

## Accepted Manuscript

Title: Biodegradable and non-retrogradable eco-films based on starch-glycerol with citric acid as crosslinking agent

Author: Paula González Seligra Carolina Medina Jaramillo  
Lucía Famá Silvia Goyanes



PII: S0144-8617(15)01131-5  
DOI: <http://dx.doi.org/doi:10.1016/j.carbpol.2015.11.041>  
Reference: CARP 10560

To appear in:

Received date: 27-8-2015  
Revised date: 16-11-2015  
Accepted date: 17-11-2015

Please cite this article as: Seligra, P. G., Jaramillo, C. M., Famá, L., and Goyanes, S., Biodegradable and non-retrogradable eco-films based on starch-glycerol with citric acid as crosslinking agent., *Carbohydrate Polymers* (2015), <http://dx.doi.org/10.1016/j.carbpol.2015.11.041>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 Biodegradable and non-retrogradable eco-films based on starch-glycerol with  
2 citric acid as crosslinking agent.

3  
4 Paula González Seligra<sup>a</sup>, Carolina Medina Jaramillo<sup>b</sup>, Lucía Famá<sup>a</sup>, Silvia  
5 Goyanes<sup>a\*</sup>

6  
7 <sup>a</sup> LP&MC, Dep. de Física – IFIBA (CONICET). FCEyN. UBA. Ciudad  
8 Universitaria (1428), CABA, Argentina.

9 <sup>b</sup> Instituto de Tecnología en Polímeros y Nanotecnología ITPN (UBA-  
10 CONICET). FCEyN. UBA. Av. Las Heras 2214, C1127AAQ, CABA,  
11 Argentina.

12  
13 \*Corresponding author. Tel: + 54 11 4576 3300 (ext. 255) e-mail addresses:  
14 goyanes@df.uba.ar

## 15 Abstract

16  
17  
18 Biodegradable and non-retrogradable starch-glycerol based films were obtained  
19 using citric acid (CA) as crosslinking agent at 75 °C. This material allowed  
20 decreasing water vapor permeability (WVP) more than 35 %, remained  
21 amorphous for at least 45 days as a result of the network formed by the CA that  
22 avoided starch retrogradation and maintained the degradability in compost,  
23 occurring only six days after the films without citric acid. A simulation of the  
24 gelatinization process of starch-glycerol with and without CA, using a  
25 differential thermal analysis device, showed that the system with CA completed  
26 the gelatinization 5 °C before than the other and, CA first reacted with glycerol  
27 and then starch-glycerol-CA reaction occurred. The temperature at which the  
28 gelatinization process was carried out was critical to obtain the best results. An  
29 increase of gelatinization process temperature at 85 °C in system with CA, led to  
30 a worsening on WVP and its integrity after a swelling process with  
31 dimethylsulphoxide (DMSO), compared to the films processed at 75 °C.

32  
33 Keywords: starch, citric acid, crosslinking, biodegradable edible films,  
34 biodegradability.

## 35 1. Introduction

36  
37  
38 In light of the global concern for the accumulation of waste, biodegradable and  
39 compostable materials were extensively investigated, in order to partially  
40 replace petroleum-based plastics. Among the many polymers used to develop  
41 biodegradable films, starch has been focus of investigations because it is a  
42 natural polymer from renewable resources, abundant and low cost, that is  
43 capable of forming continuous thermoplastic materials (Bertuzzi, Armada, &  
44 Gottifredi, 2007; Famá, Bittante, Sobral, Goyanes, & Gerschenson, 2010;  
45 Flores, Famá, Rojas, Goyanes, & Gerschenson, 2007; Gutiérrez, Pérez, Guzmán,  
46 Tapia, & Famá, 2015; Romero-Bastida et al., 2005). However, its hydrophilic  
47 character leads to materials with poor moisture barrier properties and high water  
48 sensitivity, being very dependent to ambient humidity, limiting their application  
49 as alternative packaging (Averous & Boquillon, 2004).

50 Another starch problem is its high retrogradation during storage time, causing  
51 changes in its crystallinity and thus in its performance such as texture, color, etc.  
52 (Farhat, Blanshard, & Mitchell, 2000).

53 Retrogradation process occurs in two different stages. The first and faster one is  
54 due to the amylose retrogradation, while the second and slower one is due to the  
55 formation of an ordered structure of amylopectin (Forssell, Hulleman,  
56 Myllärinen, Moates, & Parker, 1999).

57 Crosslinking reaction appears as a common approach to limit starch  
58 retrogradation and to improve its performance for various applications. This  
59 process uses multifunctional group reagents (crosslinking agents) to react with  
60 the hydroxyl groups of starch (responsible for its hydrophilicity), leading to new

61 chemical bonds between molecular starch chains, getting a different polymer  
62 network. By introducing hydrophobic ester groups to substitute hydroxyl groups  
63 of thermoplastic starch, its hydrophilic character can be reduced and  
64 retrogradation can be prevented (Ghanbarzadeh, Almasi, & Entezami, 2011; Shi  
65 et al., 2007; Zhou, Zhang, Ma, & Tong, 2008).

66 Citric acid (CA) is a natural organic acid with multi-carboxylic structure, reason  
67 why it can be used as starch crosslinking agent (Salomão Garcia et al., 2014;  
68 Zuraida, Yusliza, Anuar, & Mohd Khairul Muhaimin, 2012). There are several  
69 researches showing that the addition of CA to starch base compounds, improves  
70 their barrier properties and that the effectiveness of citric acid to crosslink the  
71 starch strongly depends on manufacturing conditions (Menzel et al., 2013;  
72 Olsson, Hedenqvist, Johansson, & Järnström, 2013a; Olsson, Hedenqvist,  
73 Johansson, & Järnström, 2013b; Reddy & Yang, 2010).

74 Several works of literature use citric acid as crosslinking agent for starch. In the  
75 case of films obtained by casting technique, there is no agreement about the  
76 protocol stage in which citric acid must be added. Some authors (Reddy &  
77 Yang, 2010; Ghanbarzadeh et al., 2011) mix citric acid with water, glycerol and  
78 starch before gelatinization process, while others, in order to avoid hydrolysis,  
79 first gelatinize starch, cool the mixture, add the CA and finally do the drying  
80 stage (Shi et al., 2008; Menzel et al., 2013; Olsson et al., 2013a; Olsson et al.,  
81 2013b). In particular, the work of Menzel et al. (2013) should be mentioned. In  
82 that investigation the authors reported that it was possible to obtain crosslinked  
83 starch gelatinizing starch at low temperature from the following protocol: first,  
84 the starch was gelatinized in boiling water bath for 45 min; then, the gel was led  
85 to room temperature and, at that moment, CA was introduced; finally, starch-CA  
86 system was dried at 70 °C for 5 h. In the cases in which it is included after starch  
87 gelatinization, the influence of the drying temperature in the crosslinking or  
88 hydrolysis of starch is one of the most discussed points in the literature.

89 However, there is agreement in the fact that high drying temperatures lead to a  
90 high hydrolysis degree (Menzel et al., 2013; Olsson et al., 2013a; Olsson et al.,  
91 2013b).

92 For the crosslinking reaction between starch and CA to occur, temperature is  
93 required. The discussion is in which stage of the development of the films this  
94 reaction occurs, and what is the order in which the different components of the  
95 films react. In the case where CA is added after the gelatinization process,  
96 obviously the crosslinking reaction occurs during the drying of the mixture.  
97 However, when CA is introduced prior to starch gelatinization, the reactions  
98 may occur at any stage of the films obtaining process. To the best of our  
99 knowledge there are no researches in the literature discussing this point.

100 Moreover, there have not been reported studies simulating the reactions between  
101 CA, starch and glycerol during gelatinization stage or drying of the films, using  
102 Differential Scanning Calorimetry (DSC) or Differential Thermal Analysis  
103 device (DTA).

104 Nor are there studies in the literature that discuss the influence of the maximum  
105 temperature at which the mixture is brought during gelatinization process.  
106 However, this step could be critical in the crosslinking process. For example  
107 Ghanbarzadeh et al. (2011) obtained films of starch crosslinked with CA,  
108 heating the mix of all components at 90 °C for 30 min and drying at 60 °C in  
109 oven. Marques et al. (2006) report crosslinked starch films with tetraethylene  
110 glycol diacrylate, heating the mix of all components at 90 °C and drying at room  
111 temperature.

112 On the other hand, low field nuclear magnetic resonance (<sup>1</sup>H NMR) is a  
113 technique used to characterize the water mobility of polymers and polymer  
114 composites (Mendes et al., 2012; Preto, Tavares, & Da Silva, 2007), rarely used  
115 in natural polymers such as chitosan and starch (Fundo et al., 2014, Gaudin et  
116 al., 1999) and there is no precedent of its application in crosslinked starch.

117 Finally, one of the most important points to be evaluated in films to be used as  
118 packaging is their biodegradability. Starch can be readily metabolized by a range  
119 of microorganisms to fermentation products such as ethanol and methane (Tang  
120 & Alavi, 2011). It is quickly attacked by microorganisms when it is buried in

121 soil. Prakash Maran et al. (2014) showed, using tapioca starch based composites  
122 that the increase of water sorption promotes the entry of soil microorganisms,  
123 which use the starch films as a source of energy for their growth. There is  
124 agreement in the literature that crosslinking starch with citric acid modifies  
125 water sorption. Moreover, Maiti, Ray, & Mitra (2013) and Imam, Cinelli,  
126 Gordon, & Chiellini (2005) showed in starch-poly(vinyl alcohol) blends  
127 crosslinked with different crosslinking agents that the starch degradation rate  
128 buried in soil is slowed when the system is crosslinked. While it is expected that  
129 the esterified or citric acid crosslinked starch do not degrade too slow, no studies  
130 in the literature reported this point.

131 Therefore, the purpose of this report was to provide a complete characterization  
132 of cassava starch crosslinked with citric acid. The focus was to show the  
133 influence that has the maximum temperature reached during starch  
134 gelatinization process on the film properties when this process is performed in  
135 presence of citric acid. Besides, the goal was to demonstrate that heating the  
136 mixture up to 75 °C is enough to obtain a completely gelatinized crosslinked  
137 material, whereas if the final temperature is 85 °C, signs of incipient hydrolysis  
138 are obtained. The manuscript also aimed to improve knowledge about the  
139 reactions between the different components (starch, CA and glycerol) during the  
140 gelatinization stage. To achieve this, the reactions between different components  
141 were simulated using a DTA. After characterization by means of Fourier  
142 transform infrared spectroscopy (FTIR) the possible esterification or crosslink  
143 reaction were identified. Furthermore, the influence of the final temperature of  
144 the gelatinization process and the presence citric acid on the mobility of water  
145 contained in the formed films were analysed by low field nuclear magnetic  
146 resonance (<sup>1</sup>H NMR). The efficiency of CA as crosslinking agent was  
147 determined by studies of swelling in dimethylsulphoxide (DMSO) and XRD,  
148 through the evaluation of the material retrogradation. Moisture content, moisture  
149 absorption and water vapor permeability were also studied. Finally  
150 biodegradability studies were performed to evaluate the effect of crosslinking in  
151 the films degradation. The combination of all these determinations allowed  
152 proposing a model that describes the interactions present in the developed  
153 materials, helping to design new materials with improved properties.

## 154 2. Experimental

### 155 2.1. Materials

156  
157 Cassava starch (18 wt.% amylose and 82 wt.% amylopectin) was provided by  
158 *Industrias del Maiz S.A, Buenos Aires Argentina*. Citric acid (Biopack) and  
159 glycerol (J. T. Baker) were of analytical grade.

### 160 2.2. Preparation of plasticized starch films

161  
162  
163 Thermoplastic starch was processed by casting as previously reported (García,  
164 Famá, Dufresne, Aranguren, & Goyanes, 2009; Medina Jaramillo, González  
165 Seligra, Goyanes, Bernal, & Famá, 2015). Four different systems using the same  
166 manufacturing protocol were developed. Table 1 provides the composition of  
167 the samples on dry basis. Two of the systems were constituted by aqueous  
168 suspensions containing cassava starch (5.0 g/100 g of system), glycerol (1.5  
169 g/100 g of system) and distillate water (93.5 g/100 g of system). The mixture  
170 was smoothly stirred during 45 minutes at room temperature and constant  
171 agitation of 250 rpm. After that, the mixture was heated at 3 °C/min under the  
172 same rpm. The heating process was completed at 75 °C (TPS75) or at 85 °C  
173 (TPS85). Then, the stirring was continued for 5 minutes at the same final  
174 temperature in each case (75 °C or 85 °C). The resulted gel was degassed with a  
175 vacuum pump for 10 min, cast in glass petri dishes and dried at 50 °C during 24  
176 h. Other two systems were prepared incorporating 0.5 g of citric acid into a  
177 similar aqueous suspension previously described, using the same processing  
178 methodology (TPS75-CA y TPS85-CA). This is CA was incorporated at the  
179  
180

181 initial stage of the process. This is CA was incorporated at the initial stage of the  
 182 process. The thickness of the obtained films was  $(0.20 \pm 0.02)$  mm.

183  
 184 Table 1. Samples composition of the different developed systems on dry basis.

185 186 187 188 189 190 191	Components		
	Dry basis (wt.%)		
	TPS75 // TPS85	TPS75-CA // TPS85-CA	
192	Starch	76.9	71.4
193	Glycerol	23.1	21.4
194	Citric Acid	0	7.2

### 192 2.3. Characterization techniques

193 The developed films were stored for 15 days, at 25 °C, over saturated solution of  
 194 NaBr (RH ~ 56 %), before being characterized.

#### 195 2.3.1. Thermal characterization

196 A simultaneous Thermogravimetric/Differential Thermal Analyzers (TGA/DTA  
 197 DTG-60 Shimadzu instrument) was used to simulate the gelatinization process  
 198 of all materials studied and to explore chemical and physical interactions  
 199 between the reagents involved in the films. For this propose, mixtures of starch,  
 200 glycerol and water (S/G/W: 10/3/20), starch, glycerol, CA and water  
 201 (S/G/CA/W: 10/3/1/20), glycerol, CA and water (G/CA/W: 3/1/20) and starch,  
 202 CA and water (S/CA/W: 10/1/20), maintaining the relations of starch, glycerol  
 203 and CA showed in Table 1, were performed. The amount of water employed in  
 204 all the cases was less than that used in casting process due to the limit mass and  
 205 volume of TGA/DTA capsule, and the sensibility of this equipment. In this  
 206 sense twice the amount of starch was used. The protocol used to carry up the  
 207 tests was similar to that of casting: systems were first stirrer at 250 rpm during  
 208 45 min at room temperature and then the simulation was made heating the  
 209 mixtures from 25 to 100 °C at 1.7 °C/min in a dry nitrogen atmosphere with  
 210 flow rate of 30 mL/min.

211 A kinetic reaction study of starch, glycerol, CA and water system, immediately  
 212 after completed the gelatinization process, was performed by isothermal DTA at  
 213 50 °C for 48 h.

#### 214 2.3.2. Attenuated total reflectance Fourier transform infrared spectroscopy 215 (ATR/FTIR)

216 Infrared spectra of the films were recorded on a Nicolet Nexus (Madison, WI)  
 217 Fourier transform infrared spectrometer using the attenuated total reflectance  
 218 (ATR) accessory. Measurements were obtained as the average of 32 scans, in  
 219 the range from 4000  $\text{cm}^{-1}$  to 600  $\text{cm}^{-1}$ , with a resolution of 2  $\text{cm}^{-1}$ .

#### 220 2.3.3. Swelling in DMSO

221 The swelling power of the films was determined according to Liu et al. (2012).  
 222 All films were immersed in 25 mL of dimethylsulphoxide (DMSO) at room  
 223 temperature ( $25 \text{ °C} \pm 2 \text{ °C}$ ) for 48 h. The insoluble part was filtered out, lightly  
 224 wiped and weighted ( $m_s$ ). For the extraction of DMSO, the swollen films were  
 225 immersed first in water and then in ethanol (for 2-3 times). After that, they were  
 226 dried at 50 °C to constant weight ( $m_d$ ). The swelling degree (SD) was calculated  
 227 from the following equation:

$$228 \quad SD = \frac{m_s - m_d}{m_d} \quad (1)$$

#### 229 2.3.4. Low field nuclear magnetic resonance ( $^1\text{H}$ NMR)

241 Transversal or spin-spin relaxation times ( $T_2$ ) were measured by time resolved  
 242 proton nuclear magnetic resonance ( $^1\text{H NMR}$ ) in a Bruker Minispec mq20  
 243 (Bruker Biospin GmbH, Rheinstetten, Germany) equipment, with a 0.47 T  
 244 magnetic field operating at a resonance frequency of 20 MHz. All the  
 245 determinations were performed in triplicate at 25 °C. Proton populations of  
 246 different mobility were measured using single pulse method: free induction  
 247 decay analysis (FID).

### 248 2.3.5. Moisture content

249  
 250 Moisture content (MC) of the different films was determined using standard  
 251 methods of analysis of the AOAC (1995). Pieces of each system ( $\square$  0.5 g) were  
 252 dried in an oven at 100 °C, for 24 h. The percentage of moisture content was  
 253 calculated as follow:

$$254 \text{MC (\%)} = \frac{m_w - m_d}{m_d} \times 100 \quad (2)$$

255  
 256 where  $m_w$  is the mass of the wet sample and  $m_d$  is the mass of the dried sample.  
 257 The reported results represent the average of five samples in each case.

### 260 2.3.6. Moisture absorption

261  
 262 Moisture absorption (MA) was measured according to Mathew & Dufresne  
 263 (2002). The specimens were cut in rectangular pieces of 1 cm x 1 cm. The  
 264 samples were first dried overnight at 100 °C, then weighted, and finally  
 265 conditioned at room temperature in desiccators containing copper sulfate (RH ~  
 266 98 %). The weight of the samples was measurement every specific time interval  
 267 until reaching constant value.

268 The moisture absorption of the samples was calculated as follows:

$$269 \text{MA (\%)} = \frac{M_t - M_0}{M_0} \times 100 \quad (3)$$

270  
 271 where  $M_0$  is the weight of the sample before its exposure to 98 % RH and  $M_t$  is it  
 272 weight in the time (min) at this RH.

### 273 2.3.7. Water vapor permeability (WVP)

274  
 275 Water vapor permeability of the films was determined at room temperature  
 276 using a modified ASTM E96-00 procedure (Famá, Gañan Rojo, Bernal, &  
 277 Goyanes, 2012). Samples were placed into circular acrylic cells containing  
 278  $\text{CaCl}_2$ , and then located in desiccators at RH of ~ 70 % and room temperature.  
 279 Water vapor transport (WVT) was determined from the weight gain of the  
 280 permeation, measuring over 24 h for 10 days. WVP were calculated as:

$$281 \text{WVP} = \frac{WWT \times e}{P_0 \times RH} \quad (4)$$

282  
 283 where  $e$  is the films thickness and  $P_0$  the saturation vapor pressure of water at  
 284 room temperature (Gennadios, Weller, & Gooding, 1994).

### 285 2.3.8. X-ray diffraction (XRD)

286  
 287 A Siemens D 5000 X-ray diffractometer was used to observe the diffraction  
 288 patterns of all the developed films. X-ray generator tension and current were 40  
 289 kV and 30 mA, respectively. The radiation was Cu  $K\alpha$  of wavelength 1.54 Å.  
 290 The diffraction patterns were obtained at room temperature in the range of  $2\theta$   
 291 between 10 ° and 35 ° by step of 0.02 °.

### 292 2.3.9. Biodegradation of the films in vegetable compost

293  
 294 The samples were cut in pieces of 2 cm x 2 cm. Vegetable compost, which was

used as soil, was sifted to remove large clumps and poured into a plastic tray up to a thickness of about 6 cm. Samples were buried below 4 cm of soil, under ambient temperature ( $\sim 25^\circ\text{C}$ ) and humidity conditions (70-80 %). Water was sprayed twice a day to sustain the moisture of the compost. The films were removed at different times and dried in a vacuum oven at  $50^\circ\text{C}$  for 24 h.

### 3. Results and discussions

#### 3.1. Thermal Analysis

The effect of citric acid in the gelatinization process of all materials was studied by its simulation using a thermogravimetric analyser (TGA). The obtained curves are shown in Fig. 1a. System with CA showed lower mass loss during gelatinization than that without citric acid. CA decreases intra and intermolecular interactions between starch-starch chains and strengthens the hydrogen bonding interactions between the hydroxyl groups of starch and those of CA (Shi et al., 2007), leading to more amount of trapped OH that cannot easily evaporate.

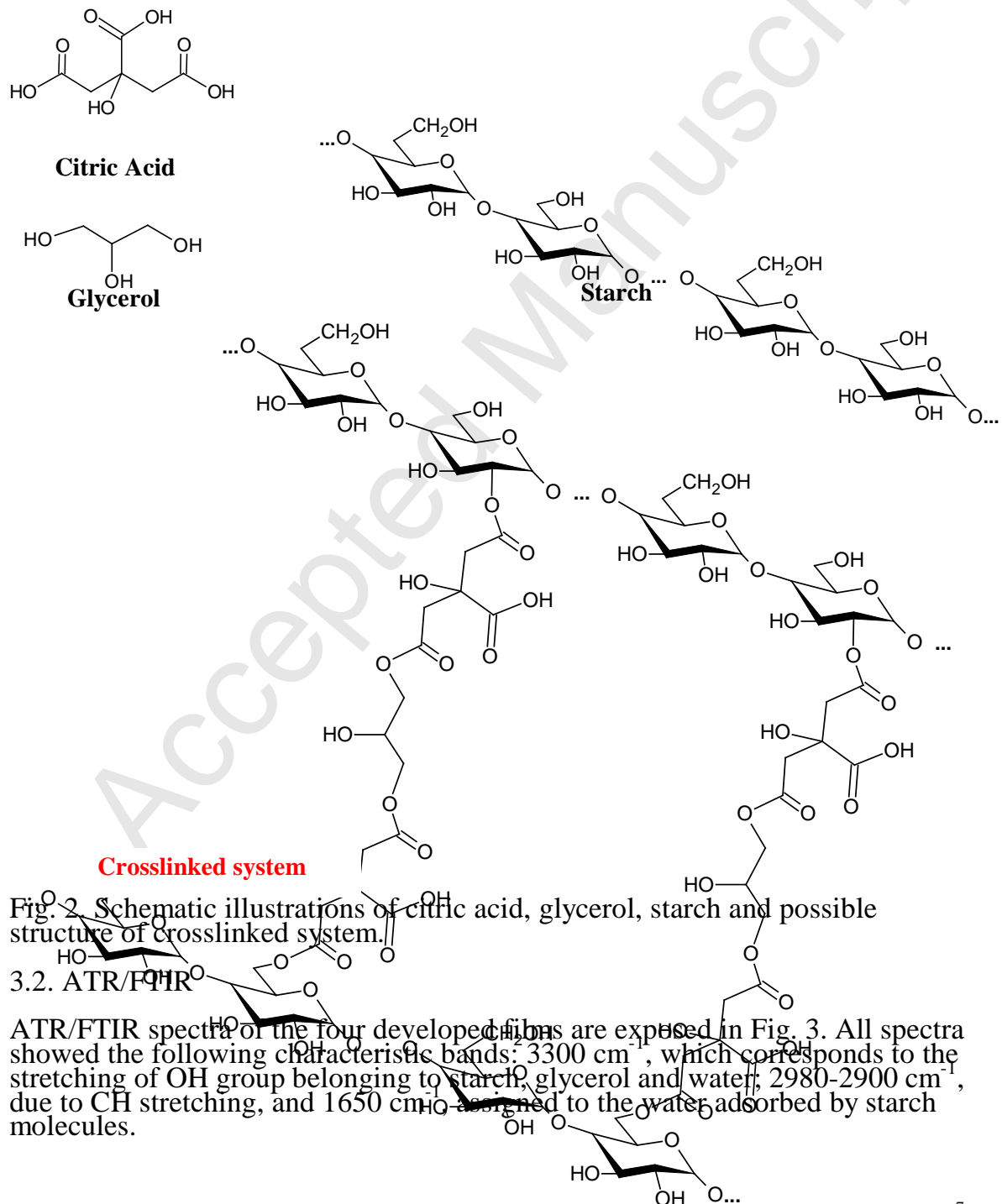
The reactions between the different components of the films, as well as the temperature at which 100 % gelatinization is obtained in each system, were determined by DTA using mixture compositions described in experimental part (Fig. 1b). As can be seen, first system showing reaction was G/CA/W, suggesting the formation of esters between glycerol and CA, while the system that reacts at higher temperature was starch, CA and water (S/CA/W). This behaviour was expected taking into account that secondary hydroxyl groups of starch are less reactive than primary OH of glycerol with respect to esterification by CA (Holser, 2008). Peak temperatures of the reaction systems used to obtain the films (S/G/CA/W and S/G/W) were similar and located between the G/CA/W and S/CA/W mixtures. Although the presence of citric acid did not affect the temperature corresponding to gelatinization peak ( $T_g \approx 52^\circ\text{C}$ ), systems with CA reached to complete gelatinization at lower temperature than that without CA. While S/G/CA/W reached 100 % of gelatinization at  $\approx 75^\circ\text{C}$ , the system without CA required  $80^\circ\text{C}$ . Then, all films without CA processed at  $85^\circ\text{C}$  will be completely gelatinized while that processed at  $75^\circ\text{C}$  will reach 82 % of gelatinization. Paes, Yakimets, & Mitchell (2008) studied the effect of the temperature on the formation of cassava starch films through light microscopy, showing that some granules were not completely swollen at  $75^\circ\text{C}$ ; they complete their expansion between  $80^\circ\text{C}$  and  $85^\circ\text{C}$ . Finally, the fact that S/G/CA/W reaction occurs at lower temperatures than that of S/CA/W, indicates that starch preferentially interacted with the ester than with CA.

With the aim to study if any reaction occurs during the drying process, an isothermal study of S/G/CA/W, immediately after complete gelatinization process, was performed at  $50^\circ\text{C}$  for 48 h. Fig. 1c shows the DTA result obtained and no reaction bands were observed. On this basis, it is conceivable the structure shown in Fig. 1d: a glycerol-citric acid gelatinized systems.

Fig.1. (a) Thermogravimetric analysis of S/G/W and S/G/CA/W, (b) Differential thermal analysis of kinetic reactions between the different components of the films, and (c) DTA result of S/G/CA/W at  $50^\circ\text{C}$  for 48 h.

The scheme of crosslinked system in Fig. 2 shows an open structure with a decrease in intra-molecular interactions of starch chains and with free OH groups very available to easily interact with the hydroxyl groups of water. Therefore, although films with citric acid should have fewer OH groups than those without CA due to the interactions between starch, glycerol and CA, they form a more open network, so the diffusion of water molecules is faster

361 explaining the acceleration of gelatinization process.  
 362 In order to confirm the proposed model for films structure and ascertain if CA  
 363 generated a crosslinked structure, three types of analysis were performed: FTIR,  
 364 swelling and  $^1\text{H}$  NMR.





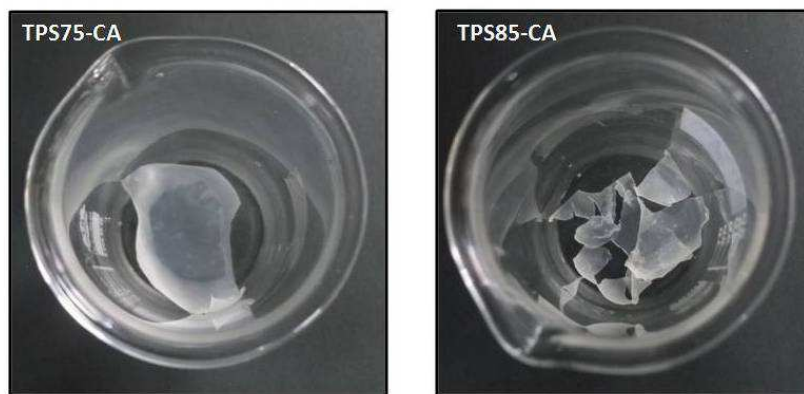
To compare the amount of available OH in the different systems, the ratio between the intensity of the peak at  $3300\text{ cm}^{-1}$  ( $I_{3300}$ ) and that at  $1149\text{ cm}^{-1}$  ( $I_{1149}$ ), associates to the stretching vibration of 'C-O' in 'C-O-H' group and previously use by Shi et al. (2007) as a reference band in modified starch with citric, were calculated. The intensity ratio ( $I_{3300}/I_{1149}$ ) of TPS-CA films was lower than TPS, indicating a smaller amount of OH available groups. This decrease is related to a decrease in the number of OH due to the esterification reaction between COOH groups of citric acid with OH groups of glycerol. Additionally, the band related to hydroxyl groups ( $3300\text{ cm}^{-1}$ ) was wider and less intense in the case of the materials with CA. This widening was both to minor and major wavenumbers with respect to that peak, indicating that there are OH that more easily vibrate and, also, there are more OH forming hydrogen bond.

On the other hand, in the films with citric acid appeared a band at  $1720\text{ cm}^{-1}$  that could be assigned to C=O (Reddy & Yang, 2010) from acid groups esters between CA and glycerol, and esters between starch and CA, suggesting that crosslinking was developed.

The effects of CA are dependents of the process temperature. In TPS85-CA, the intensity ratio ( $I_{3300}/I_{1149}$ ) was higher than in TPS75-CA films, indicating the existence of more amount of OH groups, possibly produced by the hydrolysis of starch. According to Shi et al. (2007), hydrolysis of the glycosidic linkages by citric acid can be evident by a decrease of the peak height at  $1024\text{ cm}^{-1}$  (stretching vibration of 'C-O' in 'C-O-C') with respect to that at  $1149\text{ cm}^{-1}$  (stretching vibration of 'C-O' in 'C-O-H'). FTIR results showed this effect only in the case of the films with CA heated at  $85^\circ\text{C}$ .

Olsson et al. (2013b) demonstrated that the hydrolysis of starch-glycerol-CA films was almost completely hindered at a solution with  $\text{pH} \geq 4$ . In our case, pH of the gels resulted: approximately 6.9, 6.4, 3.9 and 3.4 (TPS75, TPS85, TPS75-CA and TPS85-CA, respectively), indicating incipient hydrolysis degree in the samples with citric acid processed at  $85^\circ\text{C}$ .

Fig. 3. ATR/FTIR spectra of all the studied films: (a) Systems processed at  $75^\circ\text{C}$



crosslinked materials. DMSO in accordance (Kumar & Singh, ) and their integrity until es cause an increase swelling. However, into pieces. glycoside bonds incipient hydrolysis in

Fig. 4. Photographs of TPS75-CA and TPS85-CA after swelling process.

### 3.4. Low field nuclear magnetic resonance ( $^1\text{H}$ NMR)

Molecular mobility was evaluated by measuring the relaxation time with a low resolution magnetic resonance spectrometer. The relaxation times are affected by the presence of free OH and free volume; then, it is expectable to find differences in water mobility of TPS-AC with respect to TPS, due to the crosslinking.

The proton transverse magnetization decay curve (relaxation time  $T_2$ ), presented similar behaviour for all materials studied. As an example, in Fig. 5 is shown the curve obtained for the sample TPS75. Two different decays were observed,



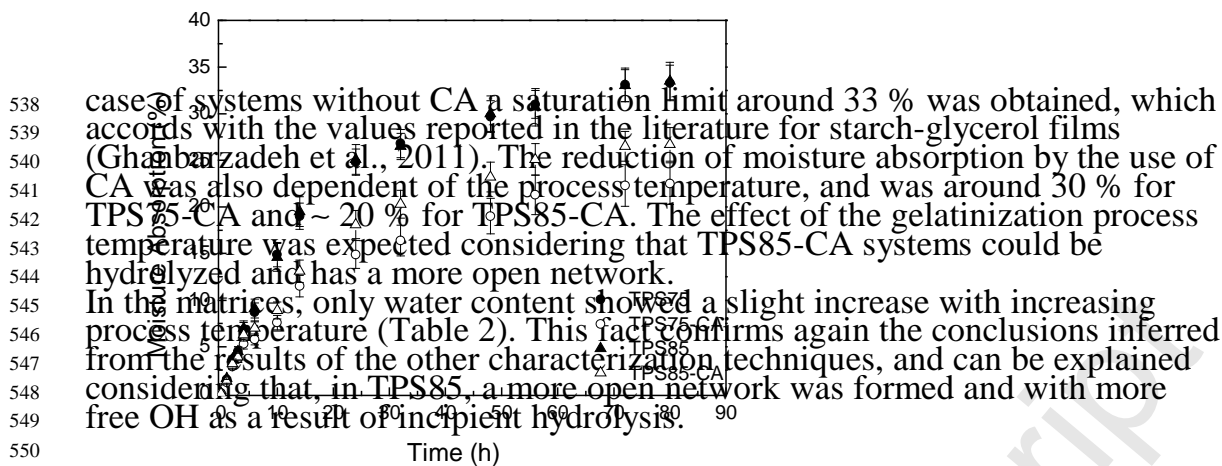


Fig. 6. Moisture absorption of the different studied films.

### 3.6. Water Vapor Permeability

The values of water vapor permeability (WVP) are reported in Table 2. WVP of samples without CA is of the same order of magnitude that those reported in the literature (García et al., 2009; Bertuzzi et al., 2007). The addition of CA led to a significant decrease in WVP of ~ 36 % for TPS75-CA and 17.2 % for TPS85-CA, confirming again the formation of a network. Considering that food packaging films are often required to limit moisture transfer between the food and the surrounding atmosphere, WVP should be as low as possible. Several authors reported that the addition of CA in starch films led to a decrease in WVP, because the hydrophilic OH groups were substituted with hydrophobic ester groups (Ghanbarzadeh et al., 2011; Reddy & Yang, 2010; Ma, Chang, Yu, & Stumborg, 2009). However, beyond this idea, this study showed that citric acid is efficient to crosslink starch at low processing temperatures. The formation of this network reduced WVP and generated other important advantages from the point of view of application of these materials for example, they avoided starch retrogradation. Starch retrogradation occurs during the starch films storage and lead to crystallization (Morales Candal Fama, Govanes & Rubiolo, 2015). Then the effect of citric acid on the films retrogradation can be studied through X-ray diffraction.

### 3.7. X-ray diffraction

Fig. 7a shows X-ray diffraction patterns (XRD) of the studied films after 15 days of storage. Films without CA exhibit mostly feature of amorphous patterns with only two small peaks at  $2\theta = 19.8^\circ$  and  $22.1^\circ$ , characteristics of B-type crystalline structure. It is well known that crystallinity of starch films occurs due to retrogradation process (Morales et al., 2015). This process, strongly dependent of water content, provokes an increase in crystallinity with the increment of hydration degree (Buléon, Véronèse, & Putaux, 2007). We demonstrated that TPS85 had higher water content than TPS75 (Table 2) and, while TPS85 completed the gelatinization process, TPS75 reached only 82 % (Fig. 1b), which means that some grains remained unbroken (crystalline). Therefore, in the case of TPS75, two effects contributed to crystallinity: retrogradation and incomplete gelatinization; while in TPS85 crystallinity was due only to starch retrogradation, which was higher than TPS75 as consequence of their greater water content.

TPS75-CA and TPS85-CA were completely amorphous at 15 days of storage which accords with the idea that CA provoked crosslinking.

In order to support this idea, XRD to all the systems with 40 days of storage were performed (Fig. 7b). The diffractograms of TPS-CA films correspond to amorphous materials, confirming the effectiveness of crosslinking reaction, while non-crosslinked films increased their crystallinity. Two new peaks at  $2\theta = 17^\circ$  and  $24^\circ$ , corresponding also to the B-crystalline structure, appeared in TPS

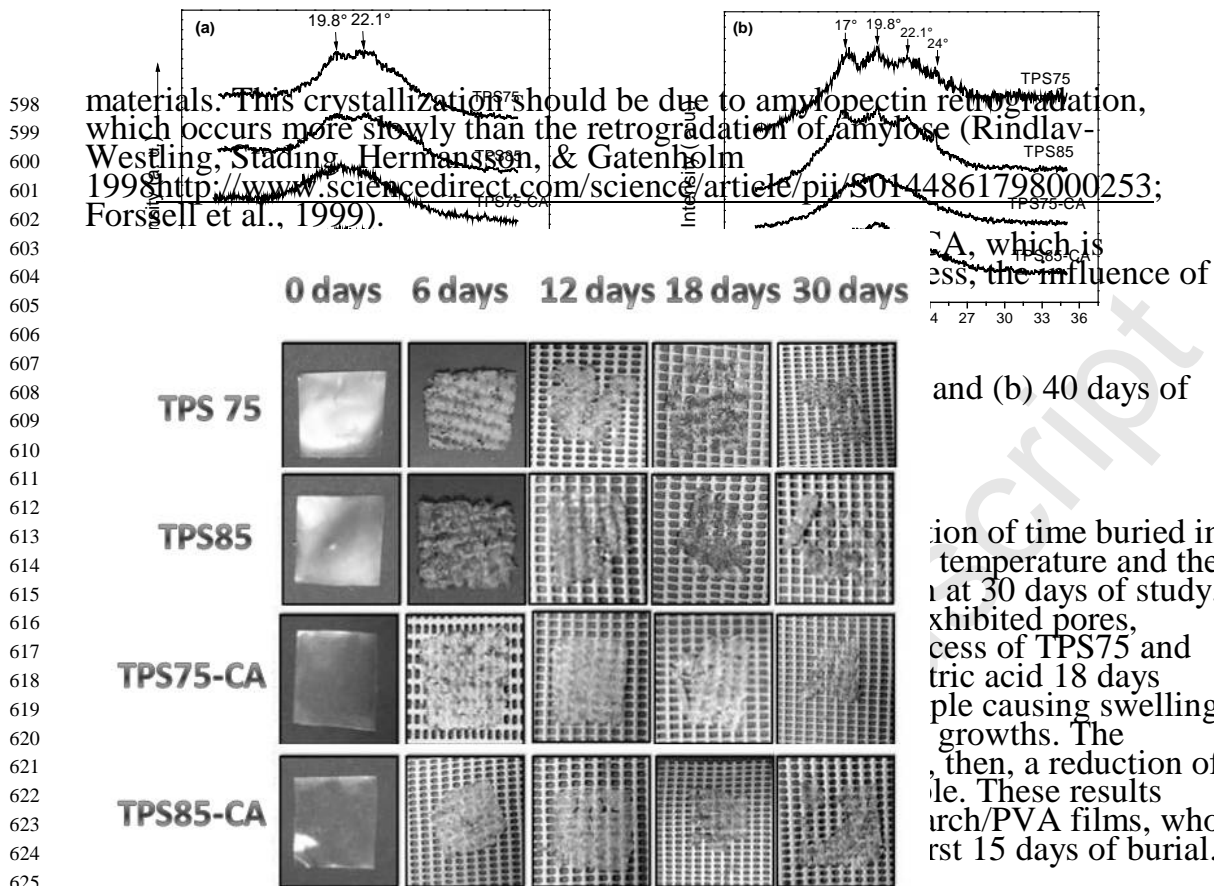


Fig. 8. Macroscopic appearance of biodegradation in soil of the different studied films.

#### 4. Conclusions

The addition of 7.2 wt.% (dry basis) of citric acid to starch/glycerol/water system modifies the gelatinization process, making it possible to be complete 5 °C before than the system without CA. The kinetic study of the reactions between the components of the films during the gelatinization process showed that the first reaction occurs between glycerol and citric acid in order to form an ester of CA and glycerol. Then, these two components together react with the starch. This study allowed predicting a structure for the material which was verified by different techniques (FTIR, <sup>1</sup>H NMR, swelling, MC, MA, WVP and XRD). As the result of the different reactions between the components of the films, the systems with CA lead to a crosslinked material after gelatinization. Gelatinization process temperature is a critical point to obtain the best results. While the material processed at 75 °C has the highest crosslink density and maintains its integrity after swelling in DMSO, the one processed at 85 °C shows a slight damage attributable to an incipient starch hydrolysis. Film with CA processed at 75 °C allows decreasing WVP more than 35 %, remains unchanged in time since it does not retrograde and degrades in compost only 6 days after the films without CA.

#### Acknowledgements

The authors wish to acknowledge the support and collaboration of the following organizations: ANPCyT -2012-1093, CONICET PIP 2014-2016 N° 11220120100508CO, Universidad de Buenos Aires UBACYT 2014-2017 N° 20020130100495BA, CAPES 2014-2015 BR/13/13.

#### References

- 658  
659 AOAC (1995). *Official methods of analysis*. Washington, DC: Association of  
660 Official Analytical Chemists.
- 661  
662 Averous, L., & Boquillon, N. (2004). Biocomposites based on plasticized starch:  
663 Thermal and mechanical behaviours. *Carbohydrate Polymers*, 56(2), 111-122.
- 664  
665 Bertuzzi, M. A., Armada, M., & Gottifredi, J. C. (2007). Physicochemical  
666 characterization of starch based films. *Journal of Food Engineering*, 82(1), 17-  
667 25.
- 668  
669 Buléon, A., Véronèse, G., & Putaux, J.-L. (2007). Self-association and  
670 crystallization of amylose. *Australian Journal of Chemistry*, 60(10), 706-718.
- 671  
672 Choi, S. G., & Kerr, W. L. (2003). <sup>1</sup>H NMR studies of molecular mobility in  
673 wheat starch. *Food Research International*, 36(4), 341-348.
- 674  
675 Famá, L., Bittante, A. M. B. Q., Sobral, P. J. A., Goyanes, S., & Gerschenson, L.  
676 N. (2010). Garlic powder and wheat bran as fillers: their effect on the  
677 physicochemical properties of edible biocomposites. *Material Science and*  
678 *Engineering C*, 30, 853-859.
- 679  
680 Famá, L., Gañan Rojo, P., Bernal, C., & Goyanes, S. (2012). Biodegradable  
681 starch based nanocomposites with low water vapor permeability and high  
682 storage modulus. *Carbohydrate Polymers*, 87(3), 1989-1993.
- 683  
684 Farhat, I. A., Blanshard, J. M. V., & Mitchell, J. R. (2000). The retrogradation of  
685 waxy maize starch extrudates: Effects of storage temperature and water content.  
686 *Biopolymers*, 53(5), 411-422.
- 687  
688 Flores, S., Famá, L., Rojas, A. M., Goyanes, S., & Gerschenson, L. (2007).  
689 Physicochemical properties of tapioca-starch edible films. Influence of  
690 gelatinization and drying technique. *Food Research International*, 40(2), 257-  
691 265.
- 692  
693 Forssell, P. M., Hulleman, S., Myllärinen, P., Moates, G. K., & Parker, R.  
694 (1999). Ageing of rubbery thermoplastic barley and oat starches. *Carbohydrate*  
695 *Polymers*, 39(1), 43-51.
- 696  
697 Fundo, J. F., Fernandes, R., Almeida, P. M., Carvalho, A., Feio, G., Silva, C. L.  
698 M., & Quintas, M. A. C. (2014). Molecular mobility, composition and structure  
699 analysis in glycerol plasticised chitosan films. *Food Chemistry*, 144, 2-8.
- 700  
701 Garcia, N. L., Famá, L., Dufresne, A., Aranguren, M., & Goyanes, S. (2009). A  
702 comparison between the physico-chemical properties of tuber and cereal  
703 starches. *Food Research International*, 42(8), 976-982.
- 704  
705 Gaudín, S., Lourdin, D., Le Botlan, D., Ilari, J. L., & Colonna, P. (1999).  
706 Plasticisation and mobility in starch-sorbitol films. *Journal of Cereal Science*,  
707 29(3), 273-284.
- 708  
709 Ghanbarzadeh, B., Almasi, H., & Entezami, A. A. (2011). Improving the barrier  
710 and mechanical properties of corn starch-based edible films: Effect of citric acid  
711 and carboxymethyl cellulose. *Industrial Crops and Products*, 33(1), 229-235.
- 712  
713 Gennadios, A., Weller, C. L., & Gooding, C. H. (1994). Measurement errors in  
714 water vapor permeability of highly permeable, hydrophilic edible films. *Journal*  
715 *of Food Engineering*, 21(4), 395-409.
- 716  
717 Gutiérrez, T. J., Pérez, E., Guzmán, R., Tapia, M.S., & Famá, L. (2015).

- 718 Structural and mechanical properties of native and modified cush-cush yam and  
719 cassava starch edible films. *Food Hydrocolloids*, 45, 211-217.
- 720  
721 Holser, R. A. (2008). Thermal analysis of glycerol citrate/starch blends. *Journal*  
722 *of Applied Polymer Science*, 110(3), 1498-1501.
- 723  
724 Hoover, R. (2000). Acid-treated starches. *Food Reviews International*, 16(3),  
725 369-392.
- 726  
727 Imam, S. H., Cinelli, P., Gordon, S. H., & Chiellini, E. (2005). Characterization  
728 of biodegradable composite films prepared from blends of poly(vinyl alcohol),  
729 cornstarch, and lignocellulosic fiber. *Journal of Polymers and the Environment*,  
730 13(1), 47-55.
- 731  
732 Kumar, A. P., & Singh, R. P. (2008). Biocomposites of cellulose reinforced  
733 starch: Improvement of properties by photo-induced crosslinking. *Bioresource*  
734 *Technology*, 99(18), 8803-8809.
- 735  
736 Krumova, M., Lopez, D., Benavente, R., Mijangos, C., & Pereña, J. M. (2000).  
737 Effect of crosslinking on the mechanical and thermal properties of poly(vinyl  
738 alcohol). *Polymer 4I*(26), 9265-9272.
- 739  
740 Leung, H. K., Steinberg, M. P., Wei, L. S., & Nelson, A. I. (1976). Water  
741 binding of macromolecules determined by pulsed NMR. *Journal of Food*  
742 *Science*, 41(2), 297-300.
- 743  
744 Liu, Z., Dong, Y., Men, H., Jiang, M., Tonga, J., & Zhou, J. (2012). Post-  
745 crosslinking modification of thermoplastic starch/PVA blend films by using  
746 sodium hexametaphosphate. *Carbohydrate Polymers*, 89(2), 473-477.
- 747  
748 Ma, X., Chang, P. R., Yu, J., & Stumborg. (2009). Properties of biodegradable  
749 citric acid-modified granular starch/thermoplastic pea starch composites.  
750 *Carbohydrate Polymers*, 75(1), 1-8.
- 751  
752 Maiti, S., Ray, D., & Mitra, D. (2012). Role of crosslinker on the biodegradation  
753 behavior of starch/polyvinylalcohol blend films. *Journal of Polymers and the*  
754 *Environment*, 20(3), 749-759.
- 755  
756 Maiti, S., Ray, D., Mitra, D., & Mukhopadhyay, A. (2013). Isolation and  
757 characterisation of starch/polyvinyl alcohol degrading fungi from aerobic  
758 compost environment. *International Biodeterioration & Biodegradation*, 82, 9-  
759 12.
- 760  
761 Marques, P. T., Lima, A. M. F., Bianco, G., Laurindo, J. B., Borsali, R., Le  
762 Meins, J.-F., & Soldi, V. (2006). Thermal properties and stability of cassava  
763 starch films cross-linked with tetraethylene glycol diacrylate. *Polymer*  
764 *Degradation and Stability*, 91(4), 726-732.
- 765  
766 Mathew, A. P., & Dufresne, A. (2002). Plasticized waxy maize starch: effect of  
767 polyols and relative humidity on material properties. *Biomacromolecules*, 3(5),  
768 1101-1108.
- 769  
770 Medina Jaramillo, C., González Seligra, P., Goyanes, S., Bernal, C., & Famá, L.  
771 (2015). Biofilms based on cassava starch containing extract of yerba mate as  
772 antioxidant and plasticizer. *Starch □ Stärke*, 67, 780-789.
- 773  
774 Mendes, L. C., Silva, D. F., & Lino, A. S. (2012). Linear low-density  
775 polyethylene and zirconium phosphate nanocomposites: Evidence from thermal,  
776 thermo-mechanical, morphological and low-field nuclear magnetic resonance  
777 techniques. *Journal of Nanoscience and Nanotechnology*, 12(12), 8867-8873.

- 778  
779 Menzel, C., Olsson, E., Plivelic, T. S., Andersson, R., Johansson, C., Kuktaite,  
780 R., Jarnstrom, L., & Koch, K. (2013). Molecular structure of citric acid cross-  
781 linked starch films. *Carbohydrate Polymers*, 96(1), 270-276.
- 782  
783 Morales, N. J., Candal, R., Famá, L., Goyanes, S., & Rubiolo, G. (2015).  
784 Improving the physical properties of starch using a new kind of water  
785 dispersible nano-hybrid reinforcement. *Carbohydrate Polymers*, 127, 291-299.
- 786  
787 Olsson, E., Hedenqvist, M. S., Johansson, C., & Järnström, L. (2013a). Influence  
788 of citric acid and curing on moisture sorption, diffusion and permeability of  
789 starch films. *Carbohydrate Polymers*, 94(2), 765-772.
- 790  
791 Olsson, E., Menzel, C., Johansson, C., Andersson, R., Koch, K., & Järnström, L.  
792 (2013b). The effect of pH on hydrolysis, cross-linking and barrier properties of  
793 starch barriers containing citric acid. *Carbohydrate Polymers*, 98(2), 1505-1513.
- 794  
795 Paes, S. S., Yakimets I., & Mitchell, J. R. (2008). Influence of gelatinization  
796 process on functional properties of cassava starch films. *Food Hydrocolloids*,  
797 22(5), 788-797.
- 798  
799 Prakash Marana, J., Sivakumara, V., Thirugnanasambandhama, K., & Sridharba,  
800 R. (2014). Degradation behavior of biocomposites based on cassava starch  
801 buried under indoor soil conditions. *Carbohydrate Polymers*, 101, 20-28.
- 802  
803 Preto, M., Tavares, M. I. B., & Da Silva, E. P. (2007). Low-field NMR study of  
804 Nylon 6/silica composites. *Polymer Testing*, 26(4), 501-504.
- 805  
806 Reddy, N., & Yang, Y. (2010). Citric acid cross-linking of starch films. *Food*  
807 *Chemistry*, 118(3), 702-711.
- 808  
809 Rindlav-Westling, A., Stading, M., Hermansson, A. M., & Gatenholm, P.  
810 (1998). Structure, mechanical and barrier properties of amylose and amylopectin  
811 films. *Carbohydrate Polymers*, 36(2-3), 217-224.
- 812  
813 Romero-Bastida, C. A., Bello-Perez, L. A., Garcia, M. A., Martino, M. N,  
814 Solorza-Feria, J., & Zarintzky, N. E. (2005). Physicochemical and  
815 microstructural characterization of films prepared by thermal and cold  
816 gelatinization from non-conventional sources of starches. *Carbohydrate*  
817 *Polymers*, 60(2), 235-244.
- 818  
819 Salomão Garcia, P., Eiras Grossmann, M. V., Ayumi Shirai, M., Lazaretti, M.  
820 M., Yamashita, F., Muller, C. M., & Mali, S. (2014). Improving action of citric  
821 acid as compatibiliser in starch/polyester blown films. *Industrial Crops and*  
822 *Products*, 52, 305-312.
- 823  
824 Shi, R., Bi, J., Zhang, Z., Zhu, A., Chen, D., Zhou, X., Zhang, L., & Tian, W.  
825 (2008). The effect of citric acid on the structural properties and cytotoxicity of  
826 the polyvinyl alcohol/starch films when molding at high temperature.  
827 *Carbohydrate Polymers*, 74(4), 763-770.
- 828  
829 Shi, R., Zhang, Z., Liu, Q., Han, Y., Zhang, L., Chen, D., & Tian, W. (2007).  
830 Characterization of citric acid/glycerol co-plasticized thermoplastic starch  
831 prepared by melt blending. *Carbohydrates Polymers*, 69(4), 748-55.
- 832  
833 Tang, X., & Alavi, S. (2011). Recent advances in starch, polyvinyl alcohol  
834 based polymer blends, nanocomposites and their biodegradability. *Carbohydrate*  
835 *Polymers*, 85, 7-16.
- 836  
837 Yu, J., Wang, N., & Ma, X. (2005). The effects of citric acid on the properties of

838 thermoplastic starch plasticized by glycerol. *Starch/Stärke*, 57(10), 494-504.

839

840 Zhou, J., Zhang, J., Ma, Y., & Tong, J. (2008). Surface photo-crosslinking of  
841 corn starch sheets. *Carbohydrate Polymers*, 74(3), 405-410.

842

843 Zuraida, A., Yusliza, Y., Anuar, H., & Mohd Khairul Muhaimin, R. (2012). The  
844 effect of water and citric acid on sago starch bio-plastics. *International Food*  
845 *Research Journal*, 19(2), 715-719.

846

847

#### 848 **Highlights**

849

850 • Starch-glycerol eco-films using citric acid (CA) as crosslinking were developed.

851

852 • CA first reacts with glycerol and then starch-glycerol-CA reaction occurs.

853

854 • Different temperatures, 75 °C and 85 °C, were used in gelatinization process.

855

856 • Biodegradable and non-retrograde starch-glycerol-CA films are obtained at 75°C.

857

858 • Films heated at 75°C show decreases in WVP and integrity after swelling in DMSO.

859

860