

PHYSICAL EVALUATION OF BIODEGRADABLE FILMS OF CALCIUM ALGINATE PLASTICIZED WITH POLYOLS

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Abstract - The influence of different polyols as plasticizers of alginate films on their physical attributes like moisture content, soluble mass in water, water uptake, water vapor permeability, opacity and mechanical properties were determined and the results discussed based on scanning electron microscopy observations and glass transition temperature. The alginate films were obtained by casting, using three different gramatures. Calcium crosslinked and non-reticulated films were considered. The films plasticized with glycerol and xylitol were more hygroscopic than the films with mannitol. The lowest water vapor permeability values were found for films plasticized with mannitol, at all studied thicknesses. The films plasticized with glycerol and xylitol showed very similar functional attributes regarding their application as food wrappings. The Ca²⁺ crosslinked mannitol films showed the highest tensile strength at rupture (>140 MPa).

Keywords: Biofilm; Alginate; Plasticizers; Xylitol; Mannitol.

INTRODUCTION

There is a growing awareness about environmental issues raised by the disposal of traditional synthetic plastics, which are still in use as packaging materials. Manufacturers are now encouraged to use biodegradable materials to replace them, while consumers are seeking better food quality with longer shelf life, conscious of the toxic or harmful consequences of the disposal of product residues in the environment.

Among the several materials investigated for the production of biodegradable films, alginate is an unique polysaccharide, due to its thermostable and tailor-made rheological and gelation characteristics. Sodium alginate is a linear polysaccharide extracted from brown algae and is composed of residues of β -D-mannuronic (M) acids and α -L-guluronic (G) acid,

joined by glycosidic (1-4) bonds and distributed in different proportions along the chain. Characteristics such as gelling and thickening action, biodegradability, biocompatibility and the absence of toxicity make sodium alginate an interesting material for a number of applications in food, pharmaceutical, textile or biomedical industries and in industrial waste treatment (Mancini *et al.*, 1996).

Crosslinking of the polymeric structure with calcium considerably reduces the solubility in water of alginate films, as well as their flexibility (Zactiti and Kieckbusch, 2009). Plasticizers are added to these films in order to depress the glass transition temperature, T_g , and therefore obtain a reduction in brittleness and an increase in flexibility, toughness, traction and impact resistance.

The choice of plasticizers to be added to the film-forming solution depends on their compatibility with

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the polymer and the solvent, i.e., they should be miscible with the polymer and in the solvent, so as to avoid premature separation during the drying process. The concentration of plasticizer used in the film preparation usually ranges from 10 to 60 g/100 g of polymer dry matter, depending on the desired flexibility of the film (Guilbert *et al.*, 1996). The more effective plasticizers usually have a molecular structure close to that of the polymer that they have to plasticize. In the case of carbohydrates the most widely used plasticizers are polyols (especially sorbitol or glycerol), because they reduce the hydrogen bonds between the chains and thus increase the free molecular volume. Other polyols like monosaccharides such as fructose, disaccharides or polyethylene glycols (PEG), are also examples of plasticizers.

The relative effectiveness of different polyols may be attributed to their different abilities to associate with water, since water also functions as a potent plasticizer of carbohydrates and proteins (Roos, 1998).

The increase in free molecular volume that the plasticizer promotes in the macromolecular structure of the film, apart from enhancing flexibility, also increases permeability to gases, vapors and added solutes (antimicrobials, drugs). Since the permeability depends on the diffusivity in the polymeric matrix (affected by the crosslinking degree and the molecular volume of the diffusing solute) and on the interfacial equilibrium solubility (affected by the moisture content), less hydrophilic plasticizers should be preferred if the objective is the reduction of water vapor permeation.

An additional problem with very soluble plasticizers is the loss by leaching when exposed to aqueous solutions during manufacturing. This problem has received little attention, but can result in films with inadequate stability characteristics (Gruetzmann and Wagner, 2005). Among the plasticizers used in edible alginate films, glycerol has proved to be the most practical and efficient one. Glycerol is abundant and of low cost, but is highly hygroscopic and infinitely soluble in water.

Table 1 compares the solubility concentration in water of simple polyols (glycerol, xylitol and mannitol) as well as their hygroscopicity, measured as water uptake in equilibrium with a 75% RH ambient. The high hygroscopicity of glycerol justifies its choice as plasticizer in carbohydrate-based structures, but its solubility in water limits the use of the films in humid ambients.

This investigation proposes to evaluate the behavior of xylitol and mannitol as plasticizers for calcium alginate films. The moisture content, water-soluble matter, water uptake degree, water vapor

permeability, mechanical resistance like strength and elongation at break, glass transition temperature, morphology, color and opacity were determined and the values found were compared to films with glycerol, seeking a compromise between flexibility and stability.

Table 1: Hydrophilic properties of selected polyols.

Polyol	Equilibrium moisture content, % (35 °C, 75% RH) ¹	Solubility concentration (%)
Glycerol	51.5	∞
Xylitol	35.9	63 (20 °C) ²
Mannitol	~0	22 (25 °C) ³

1) Cohen *et al.* (1993); 2) Bär (1991); 3) Griffin and Lynch (1980).

MATERIALS AND METHODS

Raw Materials

The films were manufactured with sodium alginate obtained from *Macrocystis Pyrifera* algae (Sigma, USA), calcium chloride dihydrate (Merck, Germany) as crosslinking agent and the plasticizers glycerol (Synth, Brazil), xylitol (Acros, USA) and mannitol (Getec, Brazil).

Film Preparation

The preparation of the films followed a two-stage process as shown in Figure 1.

In the 1st Stage a low crosslinked pre-film was formed. Sodium alginate and the polyol plasticizers (glycerol, xylitol and mannitol) were dissolved in distilled water, under constant magnetic stirring. After complete solubilization, 30 mL of the cross-linking solution was added, and the temperature raised to 70 °C. Due to the intense reaction with Ca²⁺, the cross-linking solution had to be added slowly to the alginate solution, through a peristaltic pump (Masterflex 77120-70, USA) with a maximum flow rate of 0.6 mL/minute, to avoid local gelation. Films were obtained by the casting technique, i.e., aliquots of 40, 50 or 60 g of the hot solution were poured into square Plexiglas pans (area = 225 cm²) and slowly dried at 40 °C in an oven with air recirculation (FANEM, model 099EV, Brazil) for 18 to 20 hours. The dried films were removed from the support and stored at 52% RH and 25 °C for 48 hours.

In the 2nd Stage, the films were subjected to a complementary crosslinking process by immersion in 50 mL of an aqueous solution of calcium chloride (3%) and plasticizer (3%), for a period of 15 minutes, at room temperature. The films were removed from

the bath, drained and spread over Plexiglas plates, with the edges fixed by stainless steel tube frames to prevent wrinkling and subjected to a parallel air flow at a temperature of 30 °C and RH above 60% for 4 to 5 hours.

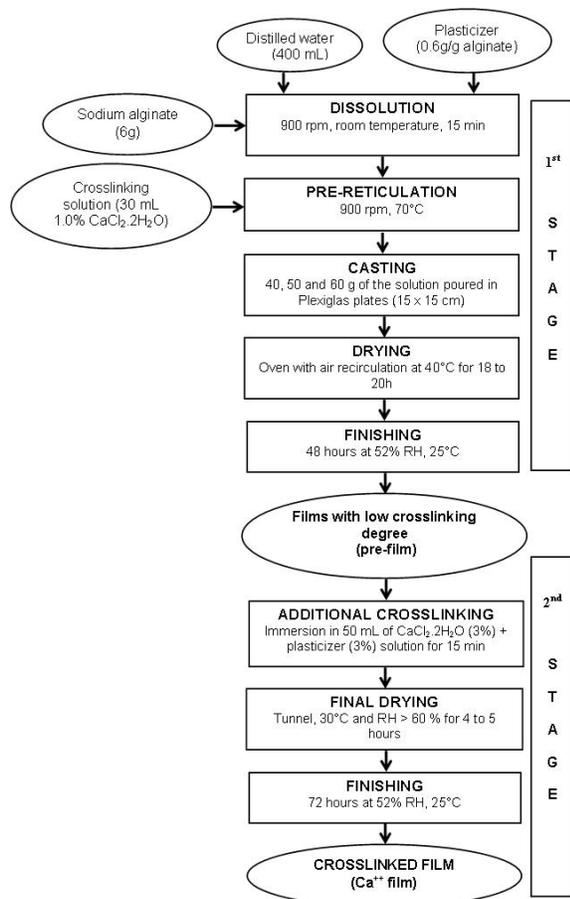


Figure 1: Flowchart for alginate film preparation (adapted from Zactiti and Kieckbusch, 2006).

All films were equilibrated at 52% relative humidity for 3 days using desiccators containing a saturated solution of $Mg(NO_3)_2$ at ambient temperature before the physical characterizations. According to the plasticizer used, glycerol, xylitol or mannitol, the films were denominated G-film, X-film or M-film, respectively.

Film Characterization

Thickness

The film thickness (δ) was determined with a digital micrometer with 0.001 mm resolution (MITUTOYO, model MDC-25S, Japan). The final thickness was calculated as the arithmetic average of

fifteen random measurements along the 8 cm disc for the water vapor permeability tests, and the mean of 10 random measurements along the 10 x 2.5 cm strip for the mechanical tests.

Moisture Content

Moisture content was determined gravimetrically using a vacuum oven (Lab-Line, Squaroid, USA) at 105 °C for 24 h according to Rhim *et al.* (2002). Moisture content was determined in quintuplicate for each film formulation and expressed as mass fraction (ω) or as percentage of total mass (100ω).

Solubilized Matter in Water (S)

The mass solubilized in water (S) was determined in quintuplicate as proposed by Irissin-Mangata *et al.* (2001). The mass (m_i) of a piece of film was determined and then immersed in 50 mL of distilled water under mild shaking action (175 rpm) at 25 °C for 24 h, in a controlled temperature water bath (*Shaker Bath Orbit*, Lab-Line, USA). After this treatment the sample was transferred to an oven (105 °C, 10 h) to determine the final dry matter, m_f . The soluble matter was expressed as a function of the initial dry matter. The values were obtained with Equation (1):

$$S = \left\{ \left[m_i (1 - \omega) - m_f \right] / \left[m_i (1 - \omega) \right] \right\} \times 100 \quad (1)$$

Water Vapor Permeability

Water vapor permeability (WVP) was gravimetrically determined at 25 °C, according to the E95-96 method (ASTM, 1995a) using a small Plexiglas cell (25 mL) completely filled with calcium chloride granules (Ecibra, Brazil) in order to maintain a 0% RH environment. The film was tightly fitted to the removable cell lid, covering a central circular opening (46.24 cm²). The cell was placed inside another hermetic Plexiglas jar, (500 mL), containing a saturated NaCl solution (Synth, Brazil) at its bottom, under intermittent agitation to keep the microenvironment at 75% RH, so as to maintain a constant difference in water vapor pressure. The rate of increase in total mass of the cell, which was monitored throughout a period of at least 72 hours, corresponds to the rate of water permeation through the film (G). Equation (2) was used to calculate the water vapor permeability (WVP):

$$WVP = \left[G\delta / A_e \Delta P_\omega \right] F \quad (2)$$

where, WVP is the water vapor permeability of the film [(g.mm)/(m².day.kPa)]; δ is the thickness of the film (mm); A_e is the area of the exposed surface of the film (m²); ΔP_w is the water partial pressure difference across the film, (kPa); G is the permeation rate (g/day) calculated by linear regression of the mass gain vs. time; F is a correction factor that takes the additional diffusion resistance in the stagnant air gap between the surface of the calcium chloride layer and the film into account. In the geometry used, this correction was close to 1.0 and therefore was not considered (McHugh *et al.*, 1993).

Water Uptake Degree

The swelling of the films are measured as Water Uptake degree (WU) and determined according to the methodology proposed by Xu *et al.* (2003). The initial mass of a film sample was measured and the sample immersed in distilled water at 25 °C. After pre-determined periods of time, the film was removed, lightly compressed between filter papers (to remove the excess surface moisture), then weighed and returned to the water bath. The water uptake (WU) was calculated as a function of the initial total mass of the sample.

Mechanical Characteristics

The mechanical resistance properties of the films were determined with a texturometer TA.XT2 (SMD Stable Microsystems, England), in an environment at a temperature of 25 ± 1 °C and relative humidity of 55 ± 3%, following the standard method D-882 of ASTM (1995b). The initial grip separation was 50 mm and the speed of the test was 1.0 cm/s. At least twelve samples of film strip measuring 10 x 2.5 cm were tested. The tensile strength, TS, at rupture (maximum strength at the moment of rupture divided by the initial transversal sectional area of the film) and the elongation at break, E, (percentage in relation to the initial length) were directly determined from the stress-strain curves by the Stable Microsystems software *Texture Expert V.1.15*.

Morphology

The surface and the cross-sectional (after fracture) morphologies of the films were observed by scanning electron microscopy (SEM). Films were dried in a vacuum oven (Lab-Line Squaroid, USA) at 70 °C for 24 h and samples were mounted onto stubs, sputter coated with gold in a vacuum chamber and photographed using a scanning electron microscope (LEICA, model DMLM Q500IW, USA).

Opacity

The opacity in the transmittance mode (Y) of the films was determined with a Color Quest II colorimeter (Hunterlab, Reston, USA), according to Hunterlab (2008). The determinations were made in triplicate after calibration of the colorimeter with standard white and standard black backgrounds. The opacity was calculated automatically through the Universal Software 3.2 (Hunter Associates Laboratory), using Equation (3).

$$Y = \left(\frac{Y_p}{Y_b} \right) \times 100 \quad (3)$$

where Y_p is the opacity of the film placed against a black background and Y_b is the opacity of the film placed against a white background.

Glass Transition Temperature

Glass transition temperatures (T_g) of the films were determined using a Dynamic Mechanical Analyser 2980 (TA Instruments, USA) in the tension mode. Film samples (4 x 1 cm rectangles) were subjected to continuous heating (2 °C/min) from -70 to 105 °C at a constant frequency (1 Hz). The oscillation amplitude was 10 μm and the initial force was 0.5N. Storage modulus (E'), loss modulus (E'') and damping coefficient ($\tan \delta = E''/E'$) were determined. The glass transition temperature was defined as the temperature associated with the peak of the $\tan \delta$ curve (Cherian *et al.*, 1995).

Statistical Analysis

All regressions were performed using Statistica V8 Software, with the Quasi-Newton method. The Analysis of Variance and the Tukey Test were used to determine statistically significant differences ($p < 0.05$) between averages.

RESULTS AND DISCUSSION

Pre-Films

The films obtained in the 1st Stage, using glycerol and xylitol as plasticizers were transparent, homogeneous, continuous and flexible and could easily be removed from the Plexiglas mold.

The pre-films, prepared with the plasticizer mannitol, showed a whitish and volumous appearance, felt rough and “dry” to the touch, similar to a sheet

of kraft paper. SEM micrography (see Figure 3(f)) confirmed the existence of intense crystal formation throughout the structure so that, at first glance, it should have been desconsidered as a potential matrix for a packaging film. After immersion in the cross-linking solution (2nd Stage), however, the films with this formulation became transparent, homogeneous, continuous and uniform.

The values obtained for moisture content, matter soluble in water, thickness, water vapor permeability, tensile strength and elongation at break of the low reticulated alginate films obtained using different hydrophilic plasticizers are shown in Table 2.

As expected, the thickness given in Table 2 increased with increasing mass of solution poured on the plate. For the transparent films (glycerol and xylitol), however, a 50% increase in mass doubled the film thickness, signaling a less dense microstructure for the thicker films. This trend correlates well with the moisture content data which also show an increase with thicker films and which, although not statistically significant, is larger for the more hygroscopic glycerol containing film than for xylitol. The combined water + polyol content exerts a strong plasticizing effect and the rather rubbery material slowed down the final drying of the thicker films (da Silva *et al.*, 2012). M-films were thicker and showed similar moisture contents, a result that cannot be explained by the low hydrophilic nature of this polyol, and should be attributed to the two-phase system formed.

The low-reticulated alginate films plasticized with glycerol, xylitol and mannitol were completely disintegrated and solubilized after treatment by 24 hour immersion in distilled water. Uncrosslinked polysaccharides are usually highly hygroscopic and they solubilize rapidly and completely in water. Zactiti and Kieckbusch (2009), studying sodium

alginate films plasticized with glycerol, also obtained a complete solubilization of this kind of films after 24 hours of immersion in water.

The values of water vapor permeability (WVP) determined for the films with different plasticizers exhibit an inverse relationship with the polyol hygroscopicities (Table 1). This trend is aggravated by the low level of reticulation with Ca²⁺ of these pre-films. Complementarily, for each type of plasticizer, the WVP increases with film thickness. In ideal homogeneous polymeric films, the permeability constants are independent of thickness. Water transport through hydrophilic films, however, is extremely complex due to non-linear moisture sorption isotherms and water content-dependent diffusivity (Schwartzberg, 1985). In the present study, the interfacial equilibration with humid air promotes an incipient structure relaxation in the outer portion of the film cross section, increasing the permeation rate in this surface layer. The thicker film reduces the relative contribution of this layer to the total resistance, and the WVP increases (Bierhalz *et al.*, 2012).

The large standard deviations found for the mechanical parameter measurements indicate poor reproducibility, since the delicate pre-films were difficult to cut uniformly and to fix between the grips of the texturometer. Nevertheless, the tensile strength (TS) at break of glycerol containing films is consistently lower than xylitol and mannitol plasticized films. The essence of the plasticizer action is measured by its effect on elongation and films with higher TS are likely to display lower elongation, a combination difficult to validate with the data in Table 2, due to the large spread of the averages. The higher E values for the M-films can be misleading since they probably resulted from the effect of crystals in the structure, which are prone to promote premature rupture points.

Table 2: Thickness (δ), matter soluble in water (S), moisture content (100 ω), water vapor permeability (WVP), tensile strength (TS) and elongation at break (E) of calcium alginate pre-films (1st Stage) containing glycerol, xylitol or mannitol as plasticizers.

Plasticizer	FS [g]	δ [μ m]	S [%]	100 ω [%]	WVP [g.mm/m ² .day.kPa]	TS [MPa]	E [%]
Glycerol	40	17 \pm 1 ^a	100	14.9 \pm 1.1 ^a	6.8 \pm 0.1 ^c	33.7 \pm 5.0 ^a	13.4 \pm 1.6 ^b
	50	26 \pm 1 ^b	100	15.7 \pm 1.3 ^a	5.3 \pm 0.1 ^{bc}	33.6 \pm 4.6 ^a	13.6 \pm 1.4 ^b
	60	32 \pm 1 ^c	100	17.6 \pm 2.9 ^a	3.7 \pm 0.2 ^b	33.6 \pm 3.6 ^a	13.6 \pm 2.8 ^b
Xylitol	40	17 \pm 1 ^a	100	12.7 \pm 1.5 ^a	3.8 \pm 0.1 ^b	49.4 \pm 3.6 ^c	10.6 \pm 2.2 ^a
	50	30 \pm 2 ^c	100	14.0 \pm 1.2 ^a	3.7 \pm 0.1 ^b	45.4 \pm 3.0 ^b	13.7 \pm 2.4 ^b
	60	34 \pm 1 ^c	100	15.7 \pm 1.1 ^a	2.3 \pm 0.1 ^a	42.4 \pm 2.7 ^b	15.9 \pm 0.9 ^{bc}
Mannitol	40	33 \pm 2 ^c	100	13.2 \pm 2.8 ^a	2.3 \pm 0.3 ^a	47.7 \pm 4.0 ^b	13.2 \pm 2.7 ^b
	50	36 \pm 4 ^{cd}	100	16.8 \pm 1.8 ^a	2.1 \pm 0.3 ^a	46.6 \pm 2.3 ^b	15.1 \pm 2.8 ^{bc}
	60	46 \pm 3 ^d	100	17.7 \pm 3.5 ^a	1.9 \pm 0.2 ^a	46.0 \pm 2.1 ^b	17.3 \pm 2.8 ^c

FS is the mass of the film forming solution poured on each plate, in the 1st Stage; Averages with the same letter in each column indicate that there is no significant difference (p<0.05) by the Tukey Test.

Therefore, the values in Table 2 indicate that glycerol and xylitol have similar capacity of plastification, and xylitol has the advantage of a higher TS and lower WVP. The WVP and the TS values given in Table 2 are prevalent in polysaccharide based non-reticulated films and can be considered suitable to be used as food wrappings (Han, 2000). Protein films usually display poorer physical attributes. Shaw *et al.* (2002) for instance, working with whey protein isolate plasticized with polyols, found WVP values about fifty times higher and the tensile strength at break was less than 1/3 of the values found here.

Crosslinked Films

As already stated, all films obtained by the 2nd Stage were transparent and firm. The results for moisture content, mass solubilized in water, thickness, water vapor permeability, tensile strength and elongation at break of the calcium-reticulated alginate films obtained with the use of different hydrophilic plasticizers are presented in Table 3.

A comparison between the film physical characteristics given in Table 3 and the values found for the low-reticulated pre-films (Table 2) reveals striking differences: the Ca²⁺ films are thinner (indicating a tighter, stronger and better-organized intermolecular structuration) and, as a consequence, they exhibit lower permeability, higher tensile strength but, unfortunately, lower elongation. The crosslinking with calcium protects the structure against disintegration by solubilisation. The matter soluble in water (S) was below 20% and the film samples, even after 24 hours immersion, showed a general appearance similar to the plain film. Unexpectedly, the matter soluble in water for the mannitol films was higher than the S values found for glycerol films, so the

hypothesis that the use of less soluble plasticizers would hinder the leaching of these solutes is not confirmed.

Turbiani and Kieckbusch (2011), studying alginate films manufactured with the same formulation used in the 1st Stage and treated by immersion for 15 minutes in a solution of 3% calcium chloride and 0.7% glycerol (2nd Stage), found moisture and solubility in water values of 18.9% and 4.5%, respectively. These values are inferior to those obtained in this paper, which received a higher amount of glycerol in the 2nd Stage. The most noticeable difference is in the S values, suggesting that the concentration of glycerol in the crosslinking solution causes the increase of film solubility.

The film thickness increased with the amount of film-forming solution poured on the plate, but is about half the thickness of the corresponding pre-film. The thicknesses of the G-films and the X-films are statistically equal ($P > 0.95$) but the M-films, despite being smooth and transparent, are thicker. This more open structure might be responsible for the higher loss in water (S) of these films.

On the other hand, the moisture content are slightly higher in the glycerol and xylitol crosslinked films and are statistically lower for the mannitol plasticized films, findings that can be attributed to the differences in hygroscopicities.

Like the pre-films, the WVP of the crosslinked films also showed a decreasing trend with thickness. The denser packing of the polymeric structure increased the resistance to migration in the more hygroscopic G and X-films and offset the lower water affinity of the mannitol films. As a result, the differences of the average WVP values measured for the three types of films with different thicknesses were statistically indistinct.

Table 3: Thickness (δ), matter soluble in water (S), moisture content (100 ω), water vapor permeability (WVP), tensile strength (TS) and elongation at break (E) of calcium alginate films (2nd Stage) containing glycerol, xylitol and mannitol as plasticizers.

Plasticizer	FS [g]	δ [μ m]	S [%]	100 ω	WVP [g.mm/m ² .day.kPa]	TS [MPa]	E [%]
Glycerol	40	10 \pm 1 ^a	13.5 \pm 2.1 ^a	19.9 \pm 1.1 ^{cd}	3.1 \pm 0.2 ^b	119.4 \pm 10.6 ^a	3.3 \pm 1.6 ^{ab}
	50	12 \pm 1 ^a	14.1 \pm 1.6 ^a	20.6 \pm 1.8 ^{cd}	2.1 \pm 0.3 ^a	118.3 \pm 7.9 ^a	4.0 \pm 0.9 ^{bc}
	60	14 \pm 1 ^a	16.3 \pm 1.9 ^{ab}	21.1 \pm 1.0 ^d	1.6 \pm 0.2 ^a	116.3 \pm 7.0 ^a	4.6 \pm 0.7 ^c
Xylitol	40	10 \pm 1 ^a	13.2 \pm 1.8 ^a	20.5 \pm 0.8 ^{cd}	2.6 \pm 0.6 ^{ab}	137.6 \pm 11.2 ^{ab}	3.7 \pm 0.6 ^{abc}
	50	14 \pm 1 ^a	17.0 \pm 1.4 ^{ab}	19.3 \pm 1.8 ^{bcd}	1.8 \pm 0.2 ^a	134.6 \pm 1.0 ^{ab}	4.8 \pm 2.0 ^c
	60	19 \pm 1 ^{ab}	21.6 \pm 2.8 ^b	18.5 \pm 1.1 ^{bcd}	1.6 \pm 0.1 ^a	118.7 \pm 4.8 ^a	5.1 \pm 0.5 ^c
Mannitol	40	17 \pm 1 ^{ab}	16.1 \pm 3.0 ^{ab}	13.3 \pm 0.6 ^a	2.0 \pm 0.2 ^a	161.6 \pm 2.1 ^c	2.6 \pm 0.8 ^d
	50	18 \pm 1 ^{ab}	16.6 \pm 2.5 ^{ab}	16.8 \pm 1.7 ^b	2.0 \pm 0.3 ^a	150.4 \pm 7.5 ^{bc}	2.9 \pm 0.8 ^{ab}
	60	21 \pm 2 ^b	19.5 \pm 3.0 ^{ab}	17.8 \pm 0.9 ^{bc}	1.7 \pm 0.2 ^a	140.4 \pm 4.6 ^b	3.3 \pm 0.5 ^{ab}

FS is the mass of films forming solution poured on the plates (Stage 1); Average \pm standard deviation; Average with the same letter in each column indicate no significant difference ($p < 0.05$) by Tukey test.

The tensile strength is the parameter enhanced most by the Ca^{2+} crosslinking procedure. On the average, a three-fold increase over the pre-film's TS was obtained. The values found for the M-films are impressive and the highest ever reported for polysaccharide-based films. According to the classification established by Krochta and De Mulder-Johnston (1997), the M-film can be considered as good in this attribute. Low density polyethylene films, for instance, have lower TS values, between 8.3 and 27.6 MPa (Andreuccetti *et al.*, 2009). The large increase in TS, however, is counterbalanced by a similar-fold decrease in elongation. A comparison of the TS and the E values in Table 3 confirms that glycerol and xylitol are more efficient plasticizers than mannitol.

The results for the mechanical resistance given in Table 3 are usually found for calcium alginate glycerol films. Zactiti and Kieckbusch (2009), characterizing alginate films plasticized with glycerol and cross-linked with a solution of 0.7% glycerol and 3.0% of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, found for the tensile strength and elongation at break values of 119.1 MPa and 4.5%, respectively, for a much thicker film (88 μm).

Kim *et al.* (2002), studying un-reticulated starch-based films plasticized with glycerol, xylitol and mannitol, found values of tensile strength and elongation at break in the range of 9.7 – 15.3 MPa and 2.6 – 7.7%; 6.8 – 13.0 MPa and 2.2 – 5.6%; 4.0 – 6.7 MPa and 2.0 – 2.4%, respectively. The range of values for the tensile strength at rupture were much lower than the value found in this paper for calcium alginate films plasticized with the same polyols. However, the ranges for the elongation at break are higher than the values obtained here.

The crosslinked films, due to the intrinsic hygroscopicity of the alginate, are able to reversibly absorb water in their polymeric structure, increasing considerably the original thickness. Swelling tests were performed with calcium reticulated films plasticized with glycerol, xylitol and mannitol, produced with 40, 50 and 60 g of film-forming solution and the results indicated that the gramature was not statistically significant.

Since the water uptake rate was similar, only the swelling curves for the films obtained with 60g aliquots of the solution are shown in Figure 2.

The shape of the curves indicates that the water uptake is extremely fast. In all cases, most of the total imbibition took place in the first minute and the process reached equilibrium in less than 5 minutes. The results indicate that the amount of water absorbed by the film samples is a direct function of the hygroscopicity of the polyols. All the WU values

obtained are extremely high and constitute an important handicap for the commercial use of these biofilms.

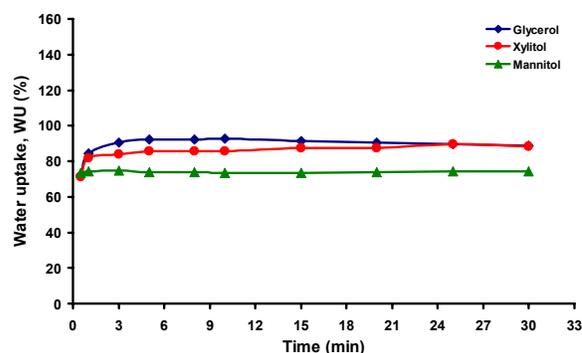


Figure 2: Water uptake of calcium alginate films containing glycerol, xylitol and mannitol as plasticizers, at 25 °C.

Additional Determinations

The opacity in the transmittance mode and the Glass Transition Temperature of the pre-films and of the calcium reticulated alginate films were determined. These studies were complemented with SEM micrograph observations. The results confirmed the subjective perception already described and are useful for quantitative comparisons.

The opacity was obtained by a colorimeter and calculated as percentages, according to Equation (3). The values given in Table 4 correspond to films elaborated with 60g of film-forming solution poured in a 15x15 cm square dish.

Table 4: Percent opacity in the transmittance mode of alginate pre-films (1st Stage) and Ca^{2+} crosslinked alginate films (2nd Stage) plasticized with glycerol, xylitol and mannitol.

Plasticizer	Pre-films (1 st Stage)	Ca^{2+} Films (2 nd Stage)
Glycerol	12.99±0.4 ^a	10.97±0.5 ^a
Xylitol	13.00±1.6 ^a	11.26±1.3 ^a
Mannitol	97.30±0.6 ^b	11.41±0.6 ^a

Average ± standard deviation; Average with the same letter in each column indicate no significant difference ($p < 0.05$) calculated by Tukey test.

The results indicate that all three Ca^{2+} films are equally transparent. The glycerol and xylitol pre-films have slightly higher opacity than the calcium treated films, probably due to the fact that they are thicker. The high % opacity of the mannitol pre-film is a quantitative confirmation of visual observations and the impressive change to a nicely transparent

film with the immersion washing procedure could also be followed by SEM micrographs.

The SEM micrographs of the surface and of the cross sections (after fracture) of the calcium alginate films plasticized with glycerol, xylitol and mannitol, before and after the crosslinking with calcium, are presented in Figure 3 and 4, respectively.

For glycerol and xylitol plasticized films, the micrographs indicate a perfectly homogeneous surface microstructure. Some surfaces present small fragmentations that may have been formed during the contact of the film forming solution with the plates, since the Plexiglas surface was not completely smooth. The cross-sectional morphology is also smooth and jellylike in these films, although an incipient stratification can be identified.

The micrographs of the pre-films plasticized with

mannitol show an intense crystal formation at the surface, as well as in the cross section, formed due to the low solubility of mannitol. These findings confirm the observations made from the visual aspect of the film. The crystals distributed over the surface impart the roughness feeling and the bulky cross section with an undulated fracture conformation differs considerably from the well-aligned arrangement in the glycerol and xylitol films.

The SEM micrographs in Figure 4 revealed similar structures for all three types of film, confirming the astonishing transformation that took place during the immersion in the crosslinking solution during the 2nd Stage. The crystals were washed out and the M-film, like the G-film and the X-film became more compact with a slightly striated cross section, typical of a strong film (Arzate-Vázquez *et al.*, 2012).

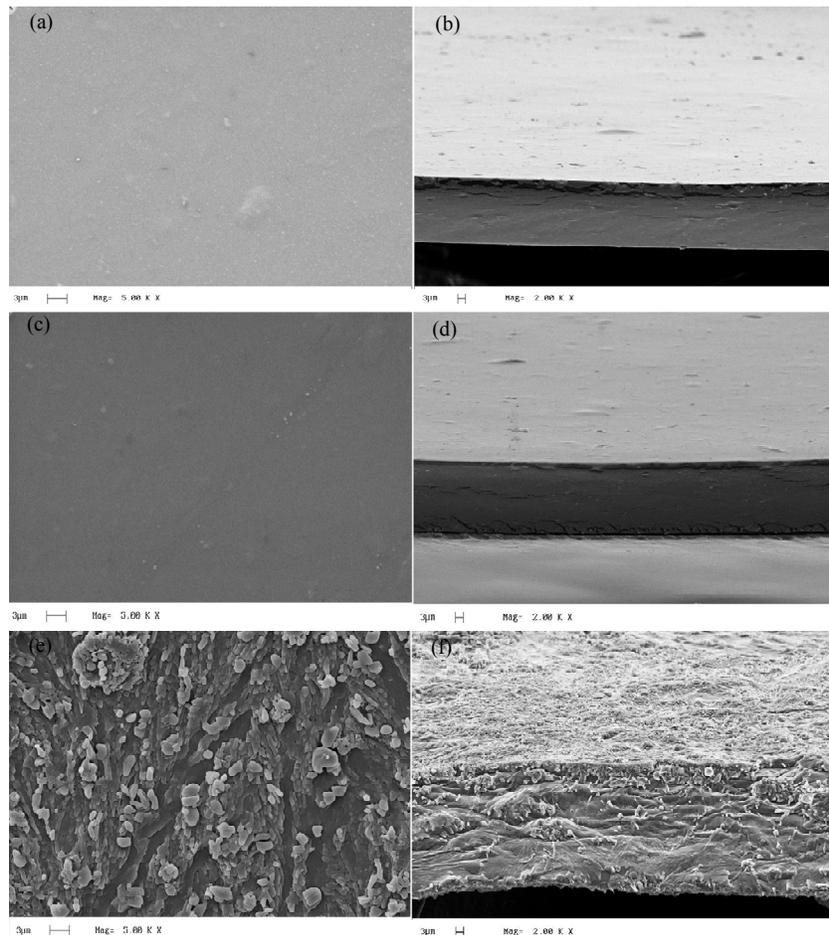


Figure 3: Micrographs of the surface ((a), (c) and (e)) and of the cross section ((b), (d) and (f)) of the alginate pre-films plasticized with glycerol, xylitol and mannitol, respectively (1st Stage).

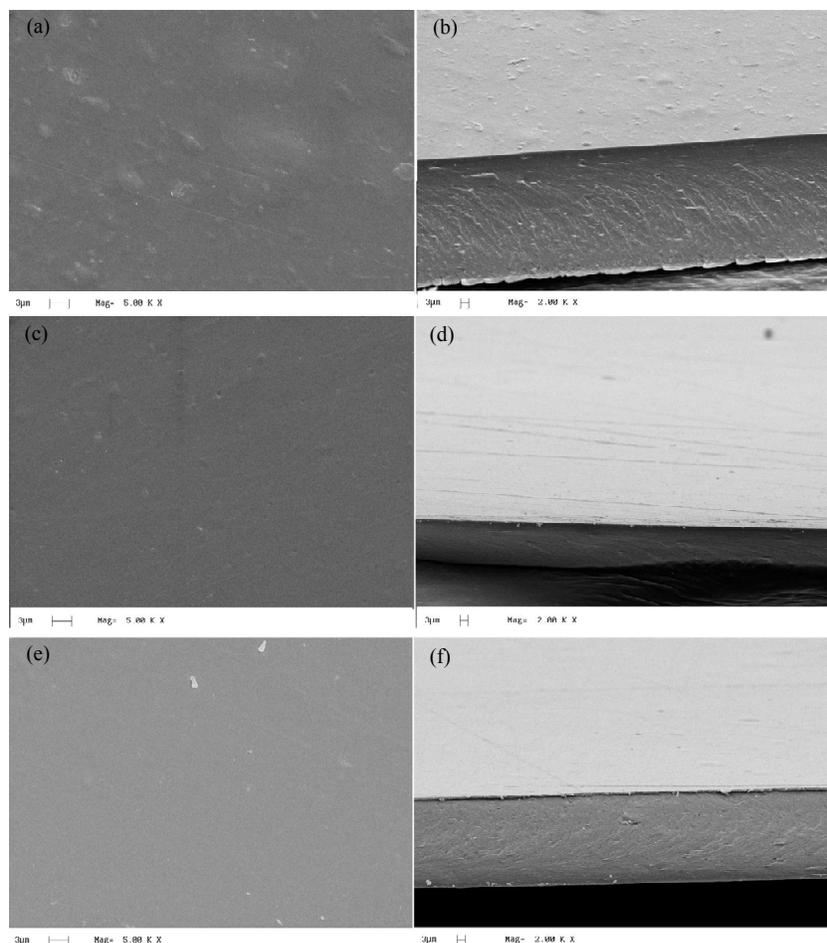


Figure 4: Micrographs of the surface ((a), (c) and (e)) and of the cross section ((b), (d) and (f)) of the calcium alginate films plasticized with glycerol, xylitol and mannitol, respectively (2nd Stage).

The Glass Transition Temperatures, T_g , of the alginate pre-films and the calcium crosslinked films plasticized with glycerol, xylitol and mannitol, obtained by thermomechanical analyses, are given in Table 5. The values correspond to the temperatures of the main peak of the curve of $\tan \delta$ vs temperature.

Table 5: Glass Transition Temperature, T_g , of the alginate films plasticized with glycerol, xylitol and mannitol.

Plasticizer	T_g , Pre-film (°C)	T_g , Ca ²⁺ film (°C)
Glycerol	-23.2	10.4
Xylitol	-11.1	4.5/18.4
Mannitol	-6.0	25.1

As an example, Figure 5 presents the DMA responses (E' , E'' , $\tan \delta$) for the two xylitol plasticized film types. The T_g peak for the pre-film (Figure 5(a)) is well defined, but for the calcium alginate film two peaks (at 4.52 °C and at 18.39 °C) were

found. $\tan \delta$ curves with multiple peaks can be obtained and they are attributed to phase separation. The film cross-sectional morphology observed in Figure 3, however, suggests no phase separation in the xylitol film. It is pertinent to assume, therefore, that the X-film T_g peak split in two due to the small drift of the E'' curve in this region (Figure 5(b)), leading to an inflection point. The true T_g would then be positioned between 4.5 and 18.4 °C.

The values of the glass transition temperature given in Table 5 indicate an excellent level of plastification in the pre-film and that the glycerol film crosslinked with calcium is more plasticized than the X-film. The mannitol plasticized film, at ambient temperature of 25 °C, on the other hand, should display an intermediate behavior, between a glassy and a rubbery material. The TS and elongation values of the three calcium reticulated films (Table 3) correlate well with the corresponding T_g given in Table 5.

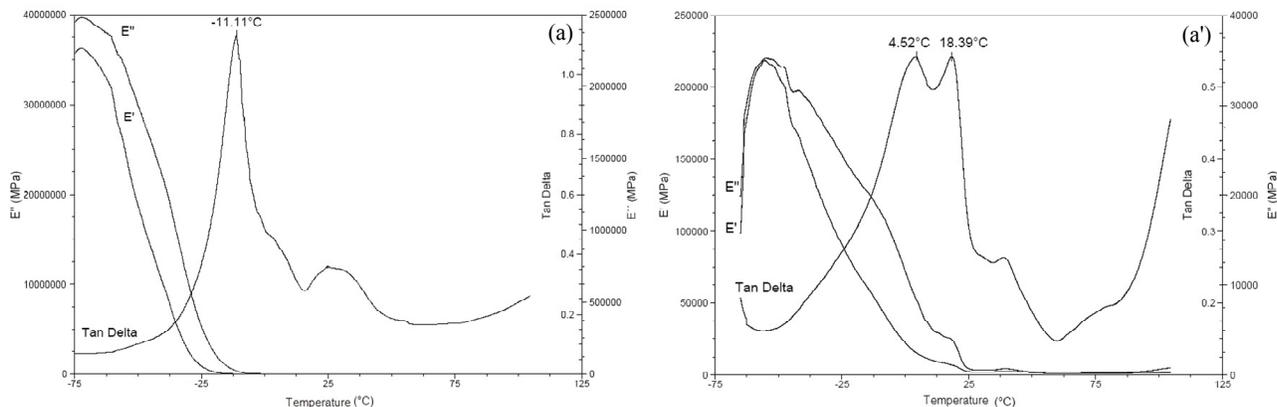


Figure 5: Thermomechanical analysis output of alginate film plasticized with xylitol: (a) pre-film (1st Stage) and (a') Ca⁺⁺ film (2nd Stage).

CONCLUSIONS

The film hydrophilicities and their glass transition temperatures are strongly affected by the plasticizer's hygroscopicity. The low-reticulated films showed acceptable WVP values and mechanical characteristics, but cannot be considered as packaging material since they disintegrated completely in an aqueous environment.

The Ca²⁺ crosslinked films were highly transparent, their WVP decreases with film thickness and the rate of water uptake was similar at all thicknesses, with fast swelling equilibration. Glycerol and xylitol showed the same plasticization potential, although xylitol promotes a higher tensile strength at rupture.

Films with mannitol as plasticizer obtained the highest values of tensile strength at rupture and, consequently, the lowest values of elongation. The use of mannitol as plasticizer is promising but limited by its low solubility in water. An adequate compromise using mixtures of glycerol and mannitol should be investigated in order to optimize film strength and elongation.

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