



Properties of starch–hydroxypropyl methylcellulose based films obtained by compression molding



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ABSTRACT

Corn starch–glycerol (1:0.3) films, containing or not citric acid (1 g/100 g starch) and HPMC (10 and 20 g/100 g starch), are obtained by compression molding. The microstructure of the films, the thermal behavior, the X-ray diffraction spectra and the physical properties (mechanical, barrier and optical) were analyzed after 1 and 5 storage weeks at 25 °C and 53% relative humidity. The bonded citric acid and film solubility were also determined. Starch–HPMC blend films showed a dispersed phase of HPMC in a continuous, starch-rich phase with lower glass transition than HPMC-free films. The addition of citric acid also provoked a decrease in glass transition in line with the partial hydrolysis of starch chains. Both components implied a decrease in the water vapour permeability while the oxygen permeability slightly increased. Although citric acid only provoked a small hardening effect in the films, it greatly decreased their extensibility (weak cross-linking effect), which seems to increase during film storage. Starch crystallization during storage was inhibited by both citric acid and HPMC.

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1. Introduction

Society's awareness of the importance of both environmental conservation and green technologies, and the growing environmental contamination caused by synthetic polymers based on petroleum, have led to an increased interest in developing environmentally friendly materials, such as biodegradable polymers based on polysaccharides. In the development of biodegradable materials, starch is the most promising of the polysaccharide polymers, due to its low cost, great processability, and abundance (Ghanbarzadeh, Almasi, & Entezami, 2011; Yoon, Chough, & Park, 2006). Starch films exhibit some good properties, such as high barrier to oxygen, carbon dioxide and lipids. However, poor water vapor permeability and mechanical properties, and the recrystallization during storage, are limitations of this material (Arif Kibar & Us, 2013; Kester & Fennema, 1986).

The deficiencies in the properties of starch films can be improved by different methods. The phenomenon of the recrystallization of starch films could be solved by combining starch with other polymers, as has been proven by other authors (Funami et al., 2005; Jiménez, Fabra, Talens, & Chiralt, 2012a). Hydroxypropyl methylcellulose (HPMC) is a cellulose-derived polymer commonly used

for obtaining edible, biodegradable films (Albert & Mittal, 2002; Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2009; Villalobos, Hernández-Muñoz, & Chiralt, 2006) with good availability and processability (Fahs, Brogly, Bistac, & Schmitt, 2010; Jiménez et al., 2012a) which have excellent film-forming properties (Villalobos et al., 2006). Compared to other polymers, HPMC films have great mechanical strength, (Jiménez, Fabra, Talens, & Chiralt, 2010, 2012a) but limited barrier properties. In this sense, Jiménez et al. (2012a) observed that the oxygen permeability of HPMC films obtained by casting was approximately 100 times higher than cornstarch films, while the water barrier properties are similar for films made from both polymers.

Nevertheless, the thermal stability of HPMC films means that they can be thermally processed using industrial equipment, and previous studies (Jiménez et al., 2012a) revealed inhibition of starch crystallization when blend films were obtained by casting the aqueous dispersions, the films showing a more amorphous character. So, HPMC–starch blends could be used to obtain thermally processed films with interesting properties, although phase separation in corn starch–HPMC films obtained by casting has been observed by scanning electron microscopy (Jiménez et al., 2012a), with the films exhibiting a starch-rich phase and a HPMC-rich phase in what is practically a bi-layer film.

The poor compatibility of these polymers can be limited by the incorporation of compatibilizer compounds, which promotes the esterification of the starch –OH groups. For this purpose,

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cross-linking agents, such as phosphorus oxychloride, sodium trimetaphosphate, sodium tripolyphosphate, epichlorohydrin, and 1,2,3,4-diepoxybutane, were used (Reddy & Yang, 2010; Seker & Hanna, 2006). These substances are relatively toxic and expensive (Reddy & Yang, 2010), so their use in food packaging is not recommendable. However, using polycarboxylic acids as cross-linking agents is a good alternative. This is especially so in the case of citric acid, a low cost organic acid widely used in the food industry, that acts as a cross-linking agent due to the presence of several carboxyl groups in its molecular structure (Ghanbarzadeh, Almasi, & Entezami, 2010). These groups can react with the hydroxyl groups of starch molecules through the formation of esters. According to Reddy and Yang (2010), the esterification reaction using polycarboxylic acids occur with the polymer hydroxyl groups, at high temperatures (about 160 °C), through the formation of anhydride groups (Yang, Wang, & Kang, 1997). This interaction can decrease the retrogradation and recrystallization of starch during storage time (Ghanbarzadeh et al., 2011). The compatibilizer effect of the citric acid in the starch–polymer blends, as well as an improvement in the properties of starch films, has been reported in several studies (Ghanbarzadeh et al., 2010, 2011; Ma, Chang, Yu, & Stumborg, 2009; Reddy & Yang, 2010; Shi et al., 2008; Yu, Wang, & Ma, 2005). The most promising results derived from the addition of citric acid to the polymeric matrix are the increase in thermal stability and the improvement in the barrier properties (Reddy & Yang, 2010; Yu et al., 2005).

The aim of this work was to study the effect of citric acid addition on the structural and physical properties of corn starch films obtained by compression molding, combined with different ratios of HPMC, using glycerol as plasticizer. The effect of storage time on the film properties was also analyzed.

2. Materials and methods

2.1. Materials

Corn starch was obtained from Roquette (Roquette Laisa España, Benifaió, Spain). Its moisture content was 10% (w/w) and amylose percentage was 14%. Glycerol was purchased from Panreac Química, S.A. (Castellar del Vallès, Barcelona, Spain). Hydroxypropyl methylcellulose (HPMC) and citric acid (CA) were provided by Fluka (Sigma–Aldrich Chemie, Steinheim, Germany).

2.2. Film preparation

Native starch and glycerol, as plasticizer, were dispersed in water. HPMC was hydrated in cold water (5%, w/w) under continuous stirring. The HPMC dispersion and the aqueous mixture of starch and glycerol were mixed in different ratios to obtain two blends with 1:0.1 and 1:0.2 starch:HPMC ratios. The polymer:glycerol ratio was 1:0.3. CA was mixed to obtain four blends with and without CA; a constant starch:CA ratio of 1:0.01 was used in every case. Blends of starch–glycerol and starch–glycerol–citric acid, in the previously described ratios, were studied as control formulations. The abbreviations used for the studied formulations were: SG:starch–glycerol; CA:citric acid; H10:10 g HPMC/100 g starch; H20:20 g HPMC/100 g starch.

The formulations were hot-mixed on a two-roll mill (Model LRM-M-100, Labtech Engineering, Thailand) at 160 °C and 8 rpm for 20 min. A visual good miscibility of HPMC, CA and starch was obtained. A trowel was used during mixing to smoothly spread the material on the rolls. The paste sheet formed was removed from the mill and conditioned at 25 °C and 53% relative humidity (RH), using a Mg(NO₃)₂ saturated solutions (Panreac Química, SA, Castellar del Vallès, Barcelona, Spain), for 48 h.

Afterwards, films were obtained by compression molding (Model LP20, Labtech Engineering, Thailand). Four grams of the pre-conditioned paste were put onto steel sheets and pre-heated on the heating unit for about 5 min. Compression molding was performed at 160 °C for 2 min at a pressure of 30 bars, followed by 6 min at 130 bars; thereafter, the cooling cycle was applied for 3 min. The films were conditioned at 25 °C and 53% RH for 1 week for the initial time characterization and for 5 weeks for the final time characterization.

2.3. Film characterization

2.3.1. Film thickness

A Palmer digital micrometer was used to measure film thickness to the nearest 0.0025 mm at six random positions around the film.

2.3.2. Scanning electron microscopy (SEM)

The microstructural analysis of the cross-sections and surface of the films was carried out by means of a scanning electron microscope (JEOL JSM-5410, Japan). The film samples were maintained in desiccators with P₂O₅ for 2 weeks to guarantee that water was not present in the sample and observations were taken in duplicate for each film sample and in two films per formulation.

Film pieces, 0.5 cm² in size, were cryofractured from films and fixed on copper stubs, gold coated, and observed using an accelerating voltage of 10 kV.

2.3.3. X-ray diffraction

A diffractometer (XRD, Bruker AXS/D8 Advance) was used to record the X-ray diffraction patterns. All the samples (equilibrated for one and five weeks at 25 °C and at 53% RH) were analyzed at 25 °C and 53% RH, between $2\theta = 5^\circ$ and $2\theta = 30^\circ$ using K α Cu radiation (λ : 1.542 Å), 40 kV and 40 mA with a step size of 0.05°. For this analysis, samples were cut into 4 cm squares. Pure citric acid was also analyzed, so as to identify its characteristic peaks.

2.3.4. Atomic force microscopy (AFM)

Samples equilibrated for 5 weeks at 25 °C and 0% RH, using P₂O₅ saturated solutions (Panreac Química, SA, Castellar del Vallès, Barcelona, Spain), were used. The surface morphology of the films was analyzed using an atomic force microscope (Multimode 8, Bruker AXS, Santa Barbara, USA) with NanoScope® V controller electronics. Measurements were taken from several areas of the film surface (20 μ m²) using the PeakForce QNM mode. Method ASME B46.1 (ASME, 1995) was used to calculate the following statistical parameters related with sample roughness: average roughness (Ra: average of the absolute value of the height deviations from a mean surface), root-mean-square roughness (Rq: root-mean-square average of height deviations taken from the mean data plane), and factor of roughness (r: ratio between the three-dimensional surface and two-dimensional area projected onto the threshold plane). The DMT modulus, derived from the Peak Force QNM Mode, was also applied to obtain the surface maps showing the heterogeneities present in the surface properties. Three replicates were considered for each sample.

2.3.5. Optical properties

The Kubelka–Munk theory for multiple scattering was applied to the reflection spectra to determine the film's transparency (Hutchings, 1999). The surface reflectance spectra were determined from 400 to 700 nm using a spectro-colorimeter CM-3600d (Minolta Co., Tokyo, Japan) on both a white and a black background. As the light passes through the film, it is partially absorbed and scattered, which is quantified by the absorption (K) and the scattering (S) coefficients. The internal transmittance (Ti) of the films

was determined using Eq. (1). In this equation, R_0 is the reflectance of the film on an ideal black background. Parameters a and b were calculated by means of Eqs. (2) and (3), where R is the reflectance of the sample layer backed by a known reflectance, R_g . Three replicates were used for each sample on the free film surface. For the purposes of the analysis, a wavelength of 450 nm was considered:

$$T_i = \sqrt{(a - R_0)^2 - b^2} \quad (1)$$

$$a = \frac{1}{2} \left(R + \frac{R_0 - R + R_g}{R_0 R_g} \right) \quad (2)$$

$$b = \sqrt{a^2 - 1} \quad (3)$$

The gloss was determined on the free film surface, at a 60° incidence angle by means of a flat surface gloss meter (Multi Gloss 268, Minolta, Germany), following the ASTM standard D523 method (ASTM, 1999). The measurements of each sample were taken in triplicate and three films were measured from each formulation. The results were expressed as gloss units (GU), relative to a highly polished surface of black glass standard with a value near to 100 GU.

2.3.6. Moisture content

The films that had been previously conditioned at 53% RH were dried for 24 h at 60 °C (J.P. Selecta, S.A. Barcelona, España) and placed in a desiccator with P_2O_5 (Panreac Quimica, S.A. Castellar Vallés, Barcelona) adjusting them to 0% RH and 25 °C for 2 weeks. The reported results represent the average of three samples.

2.3.7. Water vapour permeability (WVP)

The WVP of films was determined by means of the ASTM E96-95 (ASTM, 1995) gravimetric method, taking the modification proposed by McHugh, Avena-Bustillos, and Krochta (1993) into account. Distilled water was placed in Payne permeability cups (3.5 cm diameter, Elcometer SPRL, Hermelle/s Argenteau, Belgium) to expose the film to 100% RH on one side. Once the films were secured, each cup was placed in a relative humidity equilibrated cabinet at 25 °C, with a fan placed on the top of the cup in order to reduce resistance to water vapour transport, thus avoiding the stagnant layer effect in this exposed side of the film. The RH of the cabinets (53%) was held constant using oversaturated solutions of magnesium nitrate-6-hydrate. The cups were weighed periodically (0.0001 g) and water vapour transmission (WVTR) was determined from the slope obtained from the regression analysis of weight loss data versus time, once the steady state had been reached, divided by the film area. From WVTR data, the vapour pressure on the film's inner surface (p_2) was obtained using Eq. (4), proposed by McHugh et al. (1993), to correct the effect of concentration gradients.

$$WVTR = \frac{P \cdot D \cdot L_n[P - p_2 \setminus P - p_1]}{R \cdot T \cdot \Delta z} \quad (4)$$

where P , total pressure (atm); D , diffusivity of water through air at 25 °C (m^2/s); R , gas law constant ($82.057 \times 10^{-3} m^3 atm kmol^{-1} K^{-1}$); T , absolute temperature (K); Δz , mean stagnant air gap height (m), considering the initial and final z value; p_1 , water vapour pressure on the solution surface (atm); and p_2 , corrected water vapour pressure on the film's inner surface (atm). Water vapour permeance was calculated using Eq. (5) as a function of p_2 and p_3 (pressure on the film's outer surface in the cabinet).

$$permeance = \frac{WVTR}{p_2 - p_3} \quad (5)$$

Permeability was calculated by multiplying the permeance by film thickness.

2.3.8. Oxygen permeability (OP)

The oxygen permeation rate of the films was determined at 53% RH and 25 °C using an OX-TRAN (Model 2/21 ML Mocon Lippke, Neuwied, Germany). The samples were conditioned at the relative humidity level of the test in a desiccator using magnesium nitrate-6-hydrate saturated solutions. Three samples were placed in the equipment for analysis, and the transmission values were determined every 20 min until equilibrium was reached. The exposure area during the tests was 50 cm² for each sample. To obtain the oxygen permeability, the film thickness was considered in every case.

2.3.9. Tensile properties

A universal test Machine (TA.XTplus model, Stable Micro Systems, Haslemere, England) was used to determine the tensile strength (TS), elastic modulus (EM), and elongation (E) of the films, following ASTM standard method D882 (ASTM, 2001). EM, TS, and E were determined from the stress-strain curves, estimated from force-distance data obtained for the different films (2.5 cm wide and 5 cm long). Equilibrated samples were mounted in the film-extension grips of the testing machine and stretched at 50 mm min⁻¹ until breaking. The relative humidity of the environment was held constant, at approximately 53% during the tests, which were performed at 25 °C. At least ten replicates were obtained from each sample.

2.3.10. Film solubility and bonded citric acid

Film solubility was determined by keeping the sample in bidistilled water at a film:water ratio of 1:10, for 48 h. Three replicates were made for each formulation. After 48 h, the film samples were transferred to a convection oven (J.P. Selecta, S.A., Barcelona, Spain) for 24 h at 60 °C to remove the free water and afterwards transferred to a desiccator with P_2O_5 at 25 °C for 2 weeks to complete film drying. Film water solubility was estimated from its initial and final weights. The water solution was used to determine the bonded citric acid in the film. Free citric acid was assumed to dissolve in the aqueous phase and was determined by titration of an aliquot of the solution with NaOH (0.1 N), using phenolphthalein as indicator. Three replicates were considered for each formulation.

2.3.11. Thermal properties

A differential scanning calorimeter (DSC 1 Star^e System, Mettler-Toledo, Inc., Switzerland) was used to analyze the thermal properties. Weighted amounts of samples were placed into aluminum pans and sealed and drilled to promote the bonded moisture loss during heating. The curves were obtained using a double scan. First, a scan from 0 °C to 160 °C at 50 °C/min was used where the bonded water in the film was eliminated. Then the temperature was lowered to 0 °C at the same speed, and finally heated to 160 °C at 20 °C/min (in this scan, starch glass transition was analyzed). The initial and final weights of the pans were registered to assess water loss during the first heating.

2.3.12. Statistical analysis

Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, MD) was used to carry out statistical analyses of data through an analysis of variance (ANOVA). Fisher's least significant difference (LSD) was used at the 95% confidence level.

3. Results

3.1. Film thickness, water solubility and bonded citric acid

Constant compression molding conditions were maintained during processing in order to submit all the formulations to a

Table 1

Thickness, film solubility (g solubilized film/g initial dried film) and bonded citric acid (ratio with respect to the amount in the film) of the films equilibrated at 53% relative humidity and 25 °C and the glass transition temperature (onset: T_{go} and midpoint: T_{gm}) of dried films.

Formulation	Thickness (μm)	Film solubility (2) (g/g)	Bonded citric acid (1)	T _{go} (°C)	T _{gm} (°C)
HPMCG	–	–	–	102 (2) ^c	113 (1) ^c
SG	268 (28) ^a	0.19 (0.07) ^a	–	111 (1) ^b	125 (4) ^b
SG-CA	217 (22) ^{cd}	0.35 (0.03) ^b	0.80 (0.03) ^a	106 (2) ^c	119 (2) ^{bc}
SG-H10	257 (19) ^b	0.15 (0.01) ^a	–	105 (1) ^c	114 (7) ^{cd}
SG-H10-CA	229 (13) ^c	0.32 (0.06) ^b	0.83 (0.04) ^a	85 (5) ^e	100 (3) ^e
SG-H20	204 (18) ^d	0.18 (0.02) ^a	–	94 (1) ^d	112 (3) ^d
SG-H20-CA	158 (13) ^e	0.31 (0.05) ^b	0.89 (0.09) ^a	85 (2) ^e	105 (9) ^e

(1) At 5 storage weeks; (2) at initial time, without significant differences with respect to the final time.

Different superscript letters within the same column indicate significant differences among formulations ($p < 0.05$).

similar thermomechanical treatment. In agreement with the different composition, the differing response of the samples to the same treatment can be estimated by the film thickness. The values of this parameter obtained for every formulation are shown in Table 1. As can be observed, the film thickness decreases when HPMC was incorporated into the film (from 268 μm to 204 μm). This phenomenon is related with the increase in the blend flowability when HPMC is present in the blend, which is probably related with the decrease in the glass transition of the blend commented on below. The addition of citric acid also contributed to the decrease in the film thickness, thus indicating the promotion of the blend's flowability. Due to its cross-linking effect, citric acid has not only been reported to be a compatibilizer of biopolymers (Menzel et al., 2013; Olivato, Grossmann, Yamashita, Eiras, & Pessan, 2012; Reddy & Yang, 2010) but also a plasticizer in its free form (Ghanbarzadeh et al., 2011). Thus, this organic acid can have a relevant impact on the rheological behavior of blends during compression, which affects the final film thickness.

Table 1 also shows the solubility of the studied films in water at initial time. Values at final time (5 weeks) were not included due to the fact that no significant differences were found with respect to the initial values. The results show that the addition of citric acid is a key factor in terms of the integrity of films submitted to water action. Films containing citric acid showed higher solubility (0.31–0.35 g/g) than citric acid-free films (0.15–0.19 g/g). These results seem contradictory since the cross-linking activity of citric acid is related with an increase in the hydrophobicity as a result of the formation of ester groups. Nevertheless, the addition of citric acid is also related with a decrease in the pH values, which may give rise to a partial hydrolysis of glucosidic chains (Olsson et al., 2013). In this way, the values of bonded citric acid in films at 5 weeks of storage time (Table 1) are relevant since an important amount of citric acid remains free to contribute to the pH decrease. The contents of bonded citric acid ranged from 80% (SG-H20-CA) to 89% (SG-H10-CA) with respect to the total amount present in the film, there being no significant differences ($p > 0.05$) between samples. This indicates that this component partially reacts with the –OH groups of the polymer chains, although about 15% of the added amount is free in the matrix and could act as plasticizer.

3.2. Film microstructure

Fig. 1a and b shows SEM micrographs of the cross sections of films, containing (Fig. 1b) or not (Fig. 1a) citric acid, conditioned at 25 °C and 53% RH for 1 (initial time) or 5 weeks (final time). At the initial time, the control formulations (SGi and SG-CAi) exhibited a homogeneous structure with some cracks which are related with the more brittle nature of this formulation under the observation conditions. The low water content and the electron beam in the microscopy chamber provoked the formation of these microcracks, as observed previously for other starch films (Jiménez, Fabra, Talens, & Chiralt, 2012b). The addition of HPMC gave rise to

a two-phase structure in which this polysaccharide constitutes the dispersed phase embedded in a starch continuous phase, which can be observed both at initial and final storage times. The incompatibility of HPMC and starch has previously been observed in films obtained by casting (Jiménez et al., 2012a). Phase separation has also been reported in other polysaccharide blends such as potato maltodextrin in admixture with locust bean gum, gum arabic and carboxymethyl cellulose, and starch–chitosan blend films (Annable, Fitton, Harris, Philips, & Williams, 1994; Mathew & Abraham, 2008). The different behavior of continuous and dispersed phases in response to cryofracture is remarkable. Whereas a very hard, rigid matrix can be deduced for the starch continuous phase, HPMC particles can be inferred to be more rubbery and soft in nature, and, in some cases, they appeared greatly deformable (sample SG-H20-CAf).

Citric acid addition did not contribute to avoid the formation of microcracks as can be observed in Fig. 1b (SG-CAi), despite its plasticizing effect on the matrix (Yu et al., 2005) as deduced from the film thickness values. In HPMC-containing films, citric acid addition did not significantly affect their microstructural appearance in comparison with acid-free films and the HPMC dispersed phase was also observed in films. Nevertheless, a small increase in the polymer miscibility could be promoted, without notably affecting the visible film microstructure.

The effect of storage time on the film microstructure is also shown in Fig. 1. Starch–glycerol films, containing or not citric acid (SGf, SG-CAf), show the extensive formation of crystalline zones, as revealed by the irregular, layered fracture surface, which point to the growth of the crystalline regions. This was especially notable for CA-free starch films whereas wider amorphous regions are still observable for starch films containing CA. In this case, the advance of the crystalline front from the film surface to the inner part of the film can be observed, probably due to the easier water uptake from the environment and the subsequent increase in the molecular mobility. The greater advance of these crystalline structures in citric acid-free films suggests that, despite its apparent plasticizing effect, this organic acid contributes to partially inhibit starch recrystallization as mentioned by other authors (Shi et al., 2007). The possible starch depolymerization provoked by acid hydrolysis could also affect the crystallization advance.

In HPMC-containing films, crystalline regions are not observed to the same extent as in starch films and, although HPMC appears in a dispersed phase, its partial miscibility in starch matrix may also contribute to inhibit starch crystallization as observed by Jiménez et al. (2012a) for HPMC–corn starch films obtained by casting.

The peak force quantitative nanomechanical mapping AFM-based method is a good tool with which to analyze the nanostructure of biopolymer based films. This tool allows us to measure the Young modulus of the film at each point on its surface. Fig. 2 shows the results obtained for studied films stored for 1 week and 5 weeks under controlled conditions. Raw data were converted into 2D images and their scale is expressed as DMT modulus.

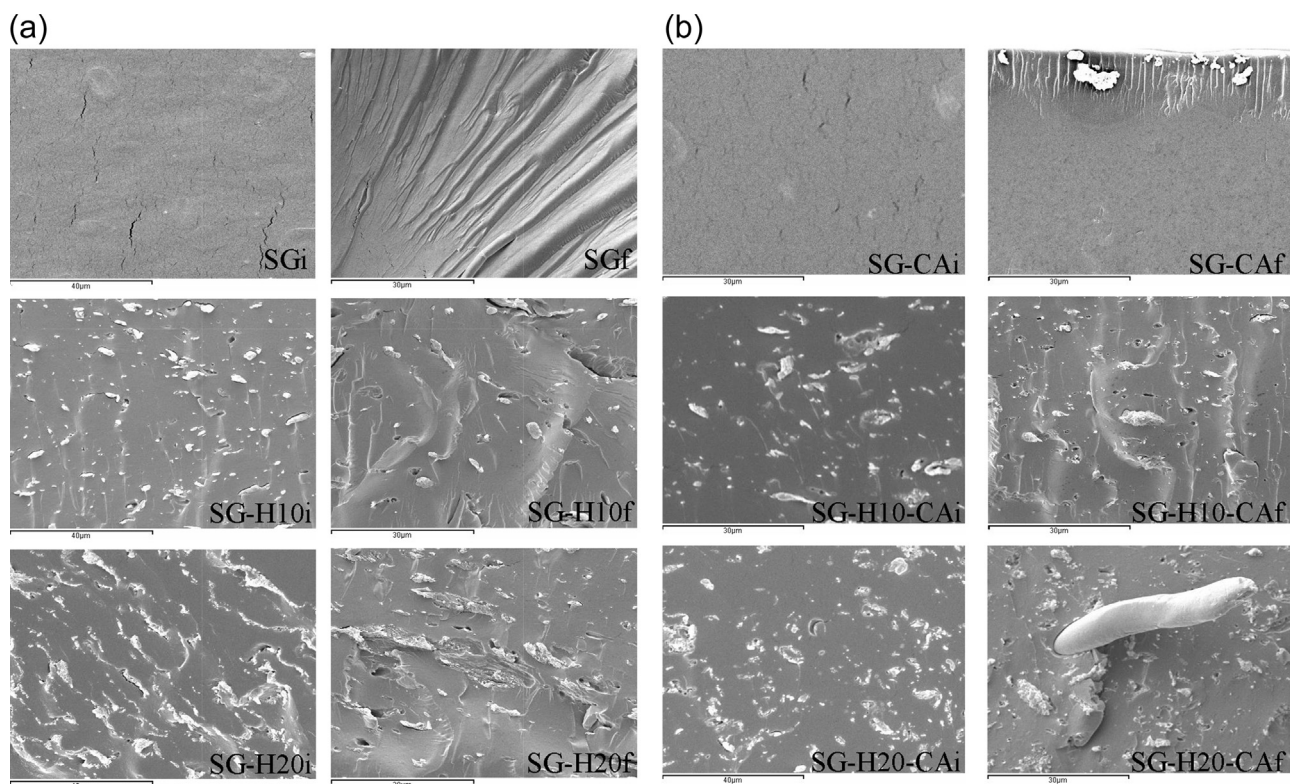


Fig. 1. SEM micrographs of the cross sections of films without (a) and with (b) citric acid after 1 (Initial time) and 5 (Final time) weeks of storage at 53% relative humidity and 25 °C.

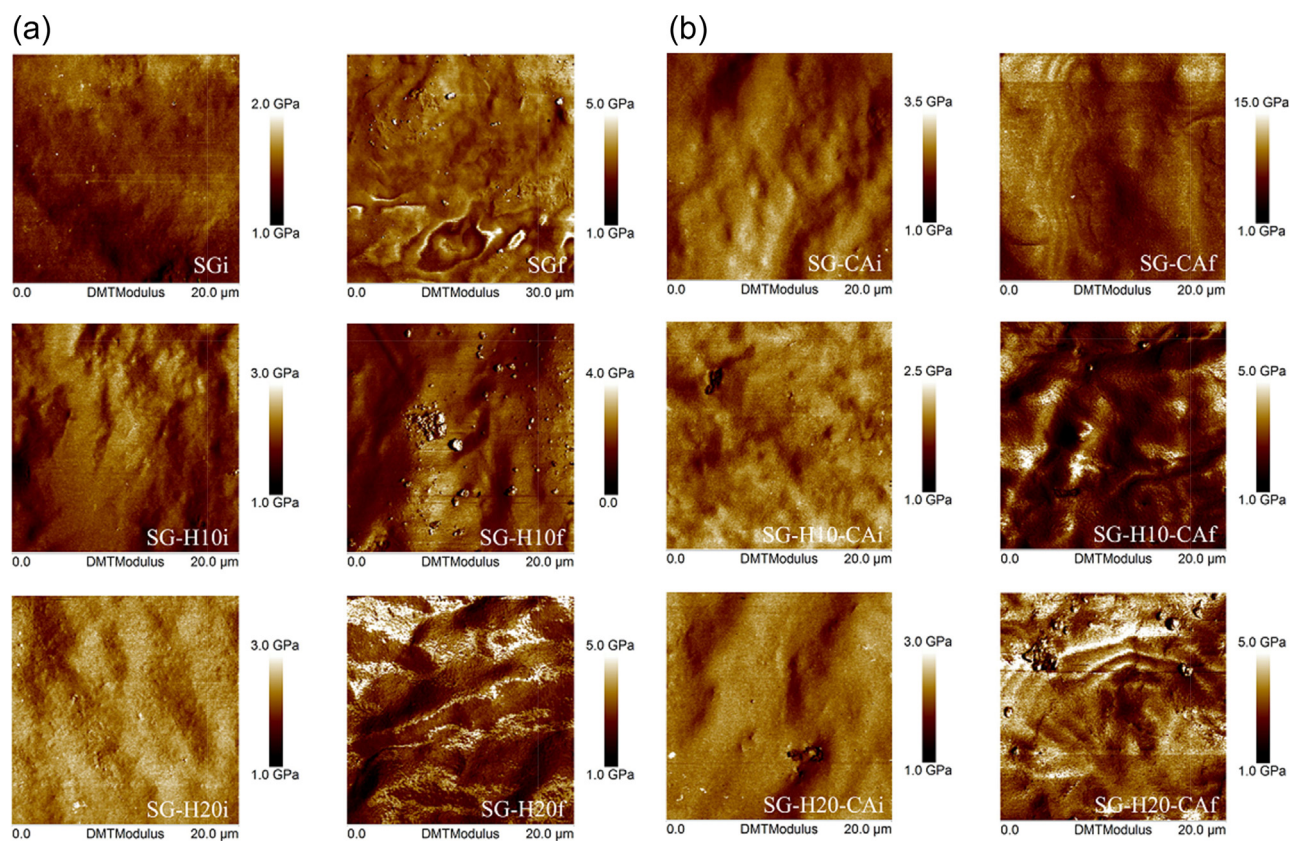


Fig. 2. AFM images (force mode) of films without (a) and with (b) citric acid after 1 (Initial time) and 5 weeks (Final time) of storage at 53% relative humidity and 25 °C.

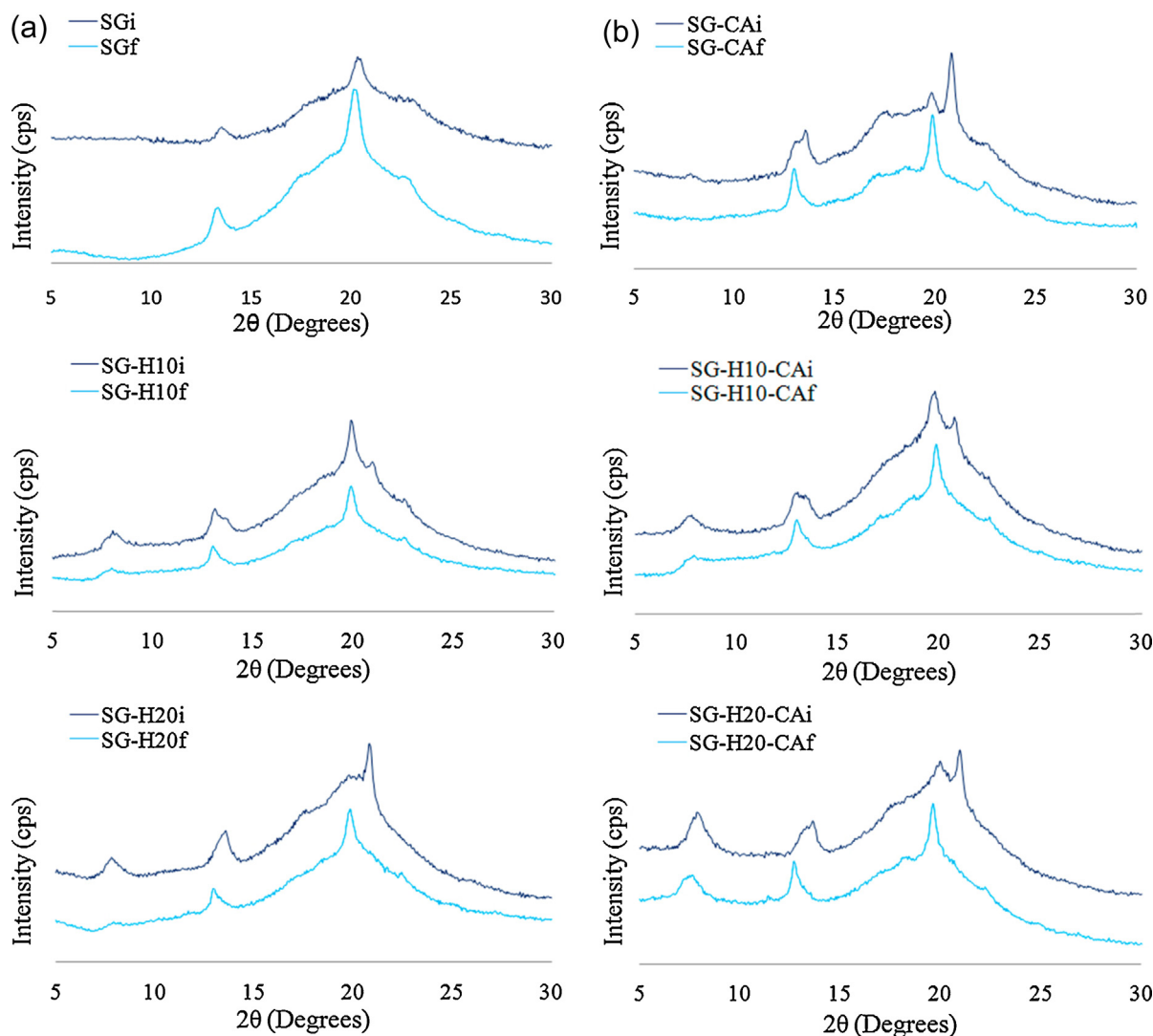


Fig. 3. X-ray diffraction patterns of films without (a) and with (b) citric acid after 1 (Initial time: i) and 5 weeks (Final time: f) of storage at 53% relative humidity and 25 °C.

At initial time, films show a more homogeneous Young's modulus (up to 3.5 GPa), since no drastic changes are observed in the surface maps. Citric acid addition implied an increase in the surface modulus in agreement with the cross-linking effect, which is less marked in films containing HPMC. Likewise, the modulus values significantly increased with storage time (up to 15 GPa in HPMC-free films with citric acid), in part due to the progress of recrystallization phenomena. In HPMC-free samples, a greater increase was observed in line with a greater ratio of crystallinity, as observed by SEM. This increase was especially marked when these samples contained citric acid (SG-CAf), probably due to the combined effect of the cross-linking, which could progress throughout time. In samples with HPMC, more heterogeneous values of surface modulus were obtained, probably due to the fact that crystal growth was inhibited in areas near to HPMC zones.

3.3. X-ray diffraction

X-ray diffraction patterns obtained for both films stored for 1 week and those aged for 5 weeks are presented in Fig. 3. Obtained patterns correspond with the formation of V-type structures, resulting from the formation of complexes of helical amylose forms with substances such as aliphatic fatty acids, surfactants, emulsifiers, alcohols, glycerol or dimethyl sulfoxide (Famá, Rojas,

Goyanes, & Gerschenson, 2005). This type of conformation may be formed when endogenous lipids form complexes within the amylose helices (Gelders, Vanderstukken, Goesart, & Delcour, 2004). These patterns have previously been observed by other authors for starch or amylose with peaks at $2\theta \sim 7^\circ$, 14° and 20° (Chen, Kuo, & Lai, 2009; Famá et al., 2005; Lesmes, Cohen, Shener, & Shimoni, 2009). Nevertheless, the addition of citric acid or HPMC to the starch films promoted the formation of polymorphs since the intensity of peaks at $2\theta 7^\circ$ and 21° is enhanced, whereas the intensity at $2\theta 20^\circ$ decreased in most of the samples stored for 1 week.

In general, the intensity of the peaks increased for stored samples thus indicating the growth in the crystal size, which was especially marked for control film (SG), as observed by SEM (Fig. 1). Nevertheless, most of the crystalline regions for the rest of the films seem to be formed during the film drying step, when the molecular mobility is still high (Rindlav-Westling, Stading, Hermansson, & Gatenholm, 1998; Stading, Rindlav-Westling, & Gatenholm, 2001).

When citric acid was added, the surface modulus obtained by peak force quantitative nanomechanical mapping significantly increased, as mentioned above. Nevertheless, this change is not only attributable to a greater increase in crystallinity, as deduced from X-ray diffraction patterns at initial and final storage times. So, the cross-linking progress or rearrangement of crystals and components throughout storage can be inferred for this sample.

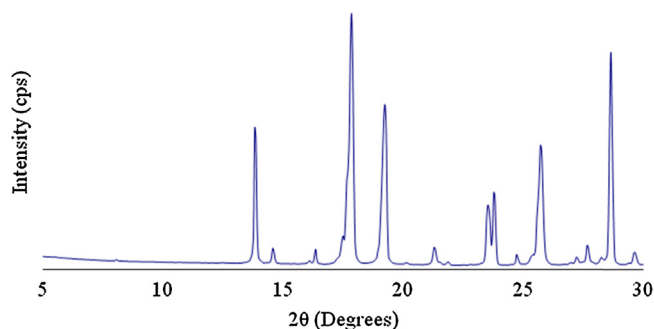


Fig. 4. X-ray diffraction patterns of pure citric acid.

In this sense, Reddy and Yang (2010) suggested that some of the amorphous regions of citric-acid cross-linked starch films may be better oriented after cross-linking, thus affecting the film mechanical behavior. In this sense, SG-CA film presented a sharp peak at 2θ 21° at initial time whose intensity decreased with storage, whereas the intensity of the 2θ 20° peaks increased. This confirms the reorganization of components and the final formation of other polymorph crystals.

Regarding the effect of HPMC addition, it seems that this component is able to limit the starch recrystallization, since no significant changes in intensity are observed in XR diffraction spectra after 5 storage weeks. Nevertheless, as observed for SG-CA film, the relative intensity of some peaks increased or decreased due to the molecular rearrangement and the formation of other polymorphic structures closer to that of starch films. Although HPMC is a totally amorphous polymer (Jiménez et al., 2012a; Kou et al., 2011), starch films containing HPMC were semicrystalline, as observed in Fig. 3. Jiménez et al. (2012a) obtained starch-HPMC films and analyzed, by wide angle X-ray diffraction, their crystallinity. This was lower as the HPMC content increased, the films being almost totally amorphous when the HPMC content was 50%.

Fig. 4 shows the X-ray diffraction pattern of pure citric acid in which the main peaks are at 2θ 14°, 17°, 19°, 24°, 26° and 29°. These are not found in the obtained films, which indicate that no crystalline citric acid is formed during film drying, even when it is non-bonded to the polymers. Similar results are obtained by Shi et al. (2007), which point to its dispersion in the polymeric matrix at molecular level.

3.4. Glass transition of films

DSC analysis of the films allows us to know the possible changes in the glass transition of components due to their interactions in the matrix. In principle, two glass transitions are expected according to the two phases (continuous-starch and dispersed-HPMC phases) observed by SEM. Different authors have also reported the presence of two different glass transitions due to the phase separation of plasticizer at high water activity, where water-polymer interactions predominate over plasticizer-polymer ones (Debeaufort & Voilley, 1997; Jiménez, Fabra, Talens, & Chiralt, 2013). Glass transition values of dry films are shown in Table 1, together with the value of HPMC with the same ratio of glycerol as that present in the films. Fig. 5 shows the obtained thermograms for the different samples in the range of glass transitions. The value for HPMC-glycerol blends was slightly lower than that obtained for starch-glycerol films (113 compared to 125 °C at the midpoint). In the blend films, only one glass transition was clearly observed, which was attributed to the starch phase which represents the greatest proportion of the film. The relatively low ratio of HPMC in the film makes the observation of its glass transition difficult. Nevertheless, the T_g value of the starch phase changed when citric acid or HPMC were added to the

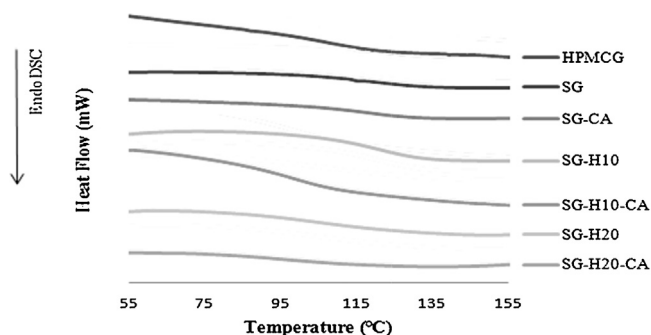


Fig. 5. Thermograms of dry films and HPMC-glycerol sample, showing glass transition.

film. Citric acid incorporation supposed a decrease of about 5 °C in T_g , which can be attributed to the plasticizing effect of the free citric acid in the matrix and/or to the degree of starch depolymerization provoked by citric acid, as deduced from the film solubility values, which reduces the overall molecular weight of the polymers. Different authors (Menzel et al., 2013) report this effect of citric acid in starch systems when it was added as a cross-linking agent. Although an anti-plasticizing effect could be expected from cross-linking due to the associated decrease in the molecular mobility (Yu et al., 2005), the overlapped hydrolysis effect masks it.

When HPMC was incorporated in the film without citric acid, a decrease in the T_g of about 10 °C was also observed, which indicates that a partial miscibility of both polymers occurs in the blend with a T_g value nearer to that of HPMC. When both, citric acid and HPMC, are present in the film, T_g value ranged between 100 and 105 °C, reflecting both the plasticizing effect of citric acid and the mixing effect of HPMC. So, phase separation between starch and HPMC was not total as deduced from these results and the continuous phase is a starch-rich phase containing glycerol, free citric acid and HPMC molecules. Both free citric acid and glycerol could also be present in the HPMC dispersed phase since their chemical structures are compatible.

The decrease in the T_g provoked by both components explains the higher flowability of the blends shown during compression-molding, commented on above.

3.5. Mechanical properties

Fig. 6 shows the stress-Hencky strain curves obtained for the films studied, conditioned at 25 °C and 53% RH for 1 week. The SG film was the most resistant and flexible, while the addition of HPMC brought about a slight reduction to the film resistance to break and to the elongation at break: the higher the HPMC content, the less resistant the film. This can be attributed to the presence of a

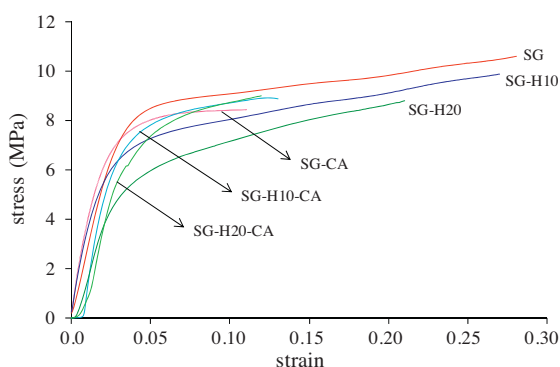


Fig. 6. Typical stress-strain curves of the different films after 1 week of storage.

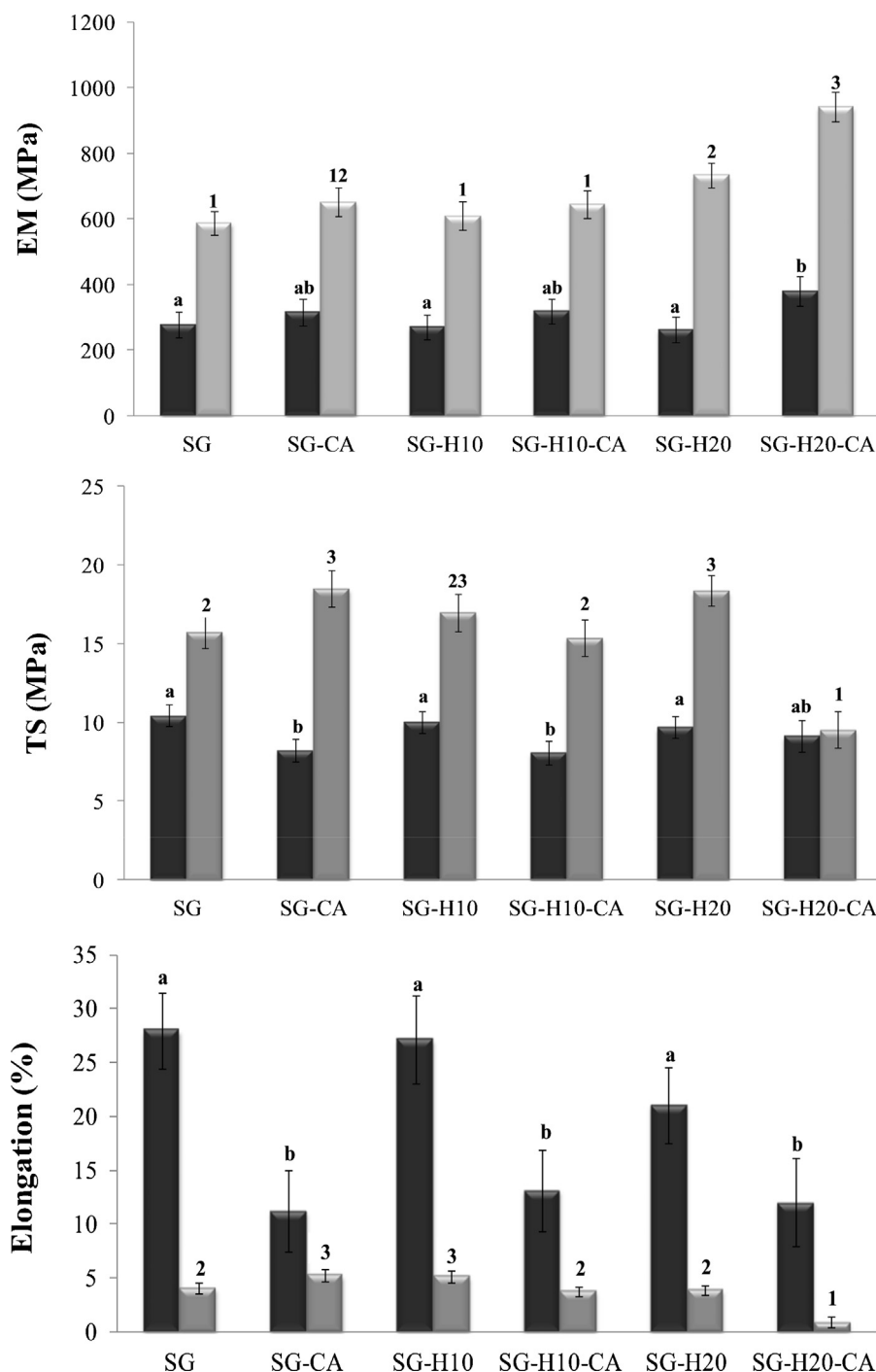


Fig. 7. Mean values of elastic modulus and stress and strain at break for the different films stored for 1 (black bars) and 5 (gray bars) weeks at 53% relative humidity and 25°C.

dispersed phase in the starch matrix (as observed in Fig. 1) which leads to a loss in the cohesion forces in the film. In this sense, a greater dispersed phase ratio lowers the cohesion forces and then the film resistance to break.

The addition of citric acid had a great impact on the film stretchability, since elongation at break was reduced by up to 60% (from 28% to 11%). The shortening of the films caused by the citric acid is coherent with the cross-linking effect, since the slippage of polymer chains during the tensile test is limited by their strong inter-chain bonds. Nevertheless, the elastic modulus only slightly increases after the addition of citric acid, this increase only being significant in the sample with 20% HPMC. Similar behavior was observed for

the tensile strength at break. These results seem to indicate that inter-chain bonding did not progress enough, as observed by other authors for higher contents of citric acid (Reddy & Yang, 2010), and that HPMC chains seem to participate in the cross-linking reactions, probably through the hydroxy-propyl group which can be more readily available for reaction.

Fig. 7 also shows the values of the mechanical parameters of the films after 5 storage weeks. For every formulation, the elongation at break significantly decreases while the elastic modulus and the tensile strength increase. There are different mechanisms responsible for this behavior. On the one hand, starch tends to recrystallize over time, as commented on above, which explains the observed

Table 2

Mean values and standard deviation of water content (Xw: g water/g dry film), water vapour and oxygen permeabilities of the different films after 1 (Initial time) and 5 (Final time) weeks of storage at 53% relative humidity and 25 °C.

Formulation	Xw		WVP (g mm kPa ⁻¹ h ⁻¹ m ⁻²)		OP (×10 ¹³ cm ³ m ⁻¹ s ⁻¹ Pa ⁻¹)	
	Initial	Final	Initial	Final	Initial	Final
SG	0.062 (0.002) ^{a1}	0.079 (0.004) ^{a2}	18 (1) ^{a1}	16 (2) ^{a1}	<D.L.	1.3 (0.5) ^a
SG-CA	0.059 (0.002) ^{ab1}	0.076 (0.002) ^{ab2}	12 (2) ^{b1}	16 (1) ^{a1}	2.6 (0.5) ^{a1}	2.81 (0.04) ^{b1}
SG-H10	0.056 (0.004) ^{b1}	0.075 (0.002) ^{ab2}	13 (1) ^{b1}	14 (2) ^{a1}	1.4 (0.3) ^{a1}	2.5 (0.5) ^{ab1}
SG-H10-CA	0.054 (0.003) ^{b1}	0.073 (0.002) ^{ab2}	11 (2) ^{b1}	11 (2) ^{b1}	2.2 (0.2) ^{a1}	4 (1) ^{c2}
SG-H20	0.055 (0.002) ^{b1}	0.071 (0.003) ^{b2}	12 (1) ^{b1}	11 (1) ^{b1}	4 (1) ^{b1}	5 (1) ^{cd1}
SG-H20-CA	0.054 (0.003) ^{b1}	0.070 (0.007) ^{b1}	9 (1) ^{c1}	10.1 (0.4) ^{b1}	5.0 (1) ^{b1}	6 (1) ^{d1}

D.L.: 0.1 cm³/(m² day).

Different superscript letters within the same column indicate significant differences among formulations ($p < 0.05$).

Different superscript numbers within the same row indicate significant differences due to storage time ($p < 0.05$).

behavior of the formulation without HPMC or citric acid (Mali, Grossmann, García, Martino, & Zaritzky, 2006). Apart from crystallization, the progressive aggregation of polymer chains throughout time has been observed for starch films and starch dispersions (Funami et al., 2005; Jiménez et al., 2012a; Rindlav-Westling et al., 1998) which provokes an increase in the matrix compactness and the subsequent changes in the tensile response. The cross linking effect of citric acid could also be extended throughout time, thus contributing to the changes in the mechanical properties. In this sense, the sample with the highest elastic modulus and lowest stretchability after 5 storage weeks is the one that contains the highest ratio of HPMC and citric acid, which also points to the fact that HPMC chains participate in the citric acid cross-linking action. This phenomenon would be associated with the non-bonded citric acid (11–20%, according to Table 1) that remains free after film formation and which is able to react with the hydroxyl groups of polymers during storage.

3.6. Barrier properties

One of the main features to take into account when biodegradable polymers are used for packaging application is their ability to avoid mass transfer mechanisms. In this way, a good barrier against aroma, water vapour and gases is generally desired. Table 2 shows the values of water vapour, oxygen permeability and water content for films conditioned for 1 week and those aged for 5 weeks. At initial time, the water vapour permeability values were significantly reduced by HPMC and the addition of citric acid, exhibiting the lowest value when both citric acid and the highest HPMC ratio were present in the film. Ghanbarzadeh et al. (2011) also found that the addition of citric acid reduced WVP when the concentrations ranged between 5 and 20%. They explained this effect in terms of the substitution of hydroxyl groups (–OH) by hydrophobic esters and by the fact that citric acid introduced a tortuous path for water molecules to pass through the films due to the cross linking effect. These authors also found that another cellulose derivative (carboxymethylcellulose) was effective at reducing the WVP and related this phenomenon with the fact that carboxymethylcellulose is less hydrophilic than starch (Ma, Chang, & Yu, 2008). Nevertheless, HPMC has been reported to produce worse water vapour barriers (Jiménez et al., 2010, 2012a). Thus, the positive effect of HPMC on the water vapour barrier of the studied films may be due to its cooperative effect on the cross-linking effect, as previously commented on.

Storing the films for 5 weeks had no effect on the WVP values, regardless of the film composition, despite the fact that a significant increase ($p < 0.05$) in the film moisture content occurred during storage. A similar result was reported by Jiménez et al. (2012c) for starch–glycerol films. They found a significant increase in the film's moisture content after 5 storage weeks, at the same time as they became more brittle, as observed in the present work. In this sense,

it is remarkable that the formation of crystalline regions implies a gain in water content, since a great amount of water molecules are bonded to the crystalline conformations (Rindlav, Hulleman, & Gatenholm, 1997). So, the water gain of the films could be associated with the water bonded to the crystalline regions which did not contribute to the plasticization of the amorphous matrix and therefore, did not affect the barrier properties.

As regards oxygen permeability, SG was the least permeable film, mainly at initial time when the equipment was not able to take measurements due to the very low quantity of oxygen molecules that passed through the film. Different studies have pointed out the very good oxygen barrier properties of starch compared with other polymers (Jiménez et al., 2012a,b; Miller & Krochta, 1997). The addition of HPMC significantly increased ($p < 0.05$) the oxygen permeability as previously observed by Jiménez et al. (2012a), which coincides with the higher oxygen permeability of this polymer. Storage time did not affect the oxygen barrier properties, unlike what occurred with the water vapour barrier.

3.7. Optical properties

Optical properties are relevant when a polymeric matrix is tested as a packaging material. Ghanbarzadeh et al. (2010) commented that film color can be an important factor in terms of the consumer acceptance of both edible and inedible films. In the same sense, Hutchings (1999) explained that the most outstanding optical properties with which to evaluate the impact on the appearance and color of coated products are opacity and gloss. Table 3 shows the gloss values (measured at 60°) and internal transmittance values (at 450 nm) for films stored for 1 or 5 weeks at 25 °C and 53% RH. The gloss values were very low for every formulation (between 10.2 GU and 12 GU), regardless of the film composition and the storage time. Higher gloss values have been found for both pure HPMC (Jiménez et al., 2010) and starch–glycerol films (Jiménez et al., 2012c) obtained by casting. Nevertheless, for starch films blended with other polymers, such as chitosan (Bonilla, Talón, Atarés, Vargas, & Chiralt, 2013) or HPMC (Jiménez et al., 2012a), similar gloss values are reported. Film gloss is directly related with the surface topography, which can be determined by atomic force microscopy. The corresponding surface roughness parameters for the obtained films are shown in Table 3, where neither a different composition nor a varying storage time led to there being any significant differences in the parameters.

Table 3 also includes the values of internal transmittance (Ti) for films after 1 and 5 storage weeks. High values of internal transmittance are associated with highly transparent films. On the contrary, more opaque films correspond with low values of internal transmittance. The Ti values ranged from 74 to 81 and were dependent on the formulation, but were not significantly affected by the storage time. Considering these results, all of the films can be considered quite transparent. It is remarkable that, whereas the incorporation

Table 3

Mean values and standard deviation of surface roughness parameters and optical properties of the different films after 1 (Initial time) and 5 (Final time) weeks of storage at 53% relative humidity and 25 °C.

Formulation	Surface roughness				Gloss 60° (GU)		T _i (450 nm)	
	Rq (nm) initial	Rq (nm) final	r (%) initial	r (%) final	Initial	Final	Initial	Final
SG	263 (63) ^{ab1}	356 (64) ^{ab1}	6 (1) ^{a1}	2.5 (0.2) ^{a2}	12.5 (0.4) ^{a1}	11.5 (0.4) ^{ab2}	77 (1) ^{ab1}	76.9 (0.5) ^{a1}
SG-CA	206 (72) ^{ab1}	302 (70) ^{b1}	2.5 (0.2) ^{ab1}	2.5 (0.2) ^{a1}	11.5 (0.4) ^{b1}	12 (1) ^{c2}	80.9 (0.5) ^{c1}	82 (1) ^{c1}
SG-H10	319 (147) ^{a1}	272 (76) ^{ab1}	2.1 (0.4) ^{ab1}	2 (1) ^{a1}	11 (1) ^{b1}	11 (1) ^{a1}	75.5 (0.4) ^{d1}	75.4 (0.5) ^{b1}
SG-H10-CA	233 (35) ^{ab1}	214 (83) ^{a1}	2 (1) ^{b1}	1.3 (0.3) ^{a1}	10.2 (0.3) ^{c1}	12 (1) ^{bc2}	78.2 (0.2) ^{a1}	79.0 (0.3) ^{d1}
SG-H20	169 (43) ^{b1}	214 (20) ^{a1}	1.2 (0.2) ^{b1}	2 (1) ^{a1}	11 (1) ^{bc1}	11.5 (0.5) ^{ab2}	74 (1) ^{d1}	74 (1) ^{b1}
SG-H20-CA	232 (42) ^{ab1}	228 (23) ^{a1}	2 (1) ^{ab1}	2 (1) ^{a1}	10.6 (0.5) ^{bc1}	11 (1) ^{a1}	76 (1) ^{b1}	77 (1) ^{a1}

Different superscript letters within the same column indicate significant differences among formulations ($p < 0.05$).

Different superscript numbers within the same row indicate significant differences due to storage time ($p < 0.05$).

of citric acid promotes a slight increase in transparency, HPMC slightly increases film opacity, in agreement with the appearance of a dispersed phase which enhances light scattering. The effect of citric acid is opposite to that observed by Olivato et al. (2012) for starch–PBAT films obtained by reactive extrusion with citric acid, where a significant increase in opacity is observed. These authors related the cross-linking activity of the acid with a higher polymeric chain compaction, which modifies the refractive index and hinders the passage of the light through the matrix. The relatively low ratio of citric acid (1% with respect to starch) in the films and the commented depolymerization effect, which gives rise to a less compact matrix, can explain the different behavior.

4. Conclusions

Corn starch films containing HPMC (10 and 20%, with respect to starch) obtained by compression molding showed a dispersed phase of HPMC in a continuous starch-rich phase with a lower glass transition than HPMC-free films. The addition of citric acid as a compatibilizer also provoked a decrease in glass transition in line with a depolymerization effect brought about by acid hydrolysis. Both components implied a decrease in the water vapour permeability but a slight increase in oxygen permeability. Although citric acid provoked a mild hardening effect in the films, it greatly decreased extensibility pointing to a weak cross-linking effect, which seems to increase during film storage to a greater extent in films with the highest ratio of HPMC. Starch crystallization was slightly modified by both components which induce the formation of other polymorphs mainly at initial time (1 storage week). Starch crystallization during storage was inhibited by both citric acid and HPMC. A greater ratio of citric acid could be interesting as a means of promoting both a more significant cross-linking effect and greater HPMC compatibility with starch.

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