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PHYSICAL AND CHEMICAL CHARACTERIZATION OF A BIOPOLYMER FILM MADE WITH CORN STARCH AND NOPAL XOCONOSTLE (*Opuntia joconsotle*) MUCILAGE

CARACTERIZACIÓN FÍSICA Y QUÍMICA DE UNA PELÍCULA BIOPOLIMÉRICA HECHA CON ALMIDÓN DE MAÍZ Y MUCÍLAGO DE NOPAL XOCONOSTLE (*Opuntia joconostle*)

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Abstract Two kinds of biopolymer films were made from mucilage nopal xoconostle (*Opuntia joconostle*), corn starch, polyvinyl alcohol, chitosan and glycerol, which were prepared by the casting/solvent evaporation method. A comparison between the formed films using the mucilage extract and the direct addition of mucilage to the polymer matrix was discussed. The FTIR analysis revealed that both biofilms possessed a similar chemical structure. The thermal behaviour of the two kinds of films resulted very similar with an initial thermal decomposition temperature around at 156 °C. Optical and SEM microscopies showed that the total solids present in the directly-added mucilage formed microphases in the bulk of the films. The mechanical properties disclosed that the higher the content of plasticisers the lower the elastic modulus and hardness values when mucilage was added directly.

Keywords: mucilage, *Opuntia joconostle*, biopolymer films, DSC-TGA, FT-IR, nanoindentation.

Resumen

Se elaboraron dos diferentes películas con mezcla de mucilago de nopal xoconostle (Opuntia joconostle), almidón de maíz, alcohol poli vinílico, quitosan y glicerol, preparadas por el método de fundición y evaporación de solvente. Se compararon las películas formadas utilizando el extracto de mucílago y la adición directa de mucílago a la matriz polimérica. El análisis FTIR reveló que ambas biopelículas poseían una estructura química similar. El comportamiento térmico de los dos tipos de películas, mostró similitud en las temperaturas de descomposición térmica inicial (156 ° C). Las microscopía óptica y SEM mostraron que los sólidos totales presentes en el mucílago añadido directamente-forman microfases en la mayor parte de las película, pero estas no afectan en su estructura. Las propiedades mecánicas evidencian que cuando se utiliza concentraciones altas de plastificante es menor el módulo de elasticidad y dureza cuando fue añadido el mucílago directamente.

Palabras clave: mucilago, *Opuntia joconostle*, biopelículas, DSC-TGS, FT-IR, nano indentación.

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1 Introduction

Recently, biofilms have drawn increasing interest mainly due to the concerns regarding the disposal of conventional synthetic plastic materials derived from petroleum products (Romero-Bastida *et al*., 2011). Degradation of petroleum plastics requires a long time, and most of these materials end up overburdening landfills. However, bio-films and bioplastics generated from renewable agricultural products are readily degraded after their disposal (Matsumoto & Taguchi, 2013; Methacanon *et al*., 2014). Among these products, mucilage-composed films are found, which are mainly extracted from *Opuntia ficus* indica nopal (Del-Valle *et al*., 2005; Espino-Díaz *et al*., 2010). Another cactus variety with high mucilage content is *O. joconostle*, which has not been used to date as an extraction source to make biopolymers for packaging purposes (Arulselvan *et al*., 2014; Morales *et al*., 2014). The composition of mucilage cladodes (nopales) includes polymers of arabinose, rhamnose, galactose, xylose and uronic acids (Matsuhiro *et al*., 2006). With molecular weight that range from $2.3 \times 10^4 - 4.3 \times 10^6$ Da. The composition and rheological characteristics of Opuntia mucilage contribute to its potential use not only as edible films and degradable plastic biofilms (Aquino *et al*., 2009; Espino-Díaz *et al*., 2010) but also as both flexible (bags) and rigid packaging (thermoformed and blisters) (Zhao *et al*., 2008). Medina-Torres *et al*., (2003) improved the elasticity of film gels made from *Opuntia ficus* indica mucilage employing carrageenan mixtures. Del Valle *et al*., (2005) increased the shelf life of strawberries using an edible film composed of mucilage. Espino-Diaz *et al*., (2010), by the solvent casting method, developed biodegradable films that possess high tensile strength and good barrier properties balanced for water and vapour.

Starch is one of the biopolymers with greater versatility in food and industrial applications (especially from corn). It is used as a thickening agent, encapsulant, texturizing, and film formation and/or biodegradable packaging (Tirado-Gallegos *et al*., 2016) that possesses thermoplastic properties (Tharanathan 1995; Tharanathan & Saroja, 2001). The preponderance of amylose (>70%) in amylomaize starches results in stronger and more flexible films (Romero-Bastida *et al*., 2011). In contrast, the branched chemical structure of amylopectin generally leads to films with poor mechanical properties (i.e., decreased tensile strength and elongation) which are moderated using formulations with poly-vinyl alcohol (PVA), glycerine, sorbitol and nitrogenous bases (Tharanathan, 2003). Bertuzzi *et al*., (2007) found that the water vapour permeability of a starch film with high amylose content was dependent upon the film's thickness, temperature and the amount of used plasticiser. HanGuo *et al*., (2008), formed a biodegradable film from a cross-linked structure of starch, PVA and nano silicon dioxide; this formulation increased the water resistance and mechanical properties but retained its susceptibility to bacteria and fungi.

Chitosan is a natural polycationic polysaccharide derived from chitin which possesses antimicrobial activity and filmogenic properties, in addition to being biocompatible and biodegradable (Martínez-Camacho *et al*., 2010; Robles-Ozuna *et al*., 2007). The exact antimicrobial mechanism of chitosan still remain unclear, but several authors have provided some proposals. The most feasible hypothesis considers the linkage of cellular proteins and other intracellular constituents caused by the interaction between the positively charged chitosan and the negatively charged microbial cell membranes (Rabea *et al*., 2003). PVA is a water-soluble synthetic polymer that has been used in many biomaterial applications due to its characteristics of easy preparation, good biodegradability, excellent chemical resistance, and good mechanical properties (Park *et al*., 2001). Chitosan contains hydroxyl and amine groups and it is potentially miscible with PVA due to the formation of hydrogen bonds (Chuang *et al*., 1999). Moreover, chitosan can increase PVA's adhesiveness and resistance to oils, fats, solvents and tears (Martinez-Camacho *et al*., 2010). However, the use of a plasticiser such as glycerol is required due to the rigidity and brittleness of chitosan (Ueoka & Katayama, 2001).

The purpose of this study was to design, develop and characterize biopolymer films with a potential application as a bio-packaging materials from the incorporation of mucilage extract and direct mucilage incorporation in the films (without previous extraction). Films with different thicknesses were synthesized from the following compounds: mucilage (*O. joconostle* FAC Weber ex Diguet, cv cuaresmeño), corn starch, polyvinyl alcohol, chitosan and glycerol. Furthermore, the changes in micromechanical properties of the films were analysed using a nanoindentation technique.

2 Materials and methods

2.1 Biological materials and reagents

The mucilage was extracted from xoconostle nopal (*O. joconostle* FAC Weber ex Diguet, cv cuaresmeño) gauge E (CODEX STAN 185) from the Municipality of San Martin de las Pirámides, State of Mexico, vintage 2013. Food grade corn starch was obtained from Droguería Cosmopolita (L3TL006, México). Polyvinyl alcohol of 89000-98000 and 99% hydrolysed. Chitosan with a 75-85% degree of deacetylation were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were used without previous purification. Glycerol with 98% purity was acquired from Hycel (D.F., México). Hydrochloric acid (HCl) of analytical grade, 63.5%, was supplied by Analytica (Monterrey, México) and ethanol with 99.5% purity was supplied by Reasol (D.F., México).

2.2 Determination of the physical properties for the cactus cladodes

The pH of the ground cladodes was determined with a pH-meter (Hanna Instruments model HI3221 pH / ORP / ISE METER). The water content was measured using an analytical balance (Ohaus MB45) with a sensitivity of ± 0.001 g. The thickness was measured with a standard micrometer (Mitutoyo series 530) with a sensitivity of ± 1 μm, registering five measurements per sample.

2.3 Mucilage extraction

Mucilage was extracted from nopal using the Medina-Torres *et al*., (2003), method, which was modified by the pectin extraction technique (Methacanon *et al*., 2014). The extraction was carried out with mucilage: ethanol ratio of 1:5 (v/v). The ethanol pH was adjusted from 5.3 to 3.5 using concentrated HCl (63.5% analytical grade). The obtained precipitate was centrifuged (Thermo Sorvall Legend RT) at 3000 rpm and 4 °C for 15 min; the obtained product was labelled as 'extracted mucilage' (EM). For the second method, 1000 g of cladodes were grinded and filtered by gravity through nylon cloth, obtaining 595 ml, which were centrifuged at 3000 rpm at 4 °C for 15 min; the supernatant was stored at 4 °C. The product obtained through this procedure was labelled as 'direct mucilage' (DM). The total solids percentage in DM was obtained by the Bidwell-Sterling method (Nielsen 2010).

2.4 Determination of the esterification degree (DE)

The esterification degree of mucilage was determined by the National Formulary 25 USA method (2007).

2.5 Biopolymer films preparation

2.5.1. Casting solutions

The components were chosen based on their film-forming properties (Laycock & Halley, 2014; Park *et al*., 2001; Del-Valle *et al*., 2005). The biofilm components were prepared as follows: corn starch was used as the film matrix; 7.2 g of this polymer were dissolved in 60 ml of a 0.25N NaOH solution (12% w/v) under constant magnetic stirring at 70 °C for 1 min. PVA was dissolved up to 6.2% w/v in distilled water under constant magnetic stirring at 80 °C for 5 min. Mucilage extract (EM) was dissolved in distilled water up to 2.2% w/v, under constant stirring at 22 °C for 24 h, while 90 ml of DM were used directly once centrifuged. Chitosan was dissolved in a 0.1 M acetic acid solution up to 2% w/v, under constant stirring at 22 °C for 24 h. Glycerol (4.7 ml) was added directly to the total formulation (5.9 g density=1.261 g/ml).

The casting solution method and evaporation process began with the addition of the PVA (6.2%), EM (2.2%), chitosan (2%) and glycerol solutions over gelatinised starch. The mixture was stirred for 5 min at 70 °C and filtered while still hot through nylon cloth in order to remove the largest particles; finally, a filter with pore sizes of 1 μm was used. The filtered mixture was stored at 4 °C for 24 h. The content of total solids in the casting solution was: 6% w/v for EM and 7.5% w/v for DM.

2.5.2. Biopolymer film preparation

The biopolymer film was obtained by the casting/solvent evaporation method (Tripathi *et al*., 2010; Pinotti *et al*., 2007; Bertuzzi *et al*., 2007) using 25, 35 and 50 ml of the casting solution to obtain three different film thicknesses. The film was prepared with EM and DM; this solution was cast into glass Petri dishes of 9 cm in diameter, getting six films. Once the dishes were filled, they were placed in a drying chamber (Lab-line Barnstead, USA) at 35 °C for 48 h; after drying, the biopolymer films were kept in polyethylene bags in a desiccator at 22 ± 2 °C. The percentages in the EM films were determined based on dry weight, obtaining the following concentrations: starch (34%), PVA (13.6%), (9.5%),

Table 1. Content percentages for EM and DM films on dry weight.

Film	Components				
	Starch	PVA	Mucilage	Chitosan	Glycerol
% EM film	34	13.6	9.5	14.8	28.1
% DM film*	27	11	27.3	11.8	22.1
% DM film**	36.8	14.3	2.5	16	30.4

* Based on total content of solids, ** based on extraction yield

chitosan (14.8%) and glycerol (28.1%). For the DM films, the total content of solids in mucilage was 8%. Then, the dry-based percentages of the different components present in the DM films can be calculated as a percentage of total solids or based on extraction yield (0.56%), as shown in Table 1.

2.6 *Films characterization*

The films microstructures were observed under an Environmental Scanning Electron Microscope, SEM, (Philips ESEM XL 30, Japan) and an optical microscopy (Olympus model BX51, Japan). Therefore, it was necessary to fracture the samples after freezing them with liquid nitrogen and coating them with gold for 40 s. A thermogravimetric analysis, TGA, (TA Instruments STD Q600, USA), was used under a nitrogen gas atmosphere in a temperature range from 30 to 500 °C (rate 10 °C/min). A Differential Scanning Calorimetry, DSC, (TA Instruments Q100, USA), was used under a nitrogen gas atmosphere in a temperature range from 30 to 250 °C (rate 10 °C/min). For the infrared analysis, FT-IR, (VERTEX70 Bruker, USA) with a 4 cm^{-1} resolution was used in Attenuated Total Reflectance mode (ATR). The mechanical properties of the films were characterized at the micrometric level using a Nano-Hardness Tester, NHT, (CSM Instruments, Switzerland). The test conditions for the mechanical properties were as follows: maximum load: 5 mN and loading and unloading rates: 15 mN/min with a 20-s pause. One Berkovich-type diamond tip was used, and load vs. indentation depth plots were obtained and analyzed by the Oliver and Pharr, (2004) method, and 15 indentations per sample were made. Micromechanical properties such as hardness and elastic modulus were obtained.

2.7 *Statistical analysis*

Analysis of variance (ANOVA) was applied using Statgraphics centurion XVII (2015 Statpoint technologies, Inc. Warrenton, Virginia) to determine significance of differences between means.

3 Results and discussion

3.1 *Physical properties of cactus cladodes*

Water is the main component of cladodes and the content of it depend of the species, humidity, soil type, water availability, age and the plant, harvesting time Cladode hydration reaches up to 95% in natural conditions based on wet weight (Bravo and Sánchez, 1991).

In this study, the water content was 90% for fresh nopal xoconostle cladodes, which is similar to the range (88-91%) reported by Pimienta (1990), Sepúlveda *et al.*, (2007) and León-Martínez *et al.*, (2010). The pH value of grinded cladode mixture for the specie *O. joconostle* species was 5.1. It is well known that the environmental conditions affect the pH of nopal (Sáenz *et al.*, 2009). According to E gauge CODEX STAN 185, 1993; the cladodes used had a length between 25-30 cm, a thickness between 3.3-4.0 cm and weighted between of 865-900 g.

3.2 *Mucilage extraction*

The EM yield in mature cladodes was 0.56%. The amount of EM obtained was similar to that obtained by Cárdenas *et al.*, (2008), who reported a 0.6% yield following alkaline extraction. The yield extraction in the present work was considerably greater than that obtained from prickly pear peel (Forni *et al.*, 1994) but lower than that reported by Sepúlveda *et al.*, (2007), who obtained 1.2% for parenchyma of *Opuntia* spp. Saag, *et al.* (1975) studied other varieties of *Opuntia* using an extraction method as the one used in the present research, they obtained a 0.53% yield for *Opuntia monocantha* and 0.48% for *Opuntia nopalea cochinillifera*; these authors noted that the yield depended on the weather conditions, the age of crops, the harvesting time and the extraction method. In the present research, the modification made to the method developed by Medina-Torres *et al*., (2003) reached for a 10-fold decrease in ethanol consumption due to ethanol acidification which allowed to adjust the ratio to 1:0.5 mucilage/ethanol (v/v).

3.3 Determination of the esterification degree

The esterification degree (DE) for the extracted mucilage of *O. joconostle* was 64 ± 0.1%, which matched the value (65%) of the commercial citrus pectin (pectin 7001) (Morris *et al*., 2000). Hoagland & Parris (1996) reported that the formation of thermally irreversible gels was enhanced when the DE was greater than 55%. Values of methoxyl groups greater than 55% are considered as high degrees of esterification; accordingly, the higher the degree of esterification the higher the gelation temperature (Liu *et al*., 2010; Cai & Corke 2000).

3.4 Film characterization

3.4.1. Film microstructure

Several continuous, semi-transparent and flexible films with different thicknesses were obtained; from 25, 35 and 50 ml of the casting solution; for both EM and DM. The thicknesses for the DM films, were 176 ± 4.5, 256 ± 3.8 and 392 ± 4 μm; and for the EM films were 171 ± 4, 243 ± 4 and 368 ± 3.5 μm, being each value the average of 5 measurements. As expected, in both cases, the thickness was directly proportional to the used volume of the casting solution and the total content of solids present in the solutions (6% w/v for EM and 7.5% w/v for DM).

Surface optical micrographs obtained for EM and DM biofilms are shown in Fig. 1. The main difference between the films was the clearly present microphase separations obtained with DM. In contrast, with the EM films which had more continuous surfaces due to better integration of the components.

SEM photomicrographs of the cross sections and surfaces of films prepared with DM and EM are shown in Fig. 2. The observed morphologies in both cases, cross sections (a, b) and surfaces (c, d) showed no cracks or pores, resulting in dense films formation. Also the films micrographs show, smooth and continuous surfaces this can be attributed to the polymer-plasticizer (glycerol and water) interactions. One of the plasticizer functions is to reduce the intermolecular forces among polymer chains, increasing their mobility and thus film flexibility (Lin *et al*., 2000). The EM film cross section (a) is rougher than the DM film cross section (b), which can be explained by the fact that the EM film has more mucilage than the DM film (9.5% vs. 2.5%) (Bagliotti *et al*., 2014). Another important difference between the two films was that in the DM film appeared, microphase separations, which can be observed in both cross sections (Fig. 2b) and surfaces (Fig. 2d); neither obvious aggregation nor microphase separations were observed in the EM film, which is in agreement with those shown in Fig. 1.

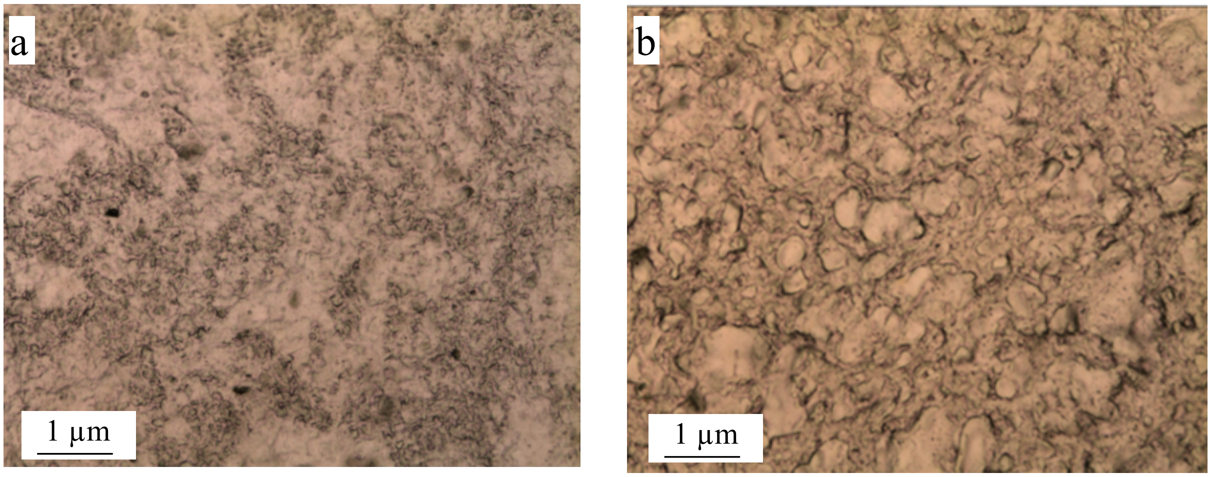

Fig. 1. Surface optical micrographs of EM (a) and DM (b) biofilms.

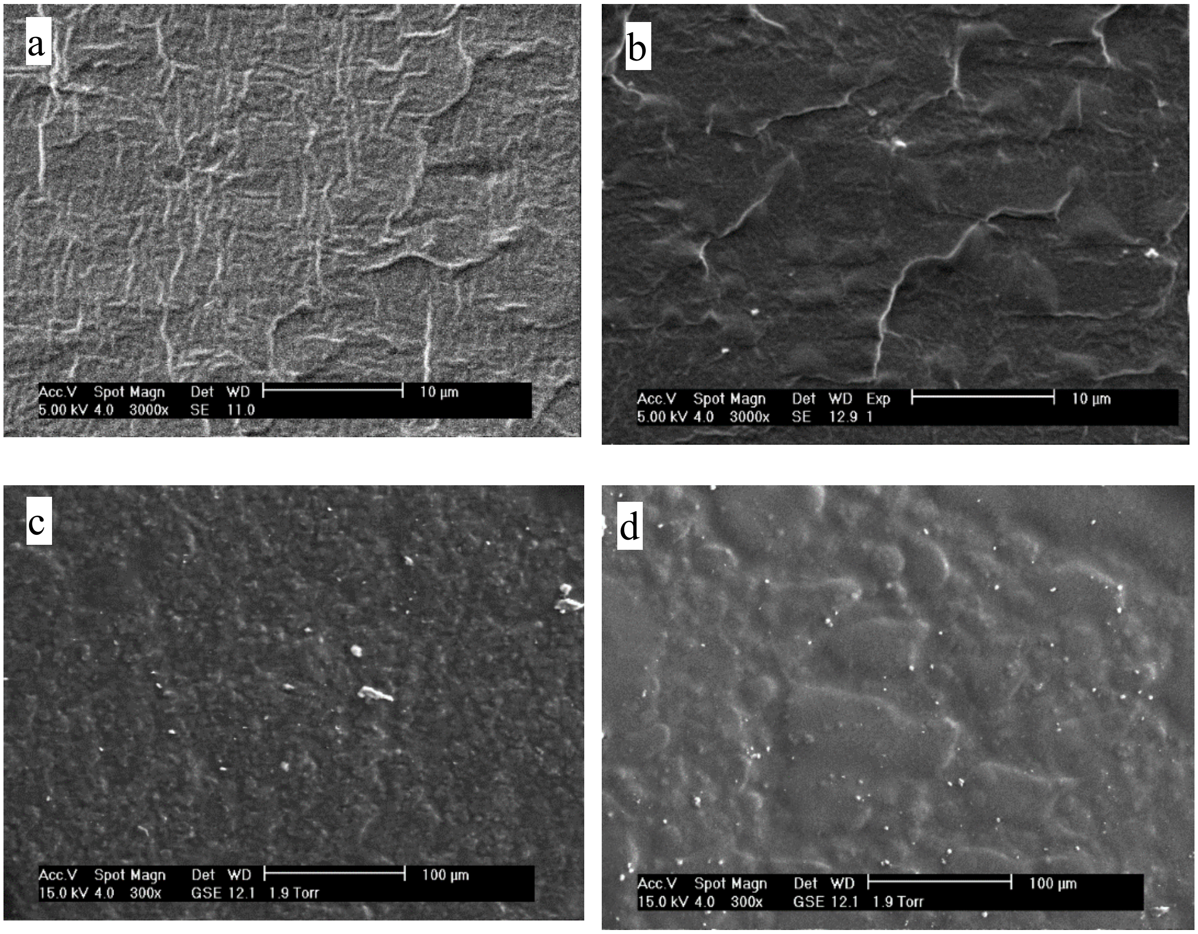

Fig. 2. SEM micrographs of the EM film: a) cross section, c) surface and DM film, b) transversal section, and d) surface.

This indicates that the dispersion of components in the EM film is more homogeneous and better than in the DM film, which increases the possibilities of component interactions, forming stronger bonds and adhesion on the interfaces among film constituents, although, at high temperature (around 70 °C), water and glycerol are known to physically break up the starch granules and disrupt the intermolecular and intramolecular hydrogen bonds; likewise, the presence of microphases in the DM film is due to the fact that it contains soluble and insoluble microfibers, mono and divalent salts, organic acids, among others. Better integration of the components, and therefore the absence of a microphase could result in films with better mechanical properties.

3.4.2. Thermal analysis

The TGA and DSC curves for starch, mucilage, PVA, chitosan and glycerol are shown in Fig. 3a, b, respectively. Mucilage, starch and chitosan presented two well-defined, weight loss stages (Fig. 3a), where the first stage corresponded to the water removal content in the following percentages: 14, 6.4 and 7.5%, respectively, although PVA and glycerol had a minimal water content <1.5%. The second stage corresponded to the decomposition process, where the initial temperature decomposition (Tin) for starch, chitosan, mucilage and PVA were relatively close, between 272 and 235 °C (Table 2). Tin of glycerol is at 158 °C and corresponded to the evaporation.

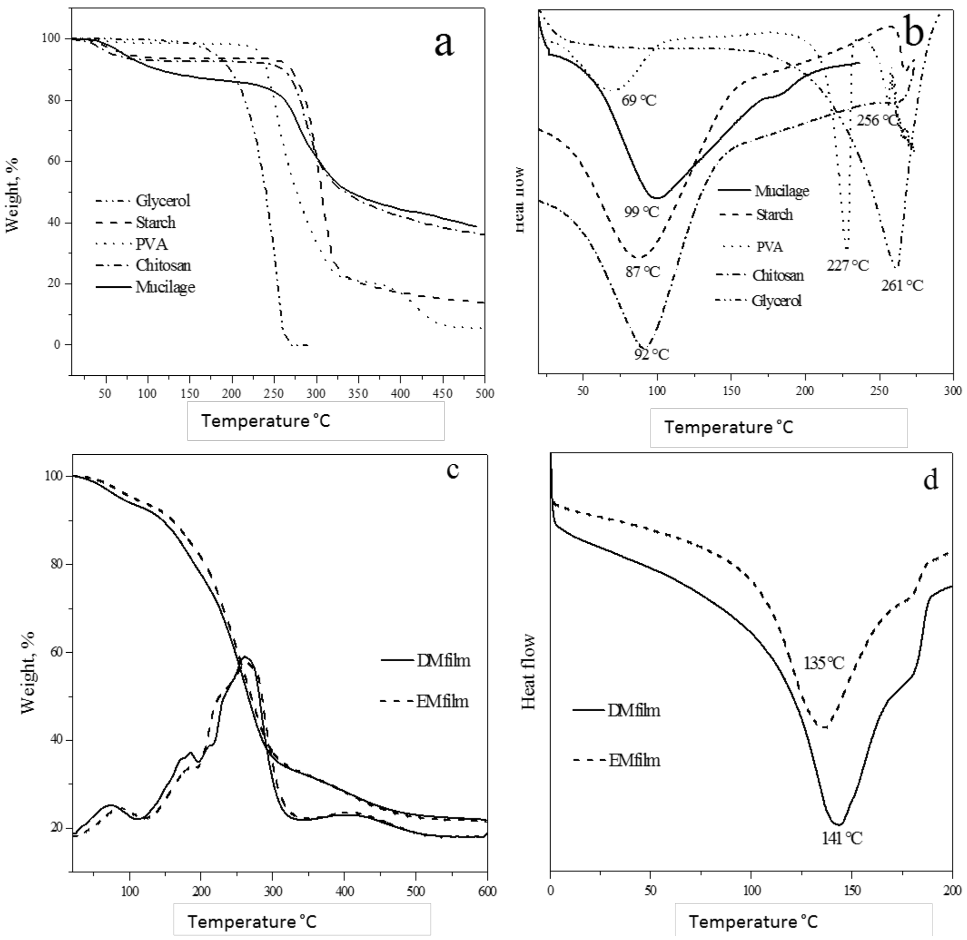

Fig. 3. TGA (a) and DSC (b) analysis of individual components, and TGA (c) and DSC (d) analysis of biofilms.

Table 2. Thermophysical characteristics of individual biofilm components.

Component	Tin, °C	Water, %	Residual weight at 500 °C, %	1st Peak DSC, °C	2nd Peak DSC, °C	3rd Peak DSC, °C
Starch	272	6.4	14	87	-	-
Chitosan	265	7.5	36	92	-	-
Mucilage	257	14	38	99	-	-
PVA	235	1.5	5	69	227	256
Glycerol	158	1	0	261	-	-

The DSC curves for mucilage, starch and chitosan presented a broad peak at around 100 °C, which is mainly related to water evaporation. The corn starch used as the film matrix is type ae du with 50% amylose and high moisture content, this is because it showed a broad signal (Matveev *et al*., 2001) (Fig. 3b). For nopal xoconostle mucilage (Fig. 3b), the glass transition temperature (Tg) was observed at 28 °C. In the mucilage of *O. joconostle* species, the Tg was lower than that found in the *Opuntia ficus indica* species, which was reported as 45 °C (León-Martínez *et al*., 2010) this is probably due to increased hygroscopicity; the compounds with lower molecular weights feature shorter chains that make them more hydrophilic groups (Cai & Corke, 2000). Chitosan (Fig. 3b) showed an endothermic signal at 92 °C due to the elimination of water vapour in the polymeric matrix. For the PVA homopolymer, the DSC displayed an endothermic glass transition temperature (Tg) at 69 °C (Fig. 3b). Another sharp and endothermic transition was observed at 227 °C, which corresponds to melting temperature (Tm). Tg and Tm values are in agreement with those reported in other studies (El-Sayed *et al*., 2011). Finally, the endothermic peak at 256 °C corresponds to the PVA thermal degradation. Glycerol (Fig. 3b) only showed a signal at 261°C which corresponds to the evaporation temperature.

The TGA and DSC curves for the EM film and the DM films are shown in Fig. 3c and d respectively. Fig. 3c shows that the thermal behaviour of the two formed biofilms was very similar, for both the weight loss curve and its derivatives. The weight loss took place in three stages; the first stage corresponded to water loss, 5.3% for the EM film and 6.6% for the DM film and the derivative curve showed; the maximum loss at 73 and 87 °C, respectively. The second stage corresponded to the output of the glycerol polymer matrix at 158 °C, this was observed clearly in the derivative curve, presenting a maximum signal at 184 °C. Finally, the third stage corresponded to the degradation process with an initial temperature of degradation at 156 °C for the EM film and at 152 °C for the DM film (Table 3). The DSC thermograms in Fig. 3d show a broad main peak at 135 °C for the EM film and at 141 °C for the DM film, these endothermic absorptions can be related to water and glycerol evaporation from the polymer matrix because their signal were very wide. The DSC presents a shoulder in the broad main peak at 184 °C and this is in accordance with the second peak seen in the derivative curve (at 184 °C), which corresponds to the decomposition of polymeric biofilms. Residual weight at 600 °C in both cases was 21%; this could mean that the content of inorganic compounds was negligible and that most of the solids percentage present in the directly added mucilage (8% w/v) in the DM film corresponded to organic materials. The main conclusion about the thermal behaviour of the two kinds of biofilms in the present study is that the use of direct mucilage or extracted mucilage to produce the biofilms has very subtle thermal differences.

3.4.3. FT-IR analysis

The FT-IR spectra of the final biopolymeric films and the three previous components additions are shown in Fig. 4. The main spectrum absorptions in the first (1st: starch and PVA), second (2nd: starch, PVA and mucilage), third (3rd: starch, PVA, mucilage and chitosan) and fourth (4th: starch, PVA, mucilage, chitosan and glycerol) component additions are listed in Table 4. The main difference among the 1st and 2nd and 3rd and 4th spectra was the presence, in these last two spectra, of well-defined and very strong absorptions at 1566 cm^{-1}, which correspond to the stretching vibration of C-N in combination with the N-H bending known as amide II, which is due to the addition of chitosan. Because of this component, it is also possible to observe an amide I absorption shoulder at 1643 cm^{-1} that corresponds to the C=O stretching. In the 1st and 2nd spectra, absorption at 1652 cm^{-1}appears, which could be attributed to the bending mode of the water molecules contained in the film (Velazquez *et al*., 2003; Detduangchan *et al*., 2014).

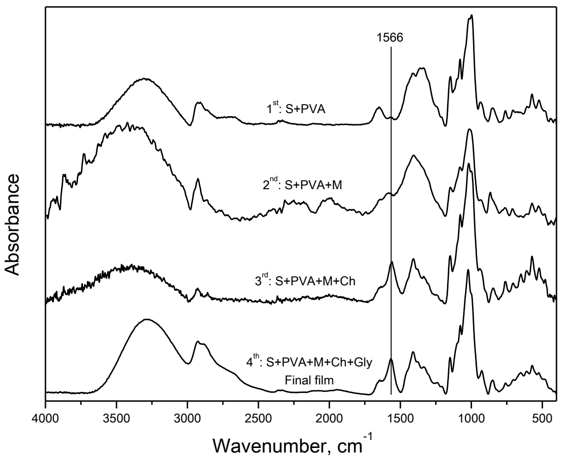

Fig. 4. FT-IR spectra of the addition components: starch (S), polyvinyl alcohol (PVA), mucilage (M), chitosan (Ch) and glycerol (Gly) in biofilm formation.

Table 3: Thermophysical properties of biofilms.

Film	Tin, °C	Water, %	1st peak deriv., °C	2nd peak deriv., °C	3rd peak deriv., °C	Residual weight at 600 °C, %	DSC peak, °C
EM	156	5.3	73	184	262	21	135
DM	152	6.6	87	184	262	21	141

Table 4. FT-IR absorption changes after adding the components for biofilm formation.

Mixtures according to Fig. 4				Vibration Type	Bond	Functional group
1st	2nd	3rd	4th			
754	754	759	765	Stretching	C-O-C	Ether Skeletal vibration
852	866	844	845	Deformation	$-CH_2-$	Methylene groups
931	929	931	925	Deformation	-OH out of plane	Carboxylic acids
1010	1009	1019	1025	Stretching	C-O-C	Ether Skeletal vibration
1078	1084	1077	1078	Stretching	C-O	Alcohols
1145	1145	1147	1149	Stretching	C-O-C	Ether Skeletal vibration
1409	1401	1412	1412	Stretching	-OH, C-O	Alcohols
		1556	1566	Stretching, bending	C-N, N-H	Amide II
1652	1652	1643	1643	Bending Stretching	-O-H C=O	H_2O Amide 1
2929	2929	2929	2927	Asymmetric stretching	$-CH_2-$	Methylene groups
3298	3392	3394	3286	Stretching -OH, N-H	Alcohols, secondary amide	and carboxylic acids

(Galicia-García *et al*., 2011; Xu *et al*., 2005; Hu, *et al*., 2013; Cárdenas *et al*., 2008; Yao, Cai *et al*., 2011; Martínez-Camacho *et al*., 2010; Tripathi *et al*., 2010)

Other common absorptions in the spectra that are characteristic of the components in the films were present: a strong and broad absorption centred between 3386-3392 cm^{-1} appertaining to the stretching vibration of -OH, N-H and intermolecular bonds of polysaccharides (Krishnapriya & Kandaswamy, 2010); the peak in 2929 cm^{-1} is due to the stretching of the C-H bond; the absorption between 1409-1412 cm^{-1} correspond to -OH vibrations, and the one between 1010-1025 cm^{-1} was assigned to the skeletal vibration involving the C-O stretching (Almeida *et al*., 2010). For two films, the EM and DM-mucilage had the same number of peaks and the position and relatively intensities of these peaks were identical.

3.4.4. Nanoindentation analysis

The elastic modulus (E) and hardness (H) values of the EM film and the DM film were calculated from the load-displacement curves and they were as follows: E= 0.20 ± 0.09 GPa and H= 19.1 ± 1.0 MPa for the DM film, and E= 0.22 ± 0.02 GPa and 22.7 ± 0.9 MPa for the EM film. Both values, E and H, in the EM film were higher than in the DM film, which can be related to the plasticizers quantity (glycerol and water) present in the components matrix of the final films. On the one hand, Table 1 shows the effective amount of glycerol in the DM film (30.4%) against the one in the ED film (28.1%), on the other hand, Table 3 shows that the water content in the DM film (6.6%) was greater than in the EM film (5.3%). Therefore, the films with fewer plasticizers were stiffer and less flexible. The increment of flexibility in the presence of plasticizers in hydrophilic films has been reported previously (Galdeano *et al*., 2009). These results confirm that the highest amount of solids (8%) present in the mucilage added to the DM films corresponds to soft materials (organic materials).

Conclusions

In this study, two kinds of films were obtained from mucilage extract and directly incorporated mucilage from fresh and mature cladodes (Category E) of nopal xoconostle (*O. joconostle*) present in the matrix of the films. The films chemical structure, morphology, thermal behaviour and mechanical properties (nanoindentation) were characterized. The dense films obtained from a high-degree-of-esterification-extracted mucilage and the direct incorporation of mucilage presented very similar signals and characteristic absorptions in infrared spectra. The thermal analysis confirmed the formation of thermally stable films, for both cases, up to 156 °C. The optical and SEM microscopies revealed the presence of other minor total solids components in the mucilage added directly in the matrix film, forming a less continuous structure compared to when the extract mucilage was used. The mechanical properties, elastic modulus and hardness disclosed that the concentration of plasticizers affect both properties. In the present study, the DM-mucilage, which had the highest concentration of glycerol and water, had slightly smaller values in both properties. This study shows the feasibility of using mucilage directly without significantly losing thermal and mechanical properties. Moreover, it avoids using large amounts of solvent for the extractions.

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