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Additional Information

1 **Properties of starch-hydroxypropyl methylcellulose based films obtained by**
2 **compression molding.**

3
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13 **ABSTRACT**

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

27

28 **Key words:** Starch, compression molding, citric acid, HPMC, crystallization

29

30 **1. INTRODUCTION**

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

42 The deficiencies in the properties of starch films can be improved by different methods.
43 The phenomenon of the recrystallization of starch films could be solved by combining
44 starch with other polymers, as has been proven by other authors (Funami, Kataoka,
45 Omoto, Goto, Asai & Nishinari, 2005; Jiménez, Fabra, Talens & Chiralt, 2012a).
46 Hydroxypropyl methylcellulose (HPMC) is a cellulose-derived polymer commonly
47 used for obtaining edible, biodegradable films (Albert & Mittal, 2002; Villalobos,
48 Hernández-Muñoz & Chiralt, 2006; Sánchez-González, Vargas, González-Martínez,
49 Chiralt & Cháfer, 2009) with good availability and processability (Fahs, Brogly, Bistac
50 & Schmitt, 2010; Jiménez *et al.*, 2012a) which have excellent film-forming properties
51 (Villalobos *et al.*, 2006). Compared to other polymers, HPMC films have great
52 mechanical strength, (Jiménez, Fabra, Talens & Chiralt, 2010; 2012a) but limited
53 barrier properties. In this sense, Jiménez *et al.* (2012a) observed that the oxygen
54 permeability of HPMC films obtained by casting was approximately 100 times higher

55 than cornstarch films, while the water barrier properties are similar for films made from
56 both polymers.

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

66 The poor compatibility of these polymers can be limited by the incorporation of
67 compatibilizer compounds, which promotes the esterification of the starch -OH groups.
68 For this purpose, cross-linking agents, such as phosphorus oxychloride, sodium
69 trimetaphosphate, sodium tripolyphosphate, epichlorohydrin, and 1,2,3,4-
70 diepoxybutane, were used (Seker & Hanna, 2006; Reddy & Yang, 2010). These
71 substances are relatively toxic and expensive (Reddy & Yang, 2010), so their use in
72 food packaging is not recommendable. However, using polycarboxylic acids as cross-
73 linking agents is a good alternative. This is especially so in the case of citric acid, a low
74 cost organic acid widely used in the food industry, that acts as a cross-linking agent due
75 to the presence of several carboxyl groups in its molecular structure (Ghanbarzadeh,
76 Almasi & Entezami, 2010). These groups can react with the hydroxyl groups of starch
77 molecules through the formation of esters. According to Reddy & Yang (2010), the
78 esterification reaction using polycarboxylic acids occur with the polymer hydroxyl
79 groups, at high temperatures (about 160 °C), through the formation of anhydride groups

80 (Yang, Wang, & Kang, 1997). This interaction can decrease the retrogradation and
81 recrystallization of starch during storage time (Ghanbarzadeh *et al.*, 2011). The
82 compatibilizer effect of the citric acid in the starch-polymer blends, as well as an
83 improvement in the properties of starch films, have been reported in several studies (Yu,
84 Wang & Ma, 2005; Shi, Bi, Zhang, Zhu, Chen, Zhou, Zhang & Tian, 2008; Ma, Chang,
85 Yu & Stumborg, 2009; Reddy & Yang, 2010; Ghanbarzadeh *et al.*, 2010; 2011). The
86 most promising results derived from the addition of citric acid to the polymeric matrix
87 are the increase in thermal stability and the improvement in the barrier properties (Yu *et*
88 *al.*, 2005; Reddy & Yang, 2010).

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

93

94 **2. MATERIALS AND METHODS**

95 **2.1. Materials**

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

101

102 **2.2. Film preparation**

103 Native starch and glycerol, as plasticizer, were dispersed in water. HPMC was hydrated
104 in cold water (5% w/w) under continuous stirring. The HPMC dispersion and the

105 aqueous mixture of starch and glycerol were mixed in different ratios to obtain two
106 blends with 1:0.1 and 1:0.2 starch:HPMC ratios. The polymer:glycerol ratio was 1:0.3.
107 CA was mixed to obtain four blends with and without CA; a constant starch:CA ratio of
108 1:0.01 was used in every case. Blends of starch-glycerol and starch-glycerol-citric acid,
109 in the previously described ratios, were studied as control formulations. The
110 abbreviations used for the studied formulations were: SG: starch-glycerol; CA: citric
111 acid; H10: 10g HPMC/100 g starch; H20: 20g HPMC/100 g starch.
112 The formulations were hot-mixed on a two-roll mill (Model LRM-M-100, Labtech
113 Engineering, Thailand) at 160 °C and 8 rpm for 20 minutes. A visual good miscibility of
114 HPMC, CA and starch was obtained. A trowel was used during mixing to smoothly
115 spread the material on the rolls. The paste sheet formed was removed from the mill and
116 conditioned at 25 °C and 53% **Relative Humidity (RH)**, using a Mg(NO₃)₂ saturated
117 solutions (Panreac Quimica, SA, Castellar del Valles, Barcelona, Spain), for 48 hours.
118 Afterwards, films were obtained by compression molding (Model LP20, Labtech
119 Engineering, Thailand). Four grams of the pre-conditioned paste were put onto steel
120 sheets and pre-heated on the heating unit for about 5 min. Compression molding was
121 performed at 160°C for 2 minutes at a pressure of 30 bars, followed by 6 minutes at
122 130 bars; thereafter, the cooling cycle was applied for 3 minutes. The films were
123 conditioned at 25°C and 53% RH for 1 week for the initial time characterization and for
124 5 weeks for the final time characterization.

125

126 **2.3. Film characterization**

127 2.3.1. Film thickness

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

130

131 2.3.2. Scanning electron microscopy (SEM)

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

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

139

140 2.3.3. X-ray diffraction

141 A diffractometer (XRD, Bruker AXS/D8 Advance) was used to record the X-ray
142 diffraction patterns. All the samples (equilibrated for one and five weeks at 25 °C and at
143 53% RH) were analyzed at 25 °C and 53% RH, between $2\theta = 5^\circ$ and $2\theta = 30^\circ$ using $K\alpha$
144 Cu radiation (λ : 1.542 Å), 40 kV and 40 mA with a step size of 0.05°. For this analysis,
145 samples were cut into 4 cm squares. Pure citric acid was also analyzed, so as to identify
146 its characteristic peaks.

147

148 2.3.4. Atomic force microscopy (AFM)

149 Samples equilibrated for five weeks at 25 °C and 0 % RH, using P₂O₅ saturated
150 solutions (Panreac Quimica, SA, Castellar del Valles, Barcelona, Spain), were used. The
151 surface morphology of the films was analyzed using an atomic force microscope
152 (Multimode 8, Bruker AXS, Santa Barbara, USA) with NanoScope[®] V controller
153 electronics. Measurements were taken from several areas of the film surface (20 μm²)
154 using the PeakForce QNM mode. Method ASME B46.1 (ASME, 1995) was used to

155 calculate the following statistical parameters related with sample roughness: average
156 roughness (Ra: average of the absolute value of the height deviations from a mean
157 surface), root-mean-square roughness (Rq: root-mean-square average of height
158 deviations taken from the mean data plane), and factor of roughness (r: ratio between
159 the three-dimensional surface and two-dimensional area projected onto the threshold
160 plane). The DMT modulus, derived from the Peak Force QNM Mode, was also applied
161 to obtain the surface maps showing the heterogeneities present in the surface properties.
162 Three replicates were considered for each sample.

163

164 2.3.5. Optical properties

165 The Kubelka-Munk theory for multiple scattering was applied to the reflection spectra
166 to determine the film's transparency (Hutchings, 1999). The surface reflectance spectra
167 were determined from 400 to 700 nm using a spectro-colorimeter CM- 3600d (Minolta
168 Co., Tokyo, Japan) on both a white and a black background. As the light passes through
169 the film, it is partially absorbed and scattered, which is quantified by the absorption (K)
170 and the scattering (S) coefficients. The internal transmittance (Ti) of the films was
171 determined using eq. (1). In this equation, R₀ is the reflectance of the film on an ideal
172 black background. Parameters *a* and *b* were calculated by means of eqs. (2) and (3),
173 where R is the reflectance of the sample layer backed by a known reflectance, R_g. Three
174 replicates were used for each sample on the free film surface. For the purposes of the
175 analysis, a wavelength of 450 nm was considered.

176

$$177 \quad T_i = \sqrt{(a - R_0)^2 - b^2} \quad (1)$$

178

$$179 \quad a = \frac{1}{2} \left(R + \frac{R_0 - R + R_g}{R_0 R_g} \right) \quad (2)$$

180

$$181 \quad b = \sqrt{a^2 - 1} \quad (3)$$

182

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

189

190 2.3.6. Moisture content

191 The films that had been previously conditioned at 53% RH were dried for 24h at 60 °C
192 (J.P. Selecta, S.A. Barcelona, España) and placed in a desiccator with P₂O₅ (Panreac
193 Quimica, S.A. Castellar Vallés, Barcelona) adjusting them to 0% RH and 25 °C for 2
194 weeks. The reported results represent the average of three samples.

195

196 2.3.7. Water vapour permeability (WVP)

197 The WVP of films was determined by means of the ASTM E96-95 (ASTM, 1995)
198 gravimetric method, taking the modification proposed by McHugh, Avena-Bustillos &
199 Krochta (1993) into account. Distilled water was placed in Payne permeability cups (3.5
200 cm diameter, Elcometer SPRL, Hermelle/s Argenteau, Belgium) to expose the film to
201 100% RH on one side. Once the films were secured, each cup was placed in a relative
202 humidity equilibrated cabinet at 25 °C, with a fan placed on the top of the cup in order
203 to reduce resistance to water vapour transport, thus avoiding the stagnant layer effect in
204 this exposed side of the film. The RH of the cabinets (53%) was held constant using

205 oversaturated solutions of magnesium nitrate-6-hydrate. The cups were weighed
 206 periodically (0.0001 g) and water vapour transmission (WVTR) was determined from
 207 the slope obtained from the regression analysis of weight loss data versus time, once the
 208 steady state had been reached, divided by the film area. From WVTR data, the vapour
 209 pressure on the film's inner surface (p_2) was obtained using eq. (4), proposed by
 210 McHugh *et al.* (1993), to correct the effect of concentration gradients.

211

$$212 \quad WVTR = \frac{P \cdot D \cdot L_n [P - p_2 \sqrt{P - p_1}]}{R \cdot T \cdot \Delta z} \quad (4)$$

213

214 where P, total pressure (atm); D, diffusivity of water through air at 25 °C (m² /s); R, gas
 215 law constant (82.057 x 10⁻³ m³ atm kmol⁻¹ K⁻¹); T, absolute temperature (K); Δz , mean
 216 stagnant air gap height (m), considering the initial and final z value; p_1 , water vapour
 217 pressure on the solution surface (atm); and p_2 , corrected water vapour pressure on the
 218 film's inner surface (atm). Water vapour permeance was calculated using eq. (5) as a
 219 function of p_2 and p_3 (pressure on the film's outer surface in the cabinet).

220

$$221 \quad permeance = \frac{WVTR}{p_2 - p_3} \quad (5)$$

222

223 Permeability was calculated by multiplying the permeance by film thickness.

224

225 2.3.8. Oxygen permeability (OP)

226 The oxygen permeation rate of the films was determined at 53% RH and 25 °C using an
 227 OX-TRAN (Model 2/21 ML Mocon Lippke, Neuwied, Germany). The samples were
 228 conditioned at the relative humidity level of the test in a desiccator using magnesium
 229 nitrate-6-hydrate saturated solutions. Three samples were placed in the equipment for

230 analysis, and the transmission values were determined every 20 min until equilibrium
231 was reached. The exposure area during the tests was 50 cm² for each sample. To obtain
232 the oxygen permeability, the film thickness was considered in every case.

233

234 2.3.9. Tensile properties

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

244

245 2.3.10. Film solubility and bonded citric acid

246 Film solubility was determined by keeping the sample in bidistilled water at a film:
247 water ratio of 1:10, for 48 h. Three replicates were made for each formulation. After
248 48h, the film samples were transferred to a convection oven (J.P. Selecta, S.A.,
249 Barcelona, Spain) for 24 h at 60 °C to remove the free water and afterwards transferred
250 to a desiccator with P₂O₅ at 25 °C for 2 weeks to complete film drying. Film water
251 solubility was estimated from its initial and final weights. The water solution was used
252 to determine the bonded citric acid in the film. Free citric acid was assumed to dissolve
253 in the aqueous phase and was determined by titration of an aliquot of the solution with

254 NaOH (0.1 N), using phenolphthalein as indicator. Three replicates were considered for
255 each formulation.

256

257 2.3.11. Thermal properties

258 A Differential Scanning Calorimeter (DSC 1 Star^e System, Mettler-Toledo Inc.,
259 Switzerland) was used to analyze the thermal properties. Weighted amounts of samples
260 were placed into aluminum pans and sealed and drilled to promote the bonded moisture
261 loss during heating. The curves were obtained using a double scan. First, a scan from 0
262 °C to 160 °C at 50 °C / min was used where the bonded water in the film was
263 eliminated. Then the temperature was lowered to 0 °C at the same speed, and finally
264 heated to 160 °C at 20 °C / min (in this scan, starch glass transition was analyzed). The
265 initial and final weights of the pans were registered to assess water loss during the first
266 heating.

267

268 2.3.12. Statistical analysis

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

272

273 **3. RESULTS**

274 **3.1. Film thickness, water solubility and bonded citric acid.**

275 Constant compression molding conditions were maintained during processing in order
276 to submit all the formulations to a similar thermomechanical treatment. In agreement
277 with the different composition, the differing response of the samples to the same
278 treatment can be estimated by the film thickness. The values of this parameter obtained

279 for every formulation are shown in Table 1. As can be observed, the film thickness
280 decreases when HPMC was incorporated into the film (from 268 μm to 204 μm). This
281 phenomenon is related with the increase in the blend flowability when HPMC is present
282 in the blend, which is probably related with the decrease in the glass transition of the
283 blend commented on below. The addition of citric acid also contributed to the decrease
284 in the film thickness, thus indicating the promotion of the blend's flowability. Due to its
285 cross-linking effect, citric acid has not only been reported to be a compatibilizer of
286 biopolymers (Reddy & Yang, 2010; Olivatto, Grossmann, Yamashita, Eiras & Pessan,
287 2012; Menzel, Olsson, Plivelic, Andersson, Johansson, Kuktaite, Järnström & Koch,
288 2013) but also a plasticizer in its free form (Ghanbarzadeh *et al.*, 2011). Thus, this
289 organic acid can have a relevant impact on the rheological behaviour of blends during
290 compression, which affects the final film thickness.

291 Table 1 also shows the solubility of the studied films in water **at initial time. Values at**
292 **final time (5 weeks) were not included due to the fact that no significant differences**
293 **were found with respect to the initial values.** The results show that the addition of citric
294 acid is a key factor in terms of the integrity of films submitted to water action. Films
295 containing citric acid showed higher solubility (0.31-0.35g/g) than citric acid-free films
296 (0.15-0.19g/g). These results seem contradictory since the cross-linking activity of citric
297 acid is related with an increase in the hydrophobicity as a result of the formation of ester
298 groups. Nevertheless, the addition of citric acid is also related with a decrease in the pH
299 values, which may give rise to a partial hydrolysis of glucosidic chains (Olsson,
300 Menzel, Johansson, Andersson, Koch & Järnström, 2013). In this way, the values of
301 bonded citric acid in films **at 5 weeks of storage time** (Table 1) are relevant since an
302 important amount of citric acid remains free to contribute to the pH decrease. The
303 contents of bonded citric acid ranged from 80 % (SG-H20-CA) to 89 % (SG-H10-CA)

304 with respect to the total amount present in the film, there being no significant
305 differences ($p>0.05$) between samples. This indicates that this component partially
306 reacts with the –OH groups of the polymer chains, although about 15 % of the added
307 amount is free in the matrix and could act as plasticizer.

308

309 **3.2. Film microstructure**

310 Figures 1a and b show SEM micrographs of the cross sections of films, containing
311 (Figure 1b) or not (Figure 1a) citric acid, conditioned at 25 °C and 53 % RH for 1
312 (initial time) or 5 weeks (final time). At the initial time, the control formulations (SGi
313 and SG-CAi) exhibited a homogeneous structure with some cracks which are related
314 with the more brittle nature of this formulation under the observation conditions. The
315 low water content and the electron beam in the microscopy chamber provoked the
316 formation of these microcracks, as observed previously for other starch films (Jiménez,
317 Fabra, Talens & Chiralt, 2012b). The addition of HPMC gave rise to a two-phase
318 structure in which this polysaccharide constitutes the dispersed phase embedded in a
319 starch continuous phase, which can be observed both at initial and final storage times.
320 The incompatibility of HPMC and starch has previously been observed in films
321 obtained by casting (Jiménez *et al.*, 2012a). Phase separation has also been reported in
322 other polysaccharide blends such as potato maltodextrin in admixture with locust bean
323 gum, gum arabic and carboxymethyl cellulose, and starch–chitosan blend films
324 (Annable, Fitton, Harris, Philips, & Williams, 1994; Mathew & Abraham, 2008). The
325 different behaviour of continuous and dispersed phases in response to cryofracture is
326 remarkable. Whereas a very hard, rigid matrix can be deduced for the starch continuous
327 phase, HPMC particles can be inferred to be more rubbery and soft in nature, and, in
328 some cases, they appeared greatly deformable (sample SG-H20-CAf).

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

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

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

353 The peak force quantitative nanomechanical mapping AFM-based method is a good tool
354 with which to analyse the nanostructure of biopolymer based films. This tool allows us
355 to measure the Young modulus of the film at each point on its surface. Figure 2 shows
356 the results obtained for studied films stored for 1 week and 5 weeks under controlled
357 conditions. Raw data were converted into 2D images and their scale is expressed as
358 DMT modulus. At initial time, films show a more homogeneous Young's modulus (up
359 to 3.5 GPa), since no drastic changes are observed in the surface maps. Citric acid
360 addition implied an increase in the surface modulus in agreement with the cross-linking
361 effect, which is less marked in films containing HPMC. Likewise, the modulus values
362 significantly increased with storage time (up to 15 GPa in HPMC-free films with citric
363 acid), in part due to the progress of recrystallization phenomena. In HPMC-free
364 samples, a greater increase was observed in line with a greater ratio of crystallinity, as
365 observed by SEM. This increase was especially marked when these samples contained
366 citric acid (SG-CAf), probably due to the combined effect of the cross-linking, which
367 could progress throughout time. In samples with HPMC, more heterogeneous values of
368 surface modulus were obtained, probably due to the fact that crystal growth was
369 inhibited in areas near to HPMC zones.

370

371 **3.3. X-ray diffraction**

372 X-ray diffraction patterns obtained for both films stored for 1 week and those aged for 5
373 weeks are presented in Figure 3. Obtained patterns correspond with the formation of V-
374 type structures, resulting from the formation of complexes of helical amylose forms
375 with substances such as aliphatic fatty acids, surfactants, emulsifiers, alcohols, glycerol
376 or dimethyl sulfoxide (Famá, Rojas, Goyanes & Gerschenson, 2005). This type of
377 conformation may be formed when endogenous lipids form complexes within the

378 amylose helices (Gelders, Vanderstukken, Goesart, & Delcour, 2004). These patterns
379 have previously been observed by other authors for starch or amylose with peaks at 2θ
380 ~ 7 , 14 and 20° (Famá *et al.*, 2005; Chen, Kuo & Lai, 2009; Lesmes, Cohen, Shener &
381 Shimoni, 2009). Nevertheless, the addition of citric acid or HPMC to the starch films
382 promoted the formation of polymorphs since the intensity of peaks at 2θ 7° and 21° is
383 enhanced, whereas the intensity at 2θ 20° decreased in most of the samples stored for 1
384 week.

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

391 When citric acid was added, the surface modulus obtained by peak force quantitative
392 nanomechanical mapping significantly increased, as mentioned above. Nevertheless,
393 this change is not only attributable to a greater increase in crystallinity, as deduced from
394 X-R diffraction patterns at initial and final storage times. So, the cross-linking progress
395 or rearrangement of crystals and components throughout storage can be inferred for this
396 sample. In this sense, Reddy & Yang (2010) suggested that some of the amorphous
397 regions of citric-acid cross-linked starch films may be better oriented after cross-linking,
398 thus affecting the film mechanical behaviour. In this sense, SG-CA film presented a
399 sharp peak at 2θ 21° at initial time whose intensity decreased with storage, whereas the
400 intensity of the 2θ 20° peaks increased. This confirms the reorganization of components
401 and the final formation of other polymorph crystals.

402 Regarding the effect of HPMC addition, it seems that this component is able to limit
403 the starch recrystallization, since no significant changes in intensity are observed in XR
404 diffraction spectra after 5 storage weeks. Nevertheless, as observed for SG-CA film, the
405 relative intensity of some peaks increased or decreased due to the molecular
406 rearrangement and the formation of other polymorphic structures closer to that of starch
407 films. Although HPMC is a totally amorphous polymer (Cai, Xu, Wang, Liu, Yang, &
408 Zhang, 2011; Jiménez *et al.*, 2012a), starch films containing HPMC were
409 semicrystalline, as observed in Figure 3. Jiménez *et al.* (2012a) obtained starch-HPMC
410 films and analysed, by wide angle X-ray diffraction, their crystallinity. This was lower
411 as the HPMC content increased, the films being almost totally amorphous when the
412 HPMC content was 50 %.

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

418

419 **3.4. Glass transition of films**

420 DSC analysis of the films allows us to know the possible changes in the glass transition
421 of components due to their interactions in the matrix. In principle, two glass transitions
422 are expected according to the two phases (continuous-starch and dispersed-HPMC
423 phases) observed by SEM. Different authors have also reported the presence of two
424 different glass transitions due to the phase separation of plasticizer at high water
425 activity, where water-polymer interactions predominate over plasticizer-polymer ones
426 (Debeaufort & Voilley, 1997; Jiménez, Fabra, Talens & Chiralt, 2013). Glass transition

427 values of dry films are shown in Table 1, together with the value of HPMC with the
428 same ratio of glycerol as that present in the films. **Figure 5 shows the obtained**
429 **thermograms for the different samples in the range of glass transitions.** The value for
430 HPMC-glycerol blends was slightly lower than that obtained for starch-glycerol films
431 (113 compared to 125 °C at the midpoint). In the blend films, only one glass transition
432 was clearly observed, which was attributed to the starch phase which represents the
433 greatest proportion of the film. The relatively low ratio of HPMC in the film makes the
434 observation of its glass transition difficult. Nevertheless, the T_g value of the starch
435 phase changed when citric acid or HPMC were added to the film. Citric acid
436 incorporation supposed a decrease of about 5 °C in T_g, which can be attributed to the
437 plasticizing effect of the free citric acid in the matrix and/or to the degree of starch
438 depolymerization provoked by citric acid, as deduced from the film solubility values,
439 which reduces the overall molecular weight of the polymers. Different authors (Menzel,
440 Olsson, Plivelic, Andersson, Johansson, Kuktaite, Järnström & Koch, 2013) report this
441 effect of citric acid in starch systems when it was added as a cross-linking agent.
442 Although an anti-plasticizing effect could be expected from cross-linking due to the
443 associated decrease in the molecular mobility (Yu, Wang & Ma, 2005), the overlapped
444 hydrolysis effect masks it.

445 When HPMC was incorporated in the film without citric acid, a decrease in the T_g of
446 about 10 °C was also observed, which indicates that a partial miscibility of both
447 polymers occurs in the blend with a T_g value nearer to that of HPMC. When both, citric
448 acid and HPMC, are present in the film, T_g value ranged between 100-105 °C,
449 reflecting both the plasticizing effect of citric acid and the mixing effect of HPMC. So,
450 phase separation between starch and HPMC was not total as deduced from these results
451 and the continuous phase is a starch-rich phase containing glycerol, free citric acid and

452 HPMC molecules. Both free citric acid and glycerol could also be present in the HPMC
453 dispersed phase since their chemical structures are compatible.

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

456

457 **3.5. Mechanical properties**

458 Figure 6 shows the stress-Hencky strain curves obtained for the films studied,
459 conditioned at 25 °C and 53 % RH for 1 week. The SG film was the most resistant and
460 flexible, while the addition of HPMC brought about a slight reduction to the film
461 resistance to break and to the elongation at break: the higher the HPMC content, the less
462 resistant the film. This can be attributed to the presence of a dispersed phase in the
463 starch matrix (as observed in Figure 1) which leads to a loss in the cohesion forces in
464 the film. In this sense, a greater dispersed phase ratio lowers the cohesion forces and
465 then the film resistance to break.

466 The addition of citric acid had a great impact on the film stretchability, since elongation
467 at break was reduced by up to 60 % (from 28 % to 11 %). The shortening of the films
468 caused by the citric acid is coherent with the cross-linking effect, since the slippage of
469 polymer chains during the tensile test is limited by their strong inter-chain bonds.
470 Nevertheless, the elastic modulus only slightly increases after the addition of citric acid,
471 this increase only being significant in the sample with 20 % HPMC. Similar behaviour
472 was observed for the tensile strength at break. These results seem to indicate that inter-
473 chain bonding did not progress enough, as observed by other authors for higher contents
474 of citric acid (Reddy & Yang (2010), and that HPMC chains seem to participate in the
475 cross-linking reactions, probably through the hydroxy-propyl group which can be more
476 readily available for reaction.

477 Figure 7 also shows the values of the mechanical parameters of the films after 5 storage
478 weeks. For every formulation, the elongation at break significantly decreases while the
479 elastic modulus and the tensile strength increase. There are different mechanisms
480 responsible for this behaviour. On the one hand, starch tends to recrystallize over time,
481 as commented on above, which explains the observed behaviour of the formulation
482 without HPMC or citric acid (Mali, Grossmann, García, Martino & Zaritzky, 2006).
483 Apart from crystallization, the progressive aggregation of polymer chains throughout
484 time has been observed for starch films and starch dispersions (Rindlav-Westling *et al.*,
485 1998; Funami *et al.*, 2005; Jiménez *et al.*, 2012a) which provokes an increase in the
486 matrix compactness and the subsequent changes in the tensile response. The cross
487 linking effect of citric acid could also be extended throughout time, thus contributing to
488 the changes in the mechanical properties. In this sense, the sample with the highest
489 elastic modulus and lowest stretchability after 5 storage weeks is the one that contains
490 the highest ratio of HPMC and citric acid, which also points to the fact that HPMC
491 chains participate in the citric acid cross-linking action. This phenomenon would be
492 associated with the non-bonded citric acid (11-20%, according to Table 1) that remains
493 free after film formation and which is able to react with the hydroxyl groups of
494 polymers during storage

495

496 **3.6. Barrier properties**

497 One of the main features to take into account when biodegradable polymers are used for
498 packaging application is their ability to avoid mass transfer mechanisms. In this way, a
499 good barrier against aroma, water vapour and gases is generally desired. Table 2 shows
500 the values of water vapour, oxygen permeability and water content for films conditioned
501 for 1 week and those aged for 5 weeks. At initial time, the water vapour permeability

502 values were significantly reduced by HPMC and the addition of citric acid, exhibiting
503 the lowest value when both citric acid and the highest HPMC ratio were present in the
504 film. Ghanbarzadeh *et al.* (2011) also found that the addition of citric acid reduced
505 WVP when the concentrations ranged between 5 and 20 %. They explained this effect
506 in terms of the substitution of hydroxyl groups (-OH) by hydrophobic esters and by the
507 fact that citric acid introduced a tortuous path for water molecules to pass through the
508 films due to the cross linking effect. These authors also found that another cellulose
509 derivative (carboxymethylcellulose) was effective at reducing the WVP and related this
510 phenomenon with the fact that carboxymethylcellulose is less hydrophilic than starch
511 (Ma, Chang & Yu, 2008). Nevertheless, HPMC has been reported to produce worse
512 water vapour barriers (Jiménez *et al.*, 2010; 2012a). Thus, the positive effect of HPMC
513 on the water vapour barrier of the studied films may be due to its cooperative effect on
514 the cross-linking effect, as previously commented on.

515 Storing the films for 5 weeks had no effect on the WVP values, regardless of the film
516 composition, despite the fact that a significant increase ($p < 0.05$) in the film moisture
517 content occurred during storage. A similar result was reported by Jiménez *et al.* (2012c)
518 for starch-glycerol films. They found a significant increase in the film's moisture
519 content after 5 storage weeks, at the same time as they became more brittle, as observed
520 in the present work. In this sense, it is remarkable that the formation of crystalline
521 regions implies a gain in water content, since a great amount of water molecules are
522 bonded to the crystalline conformations (Rindlav, Hulleman, & Gatenholm, 1997). So,
523 the water gain of the films could be associated with the water bonded to the crystalline
524 regions which did not contribute to the plasticization of the amorphous matrix and
525 therefore, did not affect the barrier properties.

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

535 **3.7. Optical properties**

536 Optical properties are relevant when a polymeric matrix is tested as a packaging
537 material. Ghanbarzadeh *et al.* (2010) commented that film colour can be an important
538 factor in terms of the consumer acceptance of both edible and inedible films. In the
539 same sense, Hutchings (1999) explained that the most outstanding optical properties
540 with which to evaluate the impact on the appearance and colour of coated products are
541 opacity and gloss. Table 3 shows the gloss values (measured at 60°) and internal
542 transmittance values (at 450 nm) for films stored for 1 or 5 weeks at 25 °C and 53 %
543 RH. The gloss values were very low for every formulation (between 10.2 GU to 12
544 GU), regardless of the film composition and the storage time. Higher gloss values have
545 been found for both pure HPMC (Jiménez *et al.*, 2010) and starch-glycerol films
546 (Jiménez *et al.*, 2012c) obtained by casting. Nevertheless, for starch films blended with
547 other polymers, such as chitosan (Bonilla, Talón, Atarés, Vargas & Chiralt, 2013) or
548 HPMC (Jiménez *et al.*, 2012a), similar gloss values are reported. Film gloss is directly
549 related with the surface topography, which can be determined by atomic force
550 microscopy. The corresponding surface roughness parameters for the obtained films are

551 shown in Table 3, where neither a different composition nor a varying storage time led
552 to there being any significant differences in the parameters.

553 Table 3 also includes the values of internal transmittance (T_i) for films after 1 and 5
554 storage weeks. High values of internal transmittance are associated with highly
555 transparent films. On the contrary, more opaque films correspond with low values of
556 internal transmittance. The T_i values ranged from 74 to 81 and were dependent on the
557 formulation, but were not significantly affected by the storage time. Considering these
558 results, all of the films can be considered quite transparent. It is remarkable that,
559 whereas the incorporation of citric acid promotes a slight increase in transparency,
560 HPMC slightly increases film opacity, in agreement with the appearance of a dispersed
561 phase which enhances light scattering. The effect of citric acid is opposite to that
562 observed by Olivatto, Grossmann, Yamashita, Eiras & Pessan (2012) for starch-PBAT
563 films obtained by reactive extrusion with citric acid, where a significant increase in
564 opacity is observed. These authors related the cross-linking activity of the acid with a
565 higher polymeric chain compaction, which modifies the refractive index and hinders the
566 passage of the light through the matrix. The relatively low ratio of citric acid (1 % with
567 respect to starch) in the films and the commented depolymerisation effect, which gives
568 rise to a less compact matrix, can explain the different behaviour

569

570

571 **4. CONCLUSIONS**

572 Corn starch films containing HPMC (10 and 20 %, with respect to starch) obtained by
573 compression molding showed a dispersed phase of HPMC in a continuous starch-rich
574 phase with a lower glass transition than HPMC-free films. The addition of citric acid as
575 a compatibilizer also provoked a decrease in glass transition in line with a

576 depolymerisation effect brought about by acid hydrolysis. Both components implied a
577 decrease in the water vapour permeability but a slight increase in oxygen permeability.
578 Although citric acid provoked a mild hardening effect in the films, it greatly decreased
579 extensibility pointing to a weak cross-linking effect, which seems to increase during
580 film storage to a greater extent in films with the highest ratio of HPMC. Starch
581 crystallization was slightly modified by both components which induce the formation of
582 other polymorphs mainly at initial time (1 storage week). Starch crystallization during
583 storage was inhibited by both citric acid and HPMC. A greater ratio of citric acid could
584 be interesting as a means of promoting both a more significant cross-linking effect and
585 greater HPMC compatibility with starch.

586

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592

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