa and 4.6×10^{-3} mol m / s m² Pa) respectively, but this barrier property was much lower than that developed by films of zein (3.2×10^{-17} mol m / s m² Pa and 1.1×10^{-16} mol m / s m² Pa) and wheat gluten (1.2×10^{-15} mol m / s m² Pa and 8.7×10^{-17} mol m / s m² Pa), respectively. In the case of mucilage films Jouki *et al.* (2014) found an O₂ permeability of 1.77×10^{-17} mol m / s m² Pa (36.81 cm³ μm/d m² kPa) but in that case the gum came from quince seeds.

The application of a biopolymeric coating on horticultural products may induce an effect of modified atmosphere, characterized by a reduction of the O₂ concentration and the elevation of CO₂ at tissue level (Falguera *et al.*, 2011). In such case, if O₂ concentration decreases below the anaerobic compensation point a fermentative metabolism can be induced (Valle-Guadarrama *et al.*, 2004), whose products (acetaldehyde and ethanol) may generate off-flavors and off-odors (Rojas-Graü *et al.*, 2009a). Therefore, it is clear that the gas permeability control must be a priority during the development of technologies based on coatings (Falguera *et al.*, 2011).

Films based on cactus mucilage have been applied on strawberry (Del-Valle *et al.*, 2005) and mango fruits (Adetunui *et al.*, 2012), with beneficial effects in the lengthening of the products shelf life. Thus, films of mucilage like those of the present work may be adequate to be used in the postharvest conservation area, since their permeability to O₂ and CO₂ allow their use without introducing harmful conditions for the product.

CONCLUSIONS

The mucilage from cactus variety Milpa Alta allows the formation of thinner films, with more transparency, and more tensile strength than mucilage from variety Toluca, although the capacity to act as barrier to water vapor, O₂, and CO₂ is similar. The addition of gelatin does not affect the thickness and transparency of such films, but causes modification of the microscopic morphology, the mechanical strength, and the barrier properties to gases and water vapor. The addition of beeswax promotes the formation of aggregates in the microscopic morphology of films and these acquire smaller thickness, higher tensile strength, and better barrier properties to O₂, CO₂, and water vapor, which gives them better potential to be used in the horticultural products postharvest conservation area, although they are less transparent. Among films based on mucilage, gelatin, and beeswax, those formulated with mucilage from cactus 'Milpa Alta' can exhibit better performance as coating of horticultural products, because they are thinner, more transparent, and the tensile strength is higher than films based on mucilage obtained from cactus 'Toluca'.

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