Development and Characterization of Edible Films Based on Mucilage of Opuntia ficus-indica (L.)

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Abstract

Mucilage of Opuntia ficus-indica (OFI) was extracted and characterized by its composition and molecular weight distribution. Mucilage film-forming dispersions were prepared under different pHs (3, 4, 5.6, 7, and 8) and calcium concentration (0% and 30% of $CaCl_2$, with respect to mucilage's weight), and their particle size determined. Mucilage films with and without calcium (MFCa and MF, respectively) were prepared. The effect of calcium and pH on mucilage films was evaluated determining thickness, color, water vapor permeability (WVP), tensile strength (TS), and percentage of elongation (%E). The average molecular weight of the different fractions of mucilage was: 3.4×10^6 (0.73%), 1×10^5 (1.46%), 1.1×10^3 (45.79%), and 2.4×10^2 Da (52.03%). Aqueous mucilage dispersions with no calcium presented particles with an average size d(0.5) of 15.4 μm, greater than the dispersions with calcium, 13.2 μm. MFCa films showed more thickness (0.13 mm) than the MF films (0.10 mm). The addition of calcium increased the WVP of the films from 109.94 to 130.45 gmm/m^2dkPa. Calcium and pH affected the mechanical properties of the films; the largest TS was observed on MF films, whereas the highest %E was observed on MFCa films. The highest differences among MF and MFCa films were observed at pHs 5.6 and 7 for TS and at pHs 4 and 8 for %E. No effect of pH and calcium was observed on luminosity

and hue angle. Chroma values were higher for MF when compared with MFCa, and increased as pH of the films increased.

Keywords: calcium, edible films, mucilage, nopal, pH.

Practical application

In this study mucilage from nopal was extracted and characterized by its ability to form edible films under different pHs, and with or without the addition of calcium. Opuntia ficus-indica mucilage had the ability to form edible films. In general, it can be considered that mucilage films without modification of pH and without the addition of calcium have the best water vapor barrier properties and tensile strength. Mucilage from nopal could represent a good option for the development of edible films in countries where nopal is highly produced at low cost, constituting a processing alternative for nopal.

Introduction

The development of edible films has received more attention in recent years, due to the growing interest for reducing environmental pollution caused by plastics, the need to extend the shelf life of foods, and the increasing demand for healthier and ecological foods.

Edible films act as a barrier between the covered product and the surrounding medium, or between different components in a food, delaying the migration of moisture, gases, and lipids. Edible films can be used as carriers of additives such as nutrients, flavors, antimicrobials, and antioxidants. Edible films help to maintain the integrity of the coated food (Brancoli and others 1997; Guilbert and others 1996; Krochta and De Mulder-Johnston 1997; Miller and Krochta 1997). The main materials used for the

formation of edible films are polysaccharides, proteins, lipids, and resins mainly obtained from plants and animals (Hern´andez-Izquierdo and Krochta 2008). Many polysaccharides have been studied for the development of edible films, however, this is not the case of mucilage, a hydrocolloid produced by nopal, a plant of the Opuntia's genus and Cactaceae family.

Mucilage from nopal could represent a good option for the development of edible films in countries, such as Mexico, where nopal is highly produced at low cost, constituting a processing alternative for nopal, and a source of economic resources for low income communities. More than 370 species of Opuntia nopal have been recognized around the world, and at least 60 of them are native from Mexico, including Opuntia ficus-indica (OFI), the most cultivated edible cactus in the world (Bravo 1978). In Mexico, there are 3 million hectares of wild nopal, and 233000 Ha of cultivated nopal, of which 150000 Ha are used for fodder and 13000 Ha are cultivated for cactus-pear fruits, with a production of 139193 Ton (SAGARPA 2007).

Mucilage of Opuntia nopal is a polysaccharide with a molecular weight in the order of 2.3×10^4 (Medina-Torres and others 2000) to 4.3×10^6 Da (Trachtenberg and Mayer 1982), and contains Dgalactose, L-arabinose, D-xylose, and L-rhamnose as the major neutral sugar units, as well as D-galacturonic acid (McGarvie and Parolis 1979; Cai and others 2008). Mucilage presents a linearmain chain with repetitions of (1→4)-linked β-D-galacturonic acid and α(1→2)-linked L-rhamnose with oligosaccharide side chains of β(1→6)-linked D-galactose attached to O(4) of L-rhamnose residues (McGarvie and Parolis 1981).

OFI mucilage forms large molecular aggregates, as shown by its shear-thinning behavior in dispersion (Cárdenas and others 1997; Medina-Torres and others 2000). Viscosity of mucilage aqueous dispersion depends on pH and Ca^{2+} ion concentration: as pH increases from acidic to alkaline conditions, the viscosity increases; on the other hand, as ionic strength increases, viscosity decreases. This latter behavior is more pronounced with divalent ions (Trachtenberg and Mayer 1982; Medina-Torres and others 2000). Since mucilage aqueous dispersions are dependent on pH and calcium, mucilage film properties could also be dependent on these variables; hence, the objective of this study was to determine the ability of mucilage to form edible films, evaluating the effect of pH and calcium content on the properties of the films.

Materials and Methods

A batch of fresh cladodes of OFI was obtained from the Center for the Research of Natural Resources (Centro de Investigación de Recursos Naturales, CIRENA), in Chihuahua, Mexico, and stored at 4 °C until mucilage extraction (period not exceeding 15 d). Harvesting of cladodes was carried out in June, after an intense period of rain.

Extraction of mucilage: Mucilage's extractionwas performed as carried out by Sepúlveda and others (2007) and Goycoolea and Cardenas (2003). Nopal cladodes were crushed, homogenized with water (1 : 1 w/w), and boiled for 20 min at 85 °C (to prevent enzymatic action). The mixture was filtered through a cheese cloth and centrifuged (4000 × g, 18 min, at 25 °C) in a Hermle Z-383 (Hermle-Labortechnik, Wehingen, Germany) centrifuge. Supernatant was then recovered and precipitated with 65% ethanol aqueous solution (v/v) for 20 h at 4 °C. After precipitation, samples were

washed twice in ethanol (95%) and freeze-dried (−12 °C for 8 hr) using a Free Zone 77540 Freeze dryer (Labconco Co., Kansas City, Mo., U.S.A.).

Characterization of mucilage: Nopal mucilage was characterized by its sugar (ISO 11292 1995), protein (Bradford 1976), moisture (AOAC 925.10, 1992), ash (AOAC 923.03, 1990), and calcium (atomic absorption spectroscopy) content.

For the determination of sugars, mucilage samples (10 mg) were hydrolyzed in 1 M HCl (6 mL) during 150 min at 100 °C. Hydrolyzed samples were filtered through a Sep Pack C-18 (Waters, Mass., U.S.A.), dried at 35 to 40 °C, washed 2 times with water and dried again. The residue was dissolved in water (0.5 mL) and filtered through a membrane of 0.45 µmpore size before being injected (20 µL) in a DIONEX DX 600 high-performance liquid chromatography (HPLC) system (Dionex Corp., N.Y., U.S.A.), equipped with an ED50 electrochemical detector and a CarboPac PA1 column (4 × 250 mm; Dionex Corp.). The mobile phase was HPLC grade water with a flow rate of 1 mL/min and 300 mM NaOH post column at 0.6 mL/min. Monosaccharides in analytical samples were identified and quantified using standard compounds. The measurements were made in duplicate.

Molecular weight distribution was determined by highperformance size exclusion chromatography (HPSEC) as proposed by Yahia and others (2009), using a ProStar HPLC system (Varian Inc.,Walnut Creek, Calif., U.S.A.), equipped with two 210 pumps and a 325 UV-Vis dual wavelength detector. Mucilage fractions were monitored at 206 nm (Fishman and others 1984). The chromatographic system included a TSK-Gel GPWXL (7.8 × 300 mm; Tosoh Bioscience, Minato-ku, Tokyo, Japan) column kept at 40°C. The mobile phase was 0.2 M phosphate buffer. The flow rate of the mobile phase

was 1 mL/min. Dextrans (Sigma Chemical, Co., Miss., U.S.A.) with a molecular weight from 1000 to 67000 Da were used as standards.

Film-forming dispersion: Film-forming dispersion consisted of mucilage, glycerol, and distilled water, at a ratio of 2 : 1 : 50, respectively. Film-forming dispersion was maintained 10 h under magnetic stirring and stored overnight at room temperature (25 °C). This procedure ensures the disintegration of mucilage aggregates to form a homogeneous dispersion.

To determine the effect of pH and calcium addition on the properties of mucilage films, calcium chloride was added to the dispersion at a concentration of 30% respect to the mucilage's weight, based on the findings by Trachtenberg andMayer in 1982, who found this concentration to be the optimal in terms of calcium addition and viscosity increase. The pH of the film forming dispersions was adjusted with hydrochloric acid or sodium hydroxide solutions (2 and 0.5M), to obtain dispersions with pH values of 3, 4, 5.6 (pH native), 7, and 8. To minimize volume differences among treatments, preliminary tests were performed to estimate the amount of solution needed. Film-forming solutions were subjected to vacuum for 20 min to eliminate trapped air.

Particle size of mucilage dispersion: The particle size distribution of the film forming dispersions was measured by laser diffraction with a Malvern Mastersizer Hydro 2000 (Malvern Instruments Ltd., Malvern, U.K.). A refractive index of 1.33 was used. The measurements were made in duplicate.

Film preparation: The process of film formation was conducted under conditions of 30% RH and 25 ± 2 °C.

The film-forming solutions were cast into a 100 mm diameter glass Petri dish coated with Teflon (Polyester film, DuPont, Hopewell, Va., U.S.A.). The plates were placed on a leveled surface at room temperature and left to dry for 24 h. All films were preconditioned before the tests in a constant temperature humidity chamber (Fisher Scientific Inc.,N.J.,U.S.A.) set at 25 °C with 50% relative humidity (RH) for 96 h to adjust the moisture content.

Film thickness: The thickness of the films was measured with a micrometer Mitutoyo 340 (Mitutoyo, Mexico). Measurements were taken at 5 different places on the film and an average value was calculated.

Color: Color parameters (L*, a*, b∗*) of mucilage films were determined using a CR-300 Minolta colorimeter (Minolta Camera Co., Osaka, Japan). Readings were obtained in the CIELAB scale; hue angle (h*) value was calculated by tan−1(*/a*), and chroma (C*) by $(a^{*2} + b^{*2})^{1/2}$. Color was determined as means of 5 measurements at different locations for each film placed on a white plate Minolta CR-200 (Y = 93.92, x = 0.3132, y = 0.3192).

Water vapor permeability: Water vapor permeability (WVP) of films was calculated as described by McHugh and others (1993), and Gennadios and others (1994). Mucilage films were cut into discs and mounted on aluminum cups (6.3 cm diameter and 1.3 cm depth) containing 18 g of anhydrous calcium sulfate (Drierite™, J.T. Baker, Mexico) as desiccant (0% RH). A rim with a gasket and screws was used to seal the films. Cups were placed in a chamber (Fisher Scientific Inc.) containing water (100% RH), equipped with fans to assure a homogeneous RH. Initial temperature of chambers was 25 °C, but use of a fan increased it to 29 °C, so temperature during test

was maintained at that level. The slope of variation in weight against time (after steady state was reached) was calculated by linear regression; correlation coefficient for all samples was 0.97 or higher. The steady transfer rate was reached after 1 h.Water vapor transmission rate (WVTR) was calculated by dividing slope by film area, and WVP as follows:

$$WVP = WVTRx/(p_3 - p_2) \quad (1)$$

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

where x is the thickness of film, p_3 the water vapor pressure inside the chamber (atm), and p_2 the corrected water vapor pressure next to the film inside the cup (atm). *P* is the total atmospheric pressure (atm), p_1 the partial pressure at the surface of the desiccant in the cup ($p_1 = 0$), R the universal gas constant (cm^3 atm/g mol K), *T* the absolute temperature during test (K), Δz the air gap inside the cup (cm), and D the diffusion coefficient (cm^2/s) (McHugh and others 1993; Gennadios and others 1994).

Mechanical properties: Tensile strength (TS) and percentage elongation (%E) at break were determined according to ASTM standard method D882-91 (ASTM 1992), with a Universal Texture Analyzer TA. XT2 (Stable MicroSystems, Godalming, U.K.), using a T-96 double clamp set (Texture Technologies, Co., U.S.A.) with a strain rate of 0.83 mm/s. TS is the maximum stress a film can withstand against applied tensile stress before the film tears, while %E at break is the percentage change in the original film length between the grips (Dogan and McHugh 2007). TS and %E were measured on rectangular strips of 70 × 15 mm. Films were previously conditioned at 50% RH. The measurements were made in duplicate.

Statistical analysis: A completely randomized design with a 4 × 2 factorial was used. Statistical analysis of data was performed through analysis of variance (ANOVA) using Minitab R.14 (Minitab Inc., State College, Pa., U.S.A.). Tukey's multiple comparison tests were used to compare treatments when statistical significance in treatment was found for general ANOVA ($P < 0.05$). The experiment was conducted in triplicate.

Results and Discussion

Mucilage characterization: Mucilage from cladodes of Opuntia ficus-indica was extracted and characterized. Mucilage yield was 0.68% (fresh weight base). HPLC analyses indicated the presence of galactose, arabinose, xylose, galacturonic acid, and glucose. Molecular size characterization indicated an average molecular weight (M_w) of the different fractions of mucilage as follows: 3.4×10^6, 1×10^5, 1.1×10^3, and 2.4×10^2 Da. Characteristics of this mucilage are presented in Table 1.

Particle size of mucilage dispersions: Aqueous mucilage dispersions containing calcium presented particles with an average size d(0.5) of 13.2 µm. This average size was smaller than the one for mucilage dispersions with no calcium, which was 15.4 µm. No significant difference was found among dispersions with different pH (Figure 1). Trachtenberg and Mayer (1981) reported that mucilage in aqueous dispersions at pHs between 4 and 10, presented a smaller size when calcium was added. According to Trachtenberg and Mayer there is a formation of intramolecular calcium links among mucilage carboxylic groups, located in the main chain of the molecule causing a reduction in the mucilage's size, whereas in the absence of calcium, the exposed

negative charges of the carboxylic groups cause an electrostatic repulsion, allowing for a wider spread configuration, with a rigid molecule of larger size.

Table 1–Chemical and physical characteristics of *Opuntia ficus-indica* mucilage.

Chemical composition		Molecular weight distribution	
Moisture	12.43	3.4×10^6 Da	0.73
Protein	1.04[a]	1.0×10^5 Da	1.46
Ash	20.08[a]	1.1×10^3 Da	45.79
Calcium	3.10[a]	2.4×10^2 Da	52.03
Fat	2.3[a]		
Carbohydrates	64.15[a]		
Galactose	5.71[a]		
Arabinose	5.32[a]		
Xylose	3.27[a]		
Galacturonic acid	0.85[a]		
Glucose	32.29[a]		

All results were obtained in triplicate.
[a]Percentage on dry weight base.

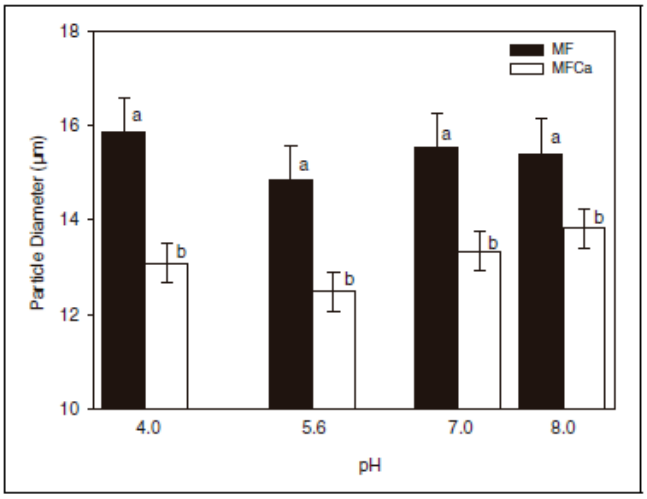

Figure 1–Effect of pH and calcium on particle size of aqueous mucilage dispersions. MF = mucilage films without calcium, MFCa = mucilage films with calcium. Error bars represent one standard deviation. Different letters indicate significant differences ($P < 0.05$).

Film preparation: Natural pH of the mucilage aqueous dispersion was 5.6. At pH 3, handling of the film was impossible, given its high adhesion and elasticity, hence, the pH was set between 4 and 8, a range in which all the films were strong enough and flexible to allow their handling. Films were uniform, smooth and translucent. Jangchud

and Chinnan (1999) reported that peanut protein films were moist and sticky when formed at low pHs.

Average thickness of mucilage films was 0.1319 mm for MFCa and 0.1095 mm for MF (Table 2). There was no significant difference ($P < 0.05$) in thickness among films due to the effect of pH. According to McHugh and Krochta (1994a) and Dangaran and others (2006), small particles have larger contact surface and form thinner films, however, in the case of MFCa, a contrary effect was presented. This is probably due to the formation of mucilage intramolecular links with calcium, reducing and tying the polysaccharide molecule and preventing the formation of a compact structure in the film. When intramolecular association is presented on polymers, a contraction of molecules is observed (Majdoub and others 2001a; Cardoso and others 2003; Maleki and others 2005). In the absence of calcium, the mucilage molecule is more extended with higher flexibility and greater contact surface among molecules, producing films with a more compact structure. According to Livney and others (2004) an open structure possesses a greater conformational flexibility than an intramolecular cross linked structure. In chitosan, intramolecular interactions between NH_{3+} and hydroxyl groups limited the molecular movement of the chitosan chain (Salleh and others 2009).

Mucilage films presented a high lightness ($L^* = 93$). Neither calcium nor pH affected this parameter ($P < 0.05$). Hue values ranged from 96.8 in acid pH to 99.6 in alkaline pH, both values are within the yellow-green area in the color scale. Hue angle was not affected by calcium addition ($P < 0.05$). However at lower pH films were lighter yellow while at higher pH films were green yellow and appeared more opaque. Chroma (C^*) was affected by both pH and calcium ($P < 0.05$), presenting a color less saturated

at pH 4 (7.9 for MFCa and 9.7 for MF), and more saturated at pH 8 (13.8 for MFCa and 16.3 for MF). Chroma values were higher on mucilage films with no calcium; this may be due to a better intermolecular bonding among molecules increasing the aggregation.

Water vapor permeability: The effect of pH and calcium on WVP of mucilage films is shown in Table 2; while the pH had no significant effect on the WVP, calcium did ($P < 0.05$). The WVP was higher in MFCa films (130.45 gmm/m^2dKPa), than in MF films (109.94 gmm/ m^2dKPa). The study of the particles in the mucilage dispersion showed a larger size in the absence of calcium (Figure 1), which could mean an extended molecule that facilitates the formation of a more compact and orderly 3-dimensional network in the film, hindering the movement of the permeate molecules through the film.

Several studies have shown the ability of calcium to form intermolecular links. With proteins, calcium may react with the carboxylic groups and improve the stability of the 3-dimensional network of the films, reducing its water solubility (Fabra and others 2010); with alginate, calcium reacts with the carboxylic groups of guluronic acid and forms films more resistant with a low water solubility (Olivas and Barbosa-Canovas 2008); with pectins, calcium may react with the carboxylic groups of galacturonic acid of the linear regions of the low-grade methylation molecule (Fang and others 2008). All these molecules have large binding areas to interact with the calcium. However, in the mucilage, whose structure is highly branched (McGarvie and Parolis 1981), similarly to the rhamnogalacturonan I fraction of pectins (Voragen and others 1995), the calcium may form intramolecular links, given the low charge density of the principal chain and the intrinsic flexibility of the molecule (Majdoub and others 2001b). This can cause an immobilization of the mucilage, avoiding the formation of a compact ordered 3-

dimensional network in the process of film formation, increasing its permeability to water vapor.

Mechanical properties: Mechanical properties of edible films are directly related to their chemical structure (Fabra and others 2010). Values of tensile strength found in mucilage films may be dependent of factors such as molecular size, intra and intermolecular associations, mucilage's molecular weight and molecular weight distribution. According to Lazaridou and others (2003) the functional properties of biopolymers will depend if they exist as disordered molecules whose interactions depend mainly on space-occupancy considerations, or as ordered structures capable of stable association into compact networks.

Table 2–Thickness, water vapor permeability, and color of mucilage films from nopal *Opuntia ficus-indica*.

pH	Thickness mm	WVP gmm/m²dKPa	Color		
			Lightness	Hue angle	Chroma
		Edible films of nopal mucilage without calcium (MF)			
4.0	0.1052 ± 0.004 a	114.81 ± 1.24 a	94.07 ± 0.71 a	97.97 ± 1.54 b	9.78 ± 3.331 a
5.6	0.1109 ± 0.003 a	98.47 ± 17.21 a	93.00 ± 1.09 a	99.58 ± 0.05 a	14.86 ± 0.936 b
7.0	0.1139 ± 0.001 a	119.04 ± 8.46 a	92.93 ± 0.52 a	97.77 ± 2.26 ab	15.91 ± 0.226 b
8.0	0.1081 ± 0.008 a	107.42 ± 13.23 a	93.09 ± 0.417 a	99.59 ± 0.27 ab	16.27 ± 0.139 b
		Edible films of nopal mucilage with calcium (MFCa)			
4.0	0.1271 ± 0.003 b	129.19 ± 8.48 b	94.07 ± 0.67 a	96.76 ± 0.09 a	7.93 ± 0.39 c
5.6	0.1319 ± 0.004 b	126.20 ± 19.99 b	93.26 ± 0.34 a	99.45 ± 0.30 b	13.20 ± 0.29 d
7.0	0.1293 ± 0.009 b	119.61 ± 18.82 b	91.97 ± 1.73 a	98.75 ± 0.46 b	13.42 ± 2.79 d
8.0	0.1391 ± 0.015 b	146.81 ± 10.45 b	92.92 ± 0.27 a	97.80 ± 0.14 c	13.84 ± 1.34 d

	Thickness mm	WVP gmm/m²dKPa	Color		
			Lightness	Hue angle	Chroma
		Comparison between MF and MFCa			
MF	0.1095 a	109.94 a	93.27 a	98.73 a	14.20 a
MFCa	0.1319 b	130.45 b	93.055 a	98.192 a	12.098 b

Each value represents ± SD average of the 3 determinations. Different letters indicate significant differences ($P < 0.05$).

Table 3 shows the mechanical properties of some edible films. Peanut protein films together with mucilage films presented the lowest TS values, and also presented the lowest molecular weights. Some studies have shown that mechanical properties are affected by molecular weight. Park and others (1993) reported that the mechanical properties of cellulose films were affected by molecular weight; showing an increase in

tensile strength as molecular weight increased. Similar findings were reported by Lazaridou and others (2003) on pullulan films.Molecular weight distribution also affects mechanical properties of the films; Van Soes (1996) found that tensile stress of starch films decreased with increasing molecular weight distribution. The low TS of the mucilage films could also be explained by the high molecularweight distribution ofmucilage films (Table 1).

The addition of calcium reduced the TS in mucilage's films (Figure 2). The highest values of TS were achieved in MF, at pH 5.6 and 7 (0.87 and 0.96MPa, respectively), while, at pHs 4 and 8, no significant difference was found among treatments ($P < 0.05$) (Figure 2). Low TS values found in MF at pHs 4 and 8 could be explained by highly (positive or negative) charged molecules, that weakened intermolecular associations between molecules due to repulsion. According to Nieto (2009) the presence of ionic groups increases the polarity of polymers, and weakens intermolecular associations between polymer chains due to repulsion. High TS values in MF at pHs 5.6 and 7 could be explained by a wider spread configuration of mucilage molecules, with less repulsion and more intermolecular hydrogen bonding, which allowed for the formation of a more ordered 3-dimensional network, producing compact and resistant films.

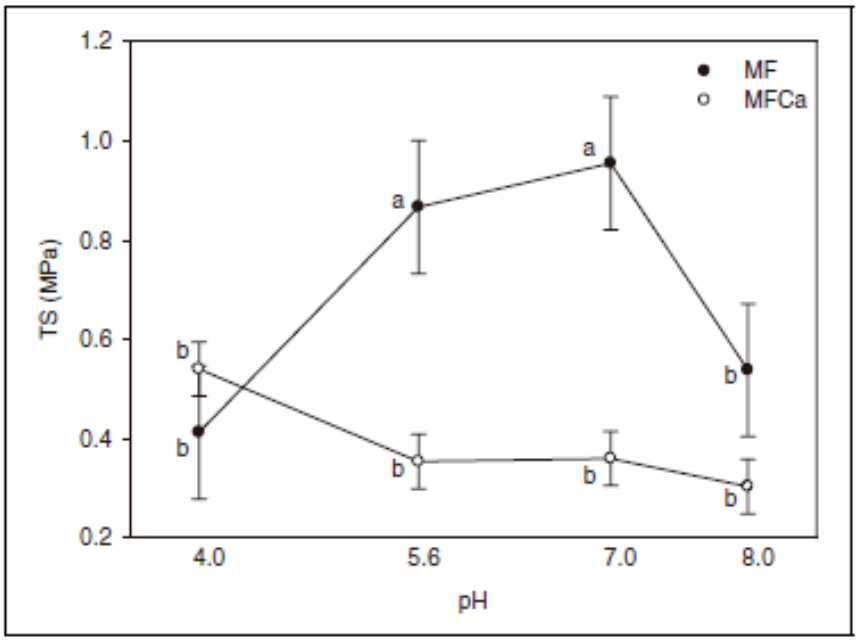

Figure 2–Effect of pH and calcium on the tensile strength of edible films of mucilage from nopal *Opuntia ficus-indica*. MF = mucilage films without calcium, MFCa = mucilage films with calcium. Error bars represent 1 SD. Different letters indicate significant differences ($P < 0.05$).

TS values of MFCa were kept low at pHs 5.6 and superior (approximately 0.38MPa), while at pH 4 a higher TS was observed (approximately 0.55 MPa). Data suggest that at pHs 5.6, 7, and 8, intramolecular bonding of mucilage is presented, due to calcium cross linkage, while at pH 4 mucilage is positively charged and intramolecular bonding by calcium may not be occurring.

The %E indicates the capacity of the film to stretch. Results showed that the pH and calcium had a significant effect on %E ($P < 0.05$). In films with a soft structure, the TS is reduced while the %E increases, and vice versa (Gennadios and others 1993). This effect is also seen in mucilage films. The %E presented a contrary effect to TS; its value was higher at pHs of 4 and 8 in MFCa (37% and 29%, respectively); whereas the values of %E at pH 5.6 and 7 were lower (14% and 23%, respectively). At pH 5.6, the

values of %E of the MF and MFCa films showed no significant difference ($P < 0.05$) (Figure 3).

Table 3 shows that mucilage films have a higher %E values than alginate-calcium films (Olivas and Barbosa-Cánovas 2008), alginate (Rhim 2004), and highly carboxymethylated starch films (HCMS) (Kim and others 2002). The %E of mucilage films was similar to some β-lactoglobulin films (Sothornvit and Krochta 2001), and lower than that of protein films, such as wheat gluten (Micard and others 2000) and peanut protein (Liu and others 2004).

Table 3–Comparison of mechanical properties of some films equilibrated at 50% relative humidity.

Edible film	Glycerol[a]	TS (MPa)	%E	M. W.[b] (Da)	Reference (Film/M.W.)
Mucilage	0.50	0.4 to 0.95	14.99	2.4×10^2 to 3.4×10^5	Present study
Mucilage-calcium	0.50	0.30 to 0.58	24.06	2.4×10^2 to 3.4×10^5	Present study
Wheat gluten	0.36	0.70 to 4.40	156.00 to 259.00	7.0×10^4 to 1.3×0^5	Gennadios and others (1993) Anjum and others (2007)
Soy protein isolate	0.60	1.30 to 3.60	34.20 to 187.00	1.2×10^4 to 1.8×10^4	Gennadios and others (1993) Cho and others (2008)
Whey protein isolate	0.43	13.90	30.80	1.43×10^4 to 1.6×10^5	McHugh and Krochta (1994b) Walstra and others (2006)
Alginate	0.43	27.00	4.86	3.3×10^4 to 2.2×10^5	Parris and others (1995) Kong and Mooney (2002)
Egg albumin	0.50	1.26	32.20	4.27×10^4	Gennadios and others (1996) Liu and others (2007)
Peanut protein	0.50	0.55 to 1.27	98.26 to 140.73	3 to 5×10^3	Liu and others (2004) Hwang and others (2010)
Wheat gluten[c]	0.24	2.40 to 7.30	170 to 386	7.0×10^4 to 1.3×0^5	Micard and others (2000)
β-lactoglobulin	—	2.70 to 10.60	24.70 to 65.80	1.93×10^4	Sothornvit and Krochta (2001) Walstra and others (2006)
HCMS[d]	0.30	9.70	7.70	5×10^5 to 1×10^6	Kim and others (2002) Wang and others (1998)
Alginate-calcium	0.50	85.90	3.80	3.3×10^4 to 2.2×10^5	Rhim (2004)
Alginate-calcium	0.40	64.70	2.88	3.3×10^4 to 2.2×10^5	Olivas and Barbosa-Cánovas (2008)

[a]Glycerol content per gram of main component of the film.
[b]Molecular weight.
[c]Equilibrated at 60% R.H.
[d]High carboxymethylated starch.

Results of TS and %E on mucilage films support the assumption that calcium forms intramolecular links that shrink and immobilize the mucilage's molecule (Trachtenberg and Mayer 1981), while in the absence of calcium a wider spread of the molecules, with greater contact zones between them, allows for the formation of a compact structure, stronger and better organized than in the presence of calcium. On

the other hand, the high proportion of mucilage with low molecular weight (Table 1) does not help the formation of a rigid 3-dimensional structure, resulting in low values of TS and relatively high %E.

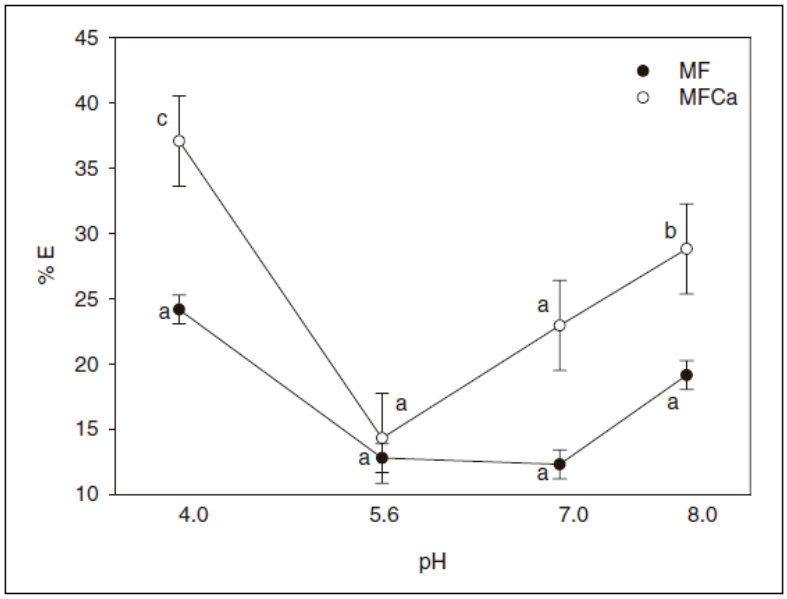

Figure 3–Effect of pH and calcium on the percentage elongation of edible films of mucilage from nopal *Opuntia ficus-indica*. MF = mucilage films without calcium, MFCa = mucilage films with calcium. Error bars represent 1 SD. Different letters indicate significant differences ($P < 0.05$).

Conclusions

Opuntia ficus-indica mucilage has the ability to form edible films at pHs between 4 and 8. Without plasticizer, films are rigid and very fragile, so it is necessary to add a plasticizer to improve the mechanical properties of the films. The possible formation of intramolecular links of calcium with carboxylic groups of the mucilage's molecule causes an adverse effect on the WVP, as well as on the TS, while improving the %E of the films. The elevated WVP of the mucilage films can be attributed to the hydrophilic properties of the mucilage. In general, it can be considered that the films with the best

water vapor barrier properties and tensile strength are obtained from mucilage without modification of pH and without the addition of calcium.

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