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

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1 Biodegradable and non-retrogradable eco-films based on starch-glycerol with
2 citric acid as crosslinking agent.
3
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15
16 Abstract
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 ºC before than the other, 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 ºC.
32
33 Keywords: starch, citric acid, crosslinking, biodegradable edible films,
34 biodegradability.
35
36 1. Introduction
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

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

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

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

For the crosslinking reaction between starch and CA to occur, temperature is required. The discussion is in which stage of the development of the films this reaction occurs, and what is the order in which the different components of the films react. In the case where CA is added after the gelatinization process, obviously the crosslinking reaction occurs during the drying of the mixture. However, when CA is introduced prior to starch gelatinization, the reactions may occur at any stage of the films obtaining process. To the best of our knowledge there are no researches in the literature discussing this point. Moreover, there have not been reported studies simulating the reactions between CA, starch and glycerol during gelatinization stage or drying of the films, using Differential Scanning Calorimetry (DSC) or Differential Thermal Analysis device (DTA).

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

On the other hand, low field nuclear magnetic resonance (^{1}H NMR) is a technique used to characterize the water mobility of polymers and polymer composites (Mendes et al., 2012; Preto, Tavares, & Da Silva, 2007), rarely used in natural polymers such as chitosan and starch (Fundo et al., 2014, Gaudin et al., 1999) and there is no precedent of its application in crosslinked starch. Finally, one of the most important points to be evaluated in films to be used as packaging is their biodegradability. Starch can be readily metabolized by a range of microorganisms to fermentation products such as ethanol and methane (Tang & Alavi, 2011). It is quickly attacked by microorganisms when it is buried in

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

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

2. Experimental

2.1. Materials

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

2.2. Preparation of plasticized starch films

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

initial stage of the process. This is CA was incorporated at the initial stage of the process. The thickness of the obtained films was (0.20 ± 0.02) mm.

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

Components	Dry basis (wt.%)	
	TPS75 // TPS85	TPS75-CA // TPS85-CA
Starch	76.9	71.4
Glycerol	23.1	21.4
Citric Acid	0	7.2

2.3. Characterization techniques

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

2.3.1. Thermal characterization

A simultaneous Thermogravimetric/Differential Thermal Analyzers (TGA/DTA DTG-60 Shimadzu instrument) was used to simulate the gelatinization process of all materials studied and to explore chemical and physical interactions between the reagents involved in the films. For this propose, mixtures of starch, glycerol and water (S/G/W: 10/3/20), starch, glycerol, CA and water (S/G/CA/W: 10/3/1/20), glycerol, CA and water (G/CA/W: 3/1/20) and starch, CA and water (S/CA/W: 10/1/20), maintaining the relations of starch, glycerol and CA showed in Table 1, were performed. The amount of water employed in all the cases was less than that used in casting process due to the limit mass and volume of TGA/DTA capsule, and the sensibility of this equipment. In this sense twice the amount of starch was used. The protocol used to carry up the tests was similar to that of casting: systems were first stirrer at 250 rpm during 45 min at room temperature and then the simulation was made heating the mixtures from 25 to 100 °C at 1.7 °C/min in a dry nitrogen atmosphere with flow rate of 30 mL/min.
A kinetic reaction study of starch, glycerol, CA and water system, immediately after completed the gelatinization process, was performed by isothermal DTA at 50 °C for 48 h.

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

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

2.3.3. Swelling in DMSO

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

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

2.3.4. Low field nuclear magnetic resonance (^{1}H NMR)

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

2.3.5. Moisture content

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

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

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

2.3.6. Moisture absorption

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

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

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

2.3.7. Water vapor permeability (WVP)

Water vapor permeability of the films was determined at room temperature using a modified ASTM E96-00 procedure (Famá, Gañan Rojo, Bernal, & Goyanes, 2012). Samples were placed into circular acrylic cells containing $CaCl_2$, and then located in desiccators at RH of ~ 70 % and room temperature. Water vapor transport (WVT) was determined from the weight gain of the permeation, measuring over 24 h for 10 days. WVP were calculated as:

$$WVP = \frac{WVT \times e}{P_0 \times RH} \qquad (4)$$

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

2.3.8. X-ray diffraction (XRD)

A Siemens D 5000 X-ray diffractometer was used to observe the diffraction patterns of all the developed films. X-ray generator tension and current were 40 kV and 30 mA, respectively. The radiation was Cu Kα of wavelength 1.54 Å. The diffraction patterns were obtained at room temperature in the range of 2 θ between 10 ° and 35 ° by step of 0.02 °.

2.3.9. Biodegradation of the films in vegetable compost

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

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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 (~ 25 °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 °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 $\square$ 52 °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 $\square$ 75 °C, the system without CA required 80 °C. Then, all films without CA processed at 85 °C will be completely gelatinized while that processed at 75 °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 °C; they complete their expansion between 80 °C and 85 °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 °C for 48 h. Fig. 1c shows the diagram obtained and no reaction bands were observed.

On this basis, it is conceivable the structure shown in Fig. 2 for starch-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 ºC 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

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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}H NMR.
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380 **Citric Acid**
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409 **Crosslinked system**
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411 Fig. 2. Schematic illustrations of citric acid, glycerol, starch and possible
412 structure of crosslinked system.
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414 3.2. ATR/FTIR
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416 ATR/FTIR spectra of the four developed films are exposed in Fig. 3. All spectra
417 showed the following characteristic bands: 3300 cm^{-1}, which corresponds to the
418 stretching of OH group belonging to starch, glycerol and water; 2980-2900 cm^{-1},
419 due to CH stretching, and 1650 cm^{-1} assigned to the water adsorbed by starch
420 molecules.

Starch

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To compare the amount of available OH in the different systems, the ratio between the intensity of the peak at 3300 cm^{-1} (I_{3300}) and that at 1149 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 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 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 cm^{-1}, (stretching vibration of 'C–O' in 'C–O–C') with respect to that at 1149 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 °C.

Olsson et al. (2013b) demonstrated that the hydrolysis of starch-glycerol-CA films was almost completely hindered at a solution with pH ≥ 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 °C.

Fig. 3. ATR/FTIR spectra of all the studied films: (a) Systems processed at 75 °C and (b) systems processed at 85 °C.
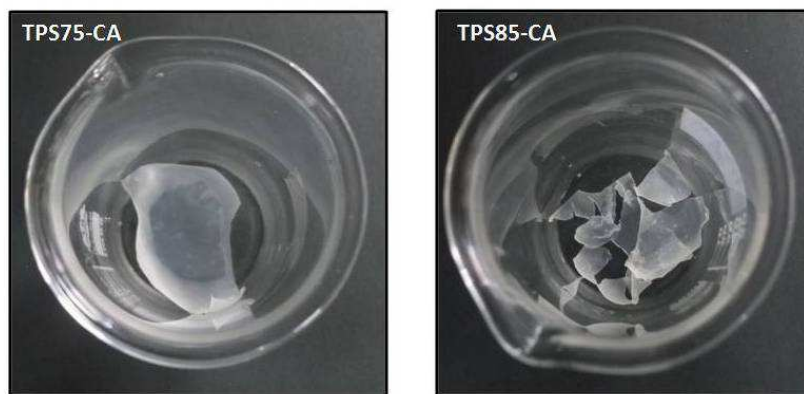
Fig. 4. Photographs of TPS75-CA and TPS85-CA after swelling process.

3.4. Low field nuclear magnetic resonance (^{1}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,

indicating the existence of two water populations of different mobility, associated with two decay times: a shorter relaxation time, T_{21}, and a longer relaxation time, T_{22} (Choi & Kerr, 2003; Leung, Steinberg, Wei, & Nelson, 1976). T_{21} is related with the water populations that are bound more tightly to the macromolecules, while T_{22} involves the less strongly linked (Leung et al., 1976).

Both decays can be adjusted by an exponential function as follow:

$$I = Ae^{\left(-\frac{t}{T_{2i}}\right)} + I_0 \quad (5)$$

The relaxation time parameters obtained from the fit are presented in Table 2. In no crossl[...] nly a slight difference in the decay time T_{22} [...] PS85. Considering that the process temperat[...] aterial structure, this result, indicated that the f[...] amount of available water than films processe[...]
In the fil[...] r than in the films without CA. This means th[...] strongly linked is more mobile. Taking in[...] led to a lower number of free OH groups a[...] mobility (FTIR), the obtained values of T_{22} [...] more open network that favours water mo[...] that CA acted as crosslinking agent of starch[...]
The incre[...] pect to TPS85 was lower compared to the systems processed at 75 °C, suggesting the existence of an additional phenomenon that could be the chain scission by starch hydrolysis in agreement with previous studies (FTIR, swelling).
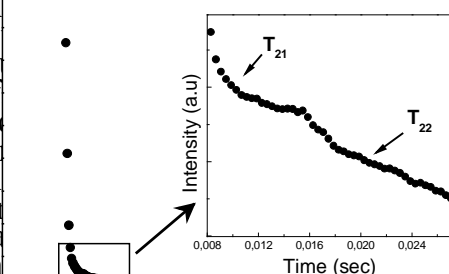

Fig. 5. Free induction decay (FID) for TPS75 system, showing two relaxation processes.

Table 2. Moisture content (MC), water vapour permeability (WVP) and relaxation times, T_{21} and T_{22}, of the developed films.

System	MC (%)	WVP (g/msPa) x 10^{-10}	T_{21} (ms)	T_{22} (ms)
TPS75	30 ± 1	2.8[a] ± 0.2	0.57 ± 0.01[a,b]	12.4 ± 0.4
TPS85	34 ± 2	2.9[a] ± 0.2	0.59 ± 0.01[a]	13.5 ± 0.4
TPS75-CA	20 ± 1	1.8 ± 0.2	0.56 ± 0.01[a,b]	15.7 ± 0.5[a]
TPS85-CA	26 ±1	2.2 ± 0.2	0.55 ± 0.01[b]	15.9 ± 0.6[a]

Values with the same letter are not significantly different (p ≤ 0.05)

3.5. Moisture content and moisture absorption

Moisture content (MC) and moisture absorption (MA) indicate the proportional amount of moisture in the film and their capacity to absorb moisture from the environment in the time, respectively. Both MC and MA of the films decreased with the addition of citric acid (Table 2 and Fig. 6). The decrease of moisture content in TPS-CA films supports the proposal about crosslinking and the consequent decrease of free OH groups, previously determined. Moisture absorption reduction of TPS75-CA and TPS85-CA was expected taking in to account the least amount of OH that they contain. Crosslinking of starch supplemented the natural intermolecular hydrogen bond, improving water resistibility (Krumova, Lopez, Benavente, Mijangos, & Pereña, 2000). The decrease in MA by the incorporation of CA was observed by several researches (Ghanbarzadeh et al., 2011; Yu, Wang, & Ma, 2005; Olsson et al., 2013a). In the
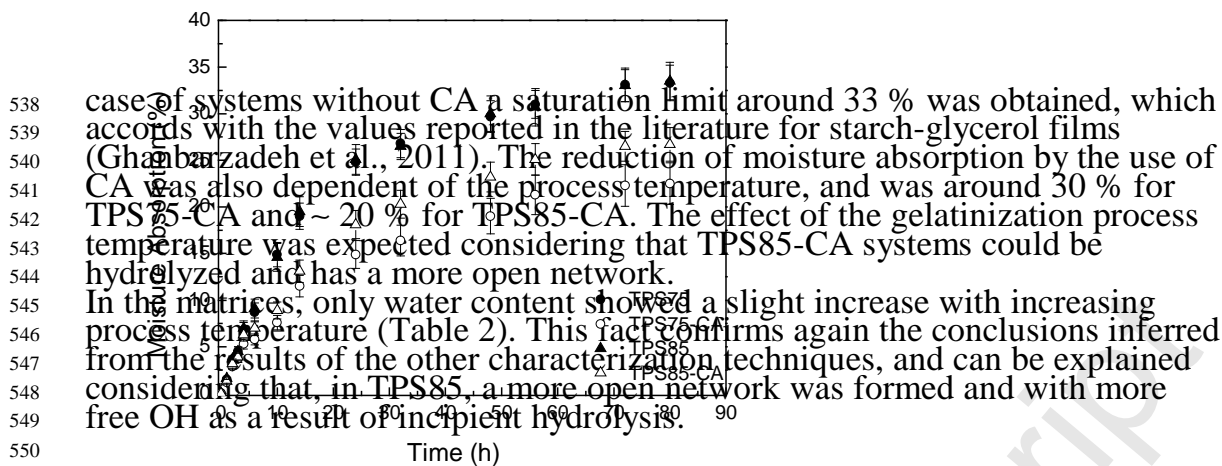
538 case of systems without CA a saturation limit around 33 % was obtained, which
539 accords with the values reported in the literature for starch-glycerol films
540 (Ghanbarzadeh et al., 2011). The reduction of moisture absorption by the use of
541 CA was also dependent of the process temperature, and was around 30 % for
542 TPS75-CA and ~ 20 % for TPS85-CA. The effect of the gelatinization process
543 temperature was expected considering that TPS85-CA systems could be
544 hydrolyzed and has a more open network.
545 In the matrices, only water content showed a slight increase with increasing
546 process temperature (Table 2). This fact confirms again the conclusions inferred
547 from the results of the other characterization techniques, and can be explained
548 considering that, in TPS85, a more open network was formed and with more
549 free OH as a result of incipient hydrolysis.
550
551
552 Fig. 6. Moisture absorption of the different studied films.
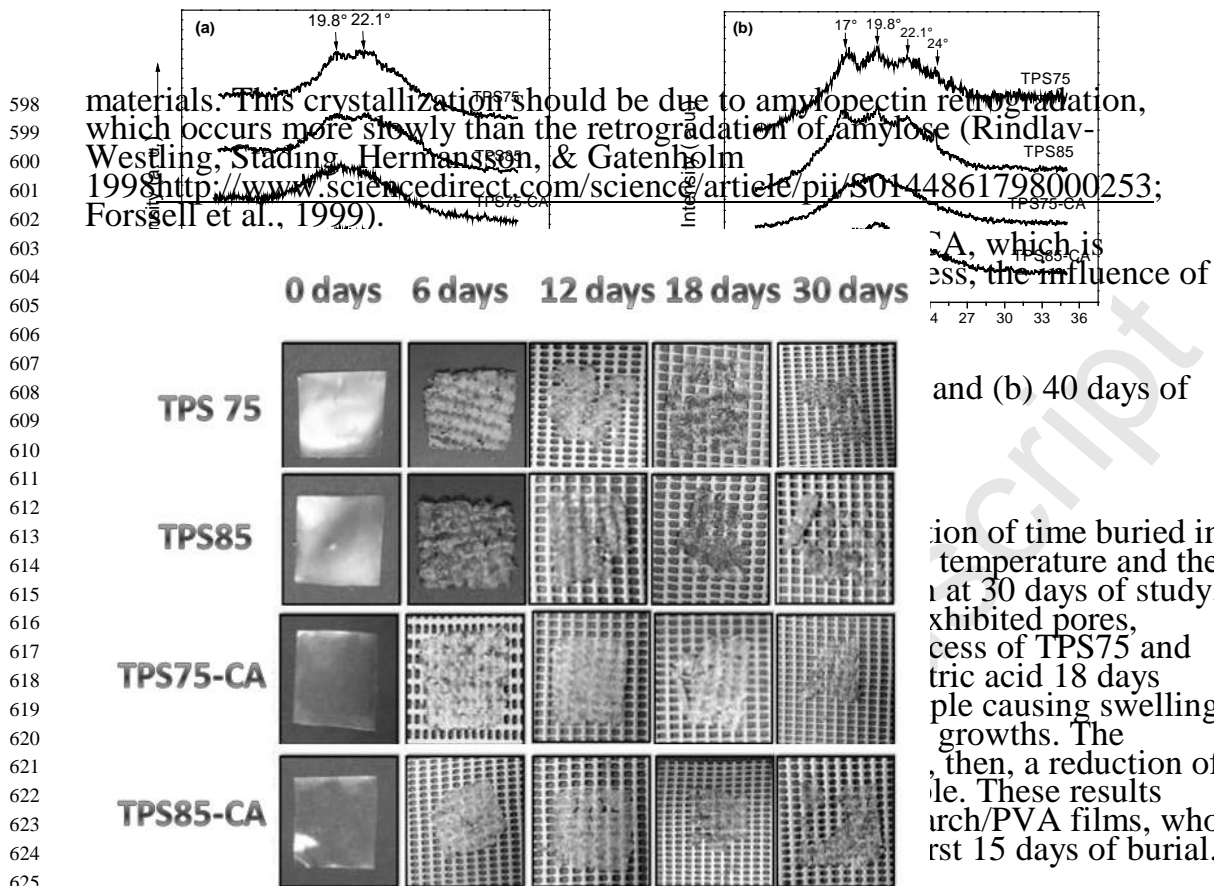553
554 3.6. Water Vapor Permeability
555
556 The values of water vapor permeability (WVP) are reported in Table 2. WVP of
557 samples without CA is of the same order of magnitude that those reported in the
558 literature (García et al., 2009; Bertuzzi et al., 2007). The addition of CA led to a
559 significant decrease in WVP of ~ 36 % for TPS75-CA and 17.2 % for TPS85-
560 CA, confirming again the formation of a network. Considering that food
561 packaging films are often required to limit moisture transfer between the food
562 and the surrounding atmosphere, WVP should be as low as possible. Several
563 authors reported that the addition of CA in starch films led to a decrease in
564 WVP, because the hydrophilic OH groups were substituted with hydrophobic
565 ester groups (Ghanbarzadeh et al., 2011; Reddy & Yang, 2010; Ma, Chang, Yu,
566 & Stumborg, 2009). However, beyond this idea, this study showed that citric
567 acid is efficient to crosslink starch at low processing temperatures. The
568 formation of this network reduced WVP and generated other important
569 advantages from the point of view of application of these materials, for example,
570 they avoided starch retrogradation. Starch retrogradation occurs during the
571 starch films storage and lead to crystallization (Morales, Candal, Famá,
572 Goyanes, & Rubiolo, 2015). Then, the effect of citric acid on the films
573 retrogradation can be studied through X-ray diffraction.
574
575 3.7. X-ray diffraction
576
577 Fig. 7a shows X-ray diffraction patterns (XRD) of the studied films after 15
578 days of storage. Films without CA exhibit mostly feature of amorphous patterns
579 with only two small peaks at 2 θ = 19.8 ° and 22.1 °, characteristics of B- type
580 crystalline structure.It is well known that crystallinity of starch films occurs due
581 to retrogradation process (Morales et al., 2015). This process, strongly
582 dependent of water content, provokes an increase in crystallinity with the
583 increment of hydration degree (Buléon, Véronèse, & Putaux, 2007). We
584 demonstrated that TPS85 had higher water content than TPS75 (Table 2) and,
585 while TPS85 completed the gelatinization process, TPS75 reached only 82 %
586 (Fig. 1b), which means that some grains remained unbroken (crystalline).
587 Therefore, in the case of TPS75, two effects contributed to crystallinity:
588 retrogradation and incomplete gelatinization; while in TPS85 crystallinity was
589 due only to starch retrogradation, which was higher than TPS75 as consequence
590 of their greater water content.
591 TPS75-CA and TPS85-CA were completely amorphous at 15 days of storage
592 which accords with the idea that CA provoked crosslinking.
593 In order to support this idea, XRD to all the systems with 40 days of storage
594 were performed (Fig. 7b). The diffractograms of TPS-CA films correspond to
595 amorphous materials, confirming the effectiveness of crosslinking reaction,
596 while non-crosslinked films increased their crystallinity. Two new peaks at 2 θ =
597 17 ° and 24 °, corresponding also to the B-crystalline structure, appeared in TPS

materials. This crystallization should be due to amylopectin retrogradation, which occurs more slowly than the retrogradation of amylose (Rindlav-Westling, Stading, Hermansson, & Gatenholm 1998http://www.sciencedirect.com/science/article/pii/S0144861798000253; Forssell et al., 1999).

... CA, which is ... ess; the influence of

0 days   6 days   12 days   18 days   30 days

... and (b) 40 days of

... ion of time buried in ... temperature and the ... n at 30 days of study. ... xhibited pores, ... cess of TPS75 and ... tric acid 18 days ... ple causing swelling ... growths. The ... , then, a reduction of ... le. These results ... rch/PVA films, who ... rst 15 days of burial.

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, ^{1}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

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848 **Highlights**
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850 • Starch-glycerol eco-films using citric acid (CA) as crosslinking were developed.
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852 • CA first reacts with glycerol and then starch-glycerol-CA reaction occurs.
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854 • Different temperatures, 75 ºC and 85 ºC, were used in gelatinization process.
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856 • Biodegradable and non-retrograde starch-glycerol-CA films are obtained at 75°C.
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858 • Films heated at 75ºC show decreases in WVP and integrity after swelling in DMSO.
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