

Influence of process (extrusion/thermo-compression, casting) and lentil protein content on physicochemical properties of starch films

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ABSTRACT

Starch films often present high water sensitivity, affecting their barrier and mechanical properties. The effects of processing technique, extrusion/thermo-compression and casting, and lentil protein concentration (0, 0.75 wt.% and 1.5 wt.%) on biodegradable starch films were investigated. Extrusion/thermo-compression process increased in 90% the mechanical resistance of starch films produced following the casting methodology and decreased their moisture content, water solubility and water vapor permeability in 35%, 23%, and 50%, respectively. In the presence of the protein, the mechanical properties (Young modulus and stress at break) and the water tolerance improved due to the crosslinking phenomenon prompted between the protein and the polymeric backbone, being these effects more pronounced on the extruded formulations. All samples resulted thermal stable until 240 °C and biodegraded in compost in 5 weeks. This work revealed that extrusion/thermo-compression process and proteins as crosslinking of starch are two alternatives to improve the drawbacks of starch-based materials.

1. Introduction

The continuous growth of the population has increased alarmingly the level of pollution and waste. One of the greatest problems related to waste management are plastics. Their difficult recycling process led in the last decades to study several alternatives for their disposal. In this context, the sustainable use of biodiversity has emerged to contribute to a less negative impact on the environment since they reduce water pollution and CO₂ emissions and due to their low cost (Chang & Nickerson, 2015; Kowalczyk, Kordowoska-Wiater, Nowak, & Baraniak, 2015; Serier & Aoufi, 2017). Polysaccharide-based materials have been investigated as an alternative to petroleum-based plastics because they come from organic sources and degraded in short periods of time by microbial or fungal action or simply by the effect of humidity and temperature (compost) (Condés, Añón, Dufresne, & Mauri, 2018; Deng et al., 2014; Etxabide, Coma, Guerrero, Gardrat, & De La Caba, 2017; Gutiérrez, Guzmán, Medina-Jaramillo, & Famá, 2016; Medina-Jaramillo, Gonzalez Seligra, Goyanes, Bernal, & Famá, 2015; Musso, Salgado, & Mauri, 2017; Pires et al., 2013).

Starch appears to be one of the most attractive biopolymers because

it is very economical, non-toxic and film forming (Azevedo et al., 2017; Müller, Laurindo, & Yamashita, 2009; Su et al., 2009). Starch films can be applied to most food products as coatings or packaging without modifying its appearance or taste because they are tasteless, odorless and colorless (Basiak, Lenart, & Debeaufort, 2016). In addition, starch materials can be enriched by antioxidants and antimicrobials in order to increase the shelf life of a food (De Lacey, López-Caballero, & Montero, 2014; Medina-Jaramillo, Ochoa-Yepes, Bernal, & Famá, 2017). Nevertheless, starch films often show some drawbacks such as their water sensitivity, adversely affecting their barrier and mechanical properties (Acosta et al., 2016). The use of crosslinking agents or copolymers with starch are interesting alternatives to improve these properties in the final thermoplastic material (Kaewtatip & Thongmee, 2013; Santacruz, Rivadeneira, & Castro, 2015). According to the literature, films obtained by the blend of starch and proteins revealed significant attributes such as better mechanical, barrier and optical properties, than those prepared with starch or proteins separately (Basiak et al., 2016; Acosta et al., 2016; Sun, Sun, & Xiong, 2013). Hydrophobic components of proteins limits the poor water vapor permeability of starch films (Primožic, Duchek, Nickerson, & Ghosh, 2017), favoring their use in

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direct contact with food to delay product deterioration by moisture and solutes migrations (Wittaya, 2012).

Films from blends of starch and proteins have been obtained through different process, such as casting, extrusion, injection or blow molding (Otoni et al., 2017; Pavlath & Orts, 2009; Teixeira et al., 2012). Most studies use a laboratory scale methodology (casting) to prepare these type of materials because it implies economical equipment and limited space (Madhumitha, Fowsiya, Mohana Roopan, & Thakur, 2018). Nevertheless, one of the major problems of casting technique is the high processing cost due to low production that it involves and that it is difficult of scale-up (industrial scale) (Hernandez-Izquierdo, Reid, McHugh, Berrios, & Krochta, 2008; Mali, Grossmann, & Yamashita, 2010). In the last years, extrusion technique for biopolymer films processing has begun to be a focus of investigations. This technique can represent a great alternative for satisfying high demands at low cost (Chevalier, Assezat, Prochazka, & Oulahal, 2018; González Seligra, Eloy Moura, Famá, Druzian, & Goyanes, 2016; Pelissari, Yamashita, & Grossmann, 2011; Xie et al., 2017). Actually, extrusion is the most used process to obtain industrial plastics in great production volume, because it achieves high temperatures in short times and its operation is very simple (von Borries-Medrano, Jaime-Fonseca, & Aguilar-Mendez, 2016). This process involves both thermal and mechanical energy (Alam, Pathania, & Sharma, 2016). The operating conditions, such as temperature profile and screw speed, strongly influences in the final material properties (González-Seligra, Guz, Ochoa-Yepes, Goyanes, & Famá, 2017; Zepón, Vieira, Soldi, Salmorla, & Kanis, 2013). Otoni et al. (2017) used extrusion following by thermo-compression process to obtained starch-proteins films with successful results. The authors observed important improvements especially in solubility and water vapor barrier properties at high protein concentrations.

In particular, lentil proteins can be easily obtained from commercial lentil by alkaline extraction and isoelectric precipitation (Jarpa-Parra et al., 2014). Although lentils have been used as staple food since ancient times, they have been limited in developed countries especially due to the lower protein digestibility, anti-nutritional factors, flatulence and poor cooking qualities (Joshi et al., 2013). Lentil proteins are rich in Glutamic acid, Aspartic acid, Leucine, Arginine and Lysine (Bamdad, Goli, & Kadivar, 2006; Hefnawy, 2011; Jarpa-Parra et al., 2014), whose functional side chain groups can interact with starch hydroxyl groups (Sun, Liu, Ji, Hou, & Dong, 2018), enabling their use as a crosslinking agent. Amino acids of lentil protein many react to carbonyl group of starch glucoses producing N-substituted glycosylamine and water (Su et al., 2012).

The aim of this investigation was to evaluate the effect of the processing technique (casting and extrusion/thermo-compression) and the addition of low lentil protein concentration as cross-linker on the morphology, physicochemical properties and biodegradability of starch films.

2. Materials and methods

2.1. Materials

Cassava starch (commercial information: 18 wt.% amylose and 82 wt.% amylopectin) was provided by Cooperativa Agrícola e Industrial San Alberto Limitada (C.A.I.S.A., Misiones, Argentina). Commercial lentils, acquired in a local market (Buenos Aires, Argentina), were used to obtain lentil protein isolate (LP). Glycerol (Aldrich) was used as plasticizer. All the other reagents used in this study were of analytical grade.

2.2. Lentil protein isolate (LP) preparation

Lentil protein isolate (LP) was prepared according to the methodology reported by Swanson (1990) and Mauri and Añón (2008) with some modifications. Commercial lentils were hydrated in water and

grounded in a processing machine to obtain a paste, which was dried at 70 °C for 24 h and grounded again until to obtain a flour. The flour was suspended in water (100 g/L) and pH was adjusted to with 1 N NaOH to extract the protein. The suspension was stirred for 40 min and stored under refrigeration at 4 °C for 4 h. Then, it was allowed to room temperature and centrifuged for 30 min at 2200 g to extract the protein content. The supernatant was adjusted to pH 4.5 with 1 N HCl and the precipitated proteins were subsequently washed with distilled water in four times, centrifuged at 2200 g for 30 min, and then suspended in water. Finally, pH was adjusted to 7 with 1 N NaOH and the dispersions were collected in glass containers to be freeze-dried (Freezone 1 Liter benchtop Freeze Dry System equipment). The resultant powder (LP) was stored in flasks under refrigeration at 4 °C until their use. The protein content of LP, measured by the Kjeldahl technique (AOAC 920.53, 1995), with a protein conversion factor of N = 6.25 (Jarpa-Parra et al., 2014), was 86 ± 1% on dry basis.

2.3. Starch-based films production

2.3.1. Casting process

Different solutions were prepared by dispersing starch (4.5 wt.%), glycerol (1.5 wt.%) and proteins (Table 1) in distilled water (94 wt.%) under magnetically stirring during 40 min at room temperature, followed by heating until 80 °C for 20 min., temperature at which the gelatinization was completed (Paes, Yakimets, & Mitchell, 2008; Ochoa-Yepes, Medina-Jaramillo, Guz, & Famá, 2018).

Afterwards the dispersions were degassed for 7 min through a vacuum pump and distributed in acrylic Petri dishes (~30 g) to dry in an oven at 50 °C for 24 h. Films were conditioned for 4 weeks in desiccators at 25 °C and 56.7% of relative humidity (RH) (equilibrium with saturated NaBr) before characterization (Morales, Candal, Famá, Goyanes, & Rubiolo, 2015). The conditioning time was selected considering the reported by Famá, Goyanes, and Gerschenson (2007), who demonstrated that between two and four weeks of storage the changes of starch film properties were highly marked, but did not from 4 weeks of conditioning at RH 56.7%.

2.3.2. Extrusion/thermo-compression process

Films developed by the extrusion/thermo-compression technique were obtained following the methodology reported by González-Seligra et al. (2017). A co-rotating twin-screw extruder (Nanjing Kerke Extrusion equipment Co., Ltd.), with screw diameter (D) of 16 mm and length to diameter ratio L/D = 40, with ten temperature-controlling zones equipped with a cylindrical die of 4 mm (Fig. 1a), was used. The ratio starch:glycerol (3:1) and LP concentrations used in this process were the same that the used in casting. Cassava starch (60 wt.%), glycerol (20 wt.%), water (20 wt.%), and the desired concentration of proteins (Table 1) were mixed at 20 rpm for 15 min in a horizontal mixer (Pulvex). After that, the mixtures were sieved with a number 10 mesh and stored for 24 h in sealed containers according with Wang and Ryu (2013). All systems were extruded at a screw speed of 80 rpm (with feeding rate of 12 g/min) and temperature profile (from the feeder to the die) of 90 °C/100 °C/110 °C/120 °C/130 °C/130 °C/140 °C/140 °C/130 °C/120 °C.

Table 1
Protein (LP) concentration and nomenclature of the different films.

Process	LP (wt.%, dry basis) ¹	Nomenclature
Casting	0	S _C
	0.75	S-LP0.75 _C
	1.5	S-LP1.5 _C
Extrusion/thermo-compression	0	S _{ET}
	0.75	S-LP0.75 _{ET}
	1.5	S-LP1.5 _{ET}

¹ Mass of the component in percentage, presented in 100% of solids.

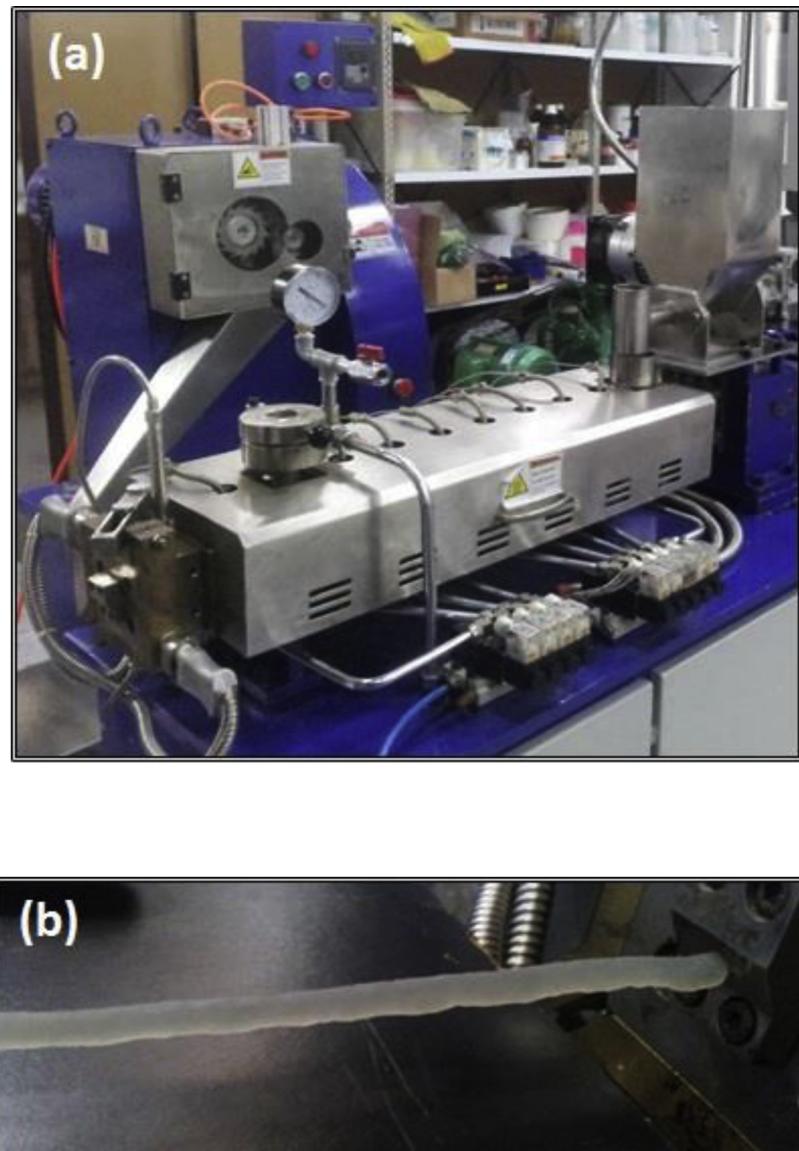


Fig. 1. (a) Co-rotating twin-screw extruder equipment and (b) a thread of starch-glycerol-water ejected by the extruder during the process.

Threads of ~ 8 mm of diameter from each system were obtained (Fig. 1b) and stabilized in a conditioned chamber at 56.7% RH (saturated NaBr) for 72 h. Then, the threads were pelletized, using an automatic pelletizer (Weinuo Technology Co., Ltd, China). Finally, the films were obtained by thermo-compression following the procedure reported by González-Seligra et al. (2017). Pellets (~ 4 g) were pressed at 130 °C and ~ 56 Kg/cm² for 15 min. The resultant films were conditioned at 56.7% RH (NaBr) up to 4 weeks from the extrusion/thermo-compression process before being characterized.

2.4. Films characterization

2.4.1. Chemical characterization

2.4.1.1. Fourier transform infrared spectroscopy (FTIR). Fourier Transform-Infrared Spectroscopy (FTIR) spectrum of all starch films and proteins were obtained using a FT/IR 4100 spectrophotometer (Jasco Inc., Japan). Transmission sampling approach was implemented. The measurement were the average of 64 scans and were recorded between 4000–400 cm⁻¹ with a resolution of 2 cm⁻¹. All tests were carried out in triplicated.

2.4.1.2. Thermogravimetric analysis (TGA). Thermogravimetric Analysis (TGA) of the protein and composites were performed using a TGA/DTA (DTG-60 Shimadzu, Kyoto, Japan) instrument. Aluminum capsules containing ~ 10 mg of sample (protein extract or film) were tested under a nitrogen flow of 30 ml/min, heating at 10 °C/min from 40 °C to 400 °C. Curves of the weight loss normalized to the initial mass in function of the temperature were reported. All tests were carried out in triplicated.

2.4.2. Morphological characterization (SEM)

Samples of all composite materials were frozen and fractured under liquid nitrogen in order to study their morphology. A scanning electron microscope with a field emission gun (FEG) Zeiss DSM982 GEMINI was used. Fractured samples were glued on a support and coated with a thin sputtered gold layer of ~ 10 nm before the analysis.

2.4.3. Susceptibility to water

2.4.3.1. Moisture content (MC). Moisture content of all films (MC) was determined through the gravimetric method proposed by the AOAC (1995). Pieces of each material (~ 0.5 g) were weight (m_i) and then subjected at 100 °C for 24 h. After that, the samples were weighed again

(m_f). Water content was calculated as:

$$MC = \frac{m_i - m_f}{m_i} \times 100 \quad (1)$$

The results represent the mean value of five sampling units taken from different films.

2.4.3.2. Water solubility (WS). Water solubility values (WS) were obtained following the method described by [Gontard, Duchez, Cuq, and Guilbert \(1994\)](#) with modifications. Disks of 2 cm of diameter were subjected in an oven at 100 °C for 24 h and then weighted to obtain the initial dry value mass (m_{si}). Other disks of 2 cm were immersed in 50 mL of distilled water for 24 h at 25 °C, and after that, they were dried at 100 °C for 24 h to obtain the final dry weight (m_{sf}).

Water solubility (%) was determined using Eq. (2):

$$WS = \frac{(m_{si} - m_{sf})}{m_{si}} \times 100 \quad (2)$$

The results are the mean and standard deviation of five replicates of each system.

2.4.3.3. Water vapor permeability (WVP). Modified ASTM E96–00, ([ASTM E96-00, 1996](#)) procedure ([Famá, Rojo, Bernal, & Goyanes, 2012](#)) was used to measure the water vapor permeability of all films (WVP). Circular acrylic cells with an exposed circular area of $3.7 \times 10^{-4} \text{ m}^2$, containing CaCl_2 as desiccant, were used. Each film sample was sealed over the circular area of the cells, thus the cells were stored at 25 °C in desiccators containing NaCl (70% of RH). Cells were weighed every 24 h for ten days, until constant mass. Changes in the weight were plotted as a function of time. WVP (g/msPa) was calculated using Eq. (3):

$$WVP = \frac{G \times e}{\Delta P \times A} \quad (3)$$

Where G (g/s) is the slope of plotted curve, e (m) the film thickness, ΔP (Pa) the saturation vapor pressure of water at ambient temperature and A (m^2) the exposed area.

The results are the mean and standard deviation of five replicates of each system.

2.4.3.4. Contact angle (θ). Contact angle (θ) measurement is an indicative of the hydrophobicity of polymeric materials. Higher the θ value, higher sample surface hydrophobicity. Contact angle measurements were carried out using a MicroView microscope (USB Digital Microscope) coupled with an image analysis software (Analysis Software 220 × 2.0 MP) and the methodology described by [Guz, Famá, Candal & Goyanes \(2017\)](#). A drop of distilled water (2 μL) was placed on the surface of each material and photographed. The contact angle was determined as the angle formed by the intersection of the liquid-solid line (drop of water-surface of the film) and the liquid-steam (tangent on the boundary of the drop). The mean of six measurements was reported.

2.4.4. Mechanical properties

2.4.4.1. Thickness measurement. Thickness of films were measured (immediately before their characterization), using a manual micrometer Micromaster IP54 (TESA-Capasystem) at 10 random positions on the films.

2.4.4.2. Uniaxial tensile. Uniaxial tensile tests were carried out in DMTA IV Rheometric Scientific equipment (Rheometric Scientific Inc., New Jersey, USA). Samples of 15 mm × 5 mm (length × wide, respectively) were studied in the Rectangular Tension mode. Nominal stress (σ)-strain (ε) curves were obtained and Young's modulus (E), tensile strength (σ_b), strain at break (ε_b), and tensile toughness (T) values were determined from 10 samples of each system.

2.5. Biodegradability

Soil burial test was employed on a laboratory scale to examine the biodegradability of the materials on qualitative form. Rectangular pieces of each system of 2 cm × 2 cm were buried in plastics containers of 22 cm × 15 cm × 8 cm containing vegetal compost at a depth of 5 cm from the surface in order to ensure the aerobic degradation. The compost was sprayed with water twice a day. At different times, a sample of each system was unearthed, dried in oven at 50 °C for 24 h and photographed to register its degradation.

2.6. Data processing and statistical analysis

Two-way ANOVA with 95% confidence level ($p < 0.05$) and Tukey post-hoc test were used to analyze the data. Results are reported as the mean and its standard deviation. To compare results “T” test was applied.

3. Results and discussion

3.1. Chemical properties (FTIR and TGA)

FTIR spectra of all films and proteins are presented in Fig. 2. The characteristic peaks for starch-based films were observed in all films at around 3300 cm^{-1} (stretching band of the OH groups corresponding to starch, water and glycerol), between 2980 - 2900 cm^{-1} (stretching of CH groups), at $\sim 1650 \text{ cm}^{-1}$ (band assigned to the absorption of water by starch molecules) ([Basiak, Lenart, & Debeaufort, 2018](#); [da Silva et al., 2012](#)) and in the 800 – 1200 cm^{-1} region that can be used as a “fingerprint” for starch (1149 , 1081 , 1019 , 931 , 850 cm^{-1} , dominated by ring vibrations overlapped by C–OH, C–C and C–H side group vibrations, and the C–O–C glycosidic bond vibration) ([Basiak et al., 2018](#); [Kačuráková, Čapek, Sasinková, Wellner, & Ebringerová, 2000](#); [da Silva, Pereira, & Druzian, 2012](#)). It can be seen that the band at around 3300 cm^{-1} resulted more intense in S_C compared to that of S_{ET} .

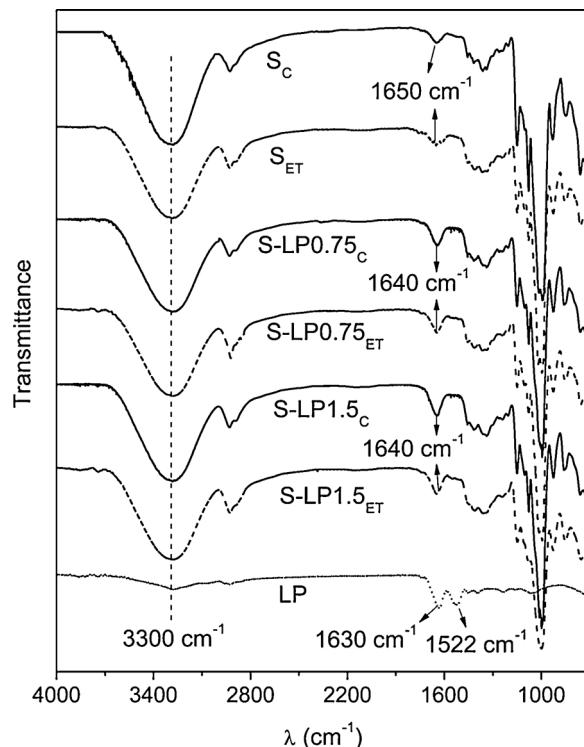


Fig. 2. FTIR spectra of all developed films and LP. The continuous line represents casting samples and the dash line extruded films.

suggesting more OH groups presented in this material. The others representative peaks of these two films did not exhibited significant differences.

Pure lentil proteins' spectra showed strong and broad band between 3100 cm^{-1} and 3600 cm^{-1} ascribed to O—H and N—H stretching vibrations of hydroxyl groups and primary and secondary amines respectively, the peak at 2921 cm^{-1} , attributed to the C—H stretching vibration, and typical protein bands at 1633, 1528, 1457, 1396 and 1238 cm^{-1} that are associated with amide I (80% C=O stretch, 10% C—N stretch), amide II (60% N—H bend, 30% C—N stretch and 10% C—C stretch), and amide III (complex bands resulting from several coordinate displacements) (Kaddour, Mondet, & Cuq, 2008; Barbana & Boye, 2013; Basú, Guha, & Ray, 2015; Carbonaro, Maselli, Dore, & Nucara, 2008). Protein bands were not clearly seen in films spectra, probably due to the low amount of this component added to film formulation. However, the band at around 3300 cm^{-1} decrease with protein incorporation, being notable in the films prepared by casting. This behavior is probably due to protein-starch interactions through hydrogen bonding that in the case of S-LP0.75_C and S-LP1.5_C it is observable because OH of proteins substituted some interactions between water and hydroxyl groups of starch that occurred in S_C due the high amount of water used in casting process, thus decreasing free OH groups. The shift of OH stretching band to lower wavenumbers, reflecting the major increase in hydrogen bond density and strength (Lu, Donner, Yada, & Liu, 2016), was not observed in this work, probably due to the low protein concentration used.

The slight peak at $\sim 1650\text{ cm}^{-1}$ observed in S_C and S_{ET} increased its intensity and occurred at $\sim 1645\text{ cm}^{-1}$ when proteins were added. According to the literature, the band at 1645 cm^{-1} could be assigned to C=N stretching vibration, ascribed to Maillard's reaction products provoked by heating (Aaslyng, Larsen, & Nielsen, 1999; Su et al., 2012), induced by protein and starch interactions (Aaslyng et al., 1999; Su et al., 2012).

Thermal curves of the protein and all developed films are exposed in Fig. 3. Proteins presented the beginning of a thermal degradation at around $230\text{ }^{\circ}\text{C}$, followed by a significant mass loss from $\sim 290\text{ }^{\circ}\text{C}$ until the end of the test ($400\text{ }^{\circ}\text{C}$), where a drop of more than 40% of weight occurred. This step is related to the split of covalent bonding of the peptides of the amino acid residues of proteins (Nanda, Lochan Nayak, & Krishna Rao, 2007). Ogale, Cunningham, Dawson, and Acton (2000) reported similar behavior for soy protein isolate and films from this raw material.

All films exhibited three mass loss steps until the end of the test, which is common in cassava starch films (dos Santos Caetano et al., 2018). One, corresponding to the evaporation of water occurred from around $90\text{ }^{\circ}\text{C}$ to $\sim 150\text{ }^{\circ}\text{C}$ (Mariani, Allganer, Oliveira, Cardoso, &

Innocentini-Mei, 2009). A slight increase in the mass loss of S_C in that step with respect to the others system can be observed, indicating higher moisture content of this film. The well-known plasticizer thermal degradation of thermoplastic starch films (Romani, Prentice-Hernández, & Martins, 2017) was also observed in all materials from $\sim 180\text{ }^{\circ}\text{C}$ to $\sim 280\text{ }^{\circ}\text{C}$, with a mass loss of around 9–11%.

Proteins addition led to a slight lower mass loss of the composites in this step, as they would favor glycerol retention in films network. Proteins' presence should favor the interactions among glycerol and films biopolymer (Azevedo et al., 2017). The process used did not led to significant differences in that temperature range except in the case of S_C in which slightly higher mass loss was observed. This behavior in the rich glycerol zone of the starch-glycerol degradation suggests that, hydrogen bonding interactions (between hydroxyl groups of starch chain and glycerol molecules) in S_C are less strong than in S_{ET} (García, Famá, Dufresne, Aranguren, & Goyanes, 2009).

The third step in the thermal curves corresponded to the starch degradation (Rodríguez-Castellanos, Martínez-Bustos, Rodríguez, & Trujillo-Barragán, 2015), and occurred from $\sim 280\text{ }^{\circ}\text{C}$ to $\sim 350\text{ }^{\circ}\text{C}$ with the highest loss weight ($\sim 65\%$). The use of proteins did not affected this thermal process when films were prepared by extrusion/thermo-compression, indicating that strong interaction between hydroxyl groups of starch and amino group of the protein chains can be occur (Moreno, Atarés, & Chiralt, 2015). However, in the case of S-LP0.75_C and S-LP1.5_C, this step occurred at slightly lower temperatures, suggesting that the proteins trend to greater interacted with the plasticizer than with starch.

3.2. Morphology (SEM)

The images of the cryogenic fracture surface micrographs (FE-SEM) of the composites obtained by both casting and extrusion/thermo-compression techniques are exposed in Fig. 4. Starch films without protein addition, S_C and S_{ET}, exhibited homogeneous and compact structures without pores and broken starch grains, independently of the methodology used (Fig. 4a and d). Both process formed matrices with completely gelatinized starch. The image of S_{ET} sample revealed that the extrusion/thermo-compression parameters resulted adequate for thermoplastic forming with great interaction between starch and glycerol.

The incorporation of the proteins in the extruded materials led to homogeneous films without holes or grains, regardless of the protein concentration (Fig. 4d and f) but with several lines, indicating less flexible fracture attributed to the starch-proteins crosslinking (Zilli et al., 2005). In the case of casting films, few broken starch grains was observed in S-LP0.75_C (Fig. 4c) and lines in the fracture surface of S-LP1.5_C (Fig. 4e). The broken starch demonstrated that the crosslinking hindered the starch gelatinization (Li, Yeh, & Fan, 2007; Parada & Aguilera, 2011; Xie, Chang, Wang, Yu, & Ma, 2011). The interaction between the starch and protein provoked lower availability water molecules to interact with the starch slightly hindering its complete gelatinization (Parada & Aguilera, 2011).

3.3. Susceptibility to water

3.3.1. Moisture content (MC), water vapor permeability (WVP) and water solubility (WS)

Table 2 exhibits moisture content (MC), water vapor permeability (WVP) and water solubility (WS) of all films. The processing methodology affected the susceptibility to water. The films prepared by extrusion/thermo-compression showed lower MC and WVP in all samples, and less water solubility in the film without proteins (S_{ET}) when compared to casting samples. Many factors made MC decreases of extruded films with respect to that prepared by casting reasonable and expected. On the one hand, the extrusion/thermo-compression process involved lower amount of water (20 wt.%) in the films preparation comparing

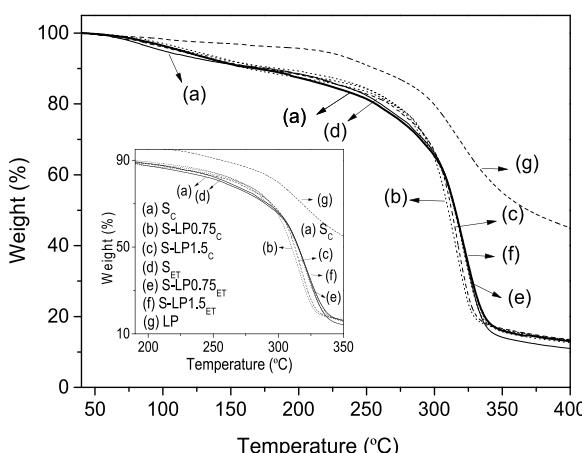


Fig. 3. Thermal degradation of: (a) S_C, (b) S-LP0.75_C, (c) S-LP1.5_C, (d) S_{ET}, (e) S-LP0.75_{ET}, (f) S-LP1.5_{ET} and (g) protein (LP).

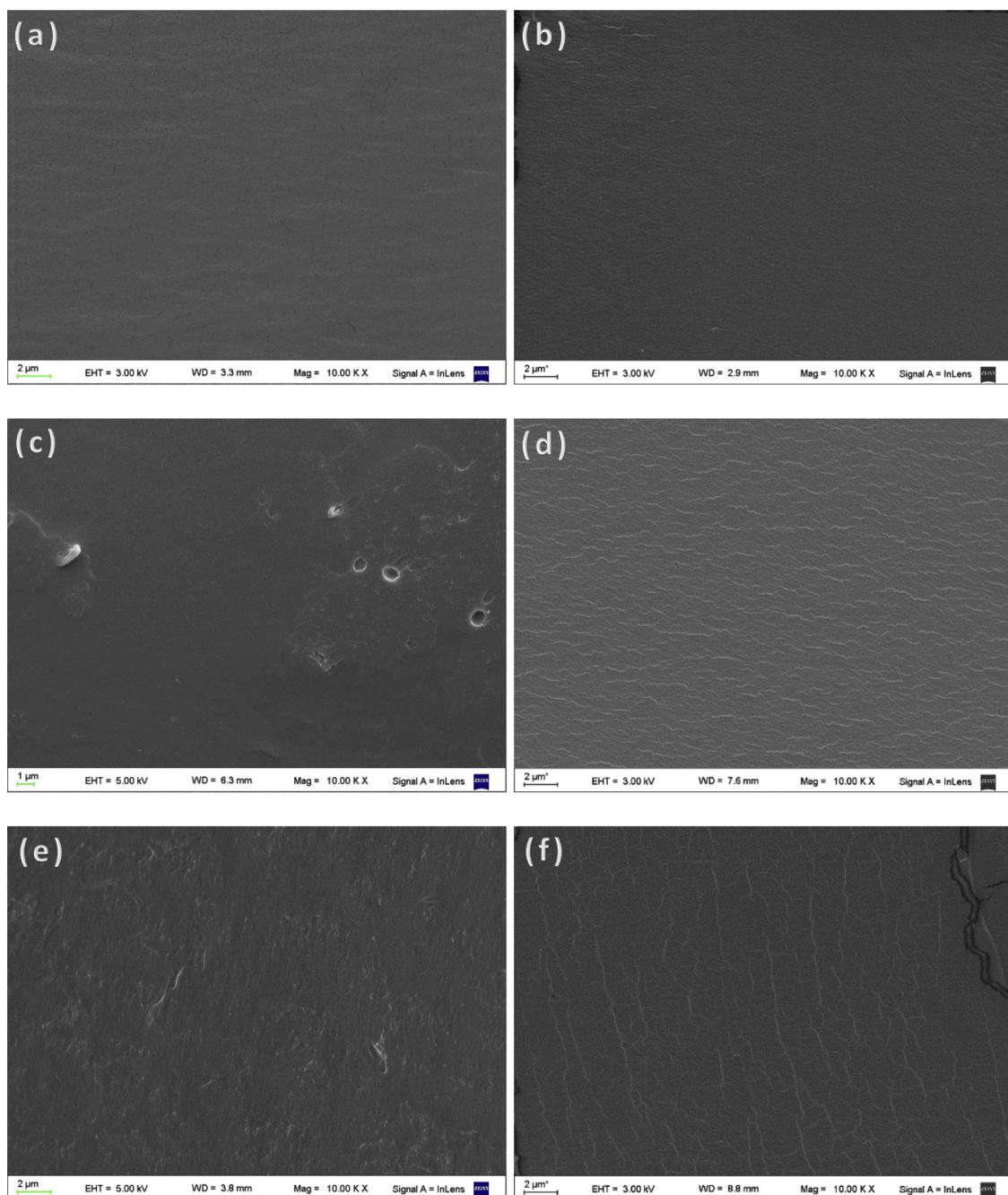


Fig. 4. FE-SEM micrographs of the cryogenic fracture surface of: (a) S_C , (b) S_{ET} , (c) $S\text{-LP}0.75_C$, (d) $S\text{-LP}0.75_{ET}$, (e) $S\text{-LP}1.5_C$, and (f) $S\text{-LP}1.5_{ET}$.

Table 2

Moisture content (MC), water solubility (WS), water vapor permeability (WVP) of all developed films.

Film	MC (%)	WS (%) [± 1]	WVP (g/msPa) $\times 10^{-10}$ [± 0.1]
S_C	30.0 ± 1.0 ^a	37 ^a	2.8 ^a
$S\text{-LP}0.75_C$	28.1 ± 0.5 ^b	33 ^b	2.3 ^b
$S\text{-LP}1.5_C$	27.4 ± 0.7 ^b	32 ^{b,c}	2.2 ^b
S_{ET}	19.1 ± 0.7 ^c	30 ^c	1.4 ^c
$S\text{-LP}0.75_{ET}$	20 ± 0.9 ^c	32 ^{b,c}	1.5 ^c
$S\text{-LP}1.5_{ET}$	18 ± 1.1 ^c	30 ^c	1.4 ^c

^{a,b,c} Different superscript letters mean significant differences ($P < 0.05$).

with that fabricated by casting methodology (94.5 wt.%). Thus, in casting, more water molecules interacted with the OH groups of the starch chains that did not evaporated during drying. In addition,

extrusion/thermo-compression processing implied higher temperatures than that of water evaporation, while the highest temperatures used in casting were 80 °C. This made that extrusion/thermo-compression methodology favored the decrease in the available water molecules. Following the literature, the increase in WVP in the samples prepared by casting may be a response to the increase in the inter-chain spacing in the films structure (Chang & Nickerson, 2015) as consequence of higher MC. It is known that water is an important plasticizer (Famá, Rojas, Goyanes, & Gerschenson, 2005) and plasticizers reduce internal hydrogen bonding between polymer chains, increasing molecular volume (Mali, Grossmann, García, Martino, & Zaritzky, 2006). Higher MC of C led to increase its plasticizer concentration and, as consequence, to the effective diffusion coefficient of water vapor in the films (Kamper & Fennema, 1984; Karbowiak et al., 2006), then an increase in WVP occurred (Balqis, Khaizura, Russly, & Hanani, 2017; Guo et al., 2012).

In the case of water solubility, the increase in casting sample (S_C) compared to S_{ET} , was expectable due to the presence of more availability OH groups in the casting film that act as plasticizer, as it was previously explained. Increasing the hydrophilic plasticizer enhanced the film solubility in water, as it was reported by other authors (Bourtoom, 2008; Seligra, Jaramillo, Famá, & Goyanes, 2016).

The addition of proteins did not affect the susceptibility to water parameters when the films were prepared by the extrusion/thermo-compression methodology. According to FTIR behavior, proteins cross-linked the starch by reactive side groups such as NH_2 and/or OH. Taking into account the extrusion results, the crosslinking reaction occurred without replacing the interactions produced between water molecules and starch chains, probably due to the low concentration of water used in this processing.

Contrary, the use of proteins in casting systems led to decreases in all susceptibility to water parameters regardless of the protein concentration. The decrease in MC is associated with that, during cross-linking, OH reactive groups of proteins substituted some interactions between water and hydroxyl groups of starch that occurred in S_C due the high amount of water used during the process, decreasing the hydroxyl groups capable to interact with water molecules (Flores, Costa, Yamashita, Gerschenson, & Grossmann, 2010). Water solubility reduction of $S\text{-LP}0.75_C$ and $S\text{-LP}1.5_C$ is related to the least amount of free OH that could interact to water and to the crosslinking of starch, which supplemented some natural intermolecular hydrogen bond, slightly improving water resistibility (Krumova, Lopez, Benavente, Mijangos, & Pereña, 2000; González Seligra et al., 2016).

Finally, taking into account that water vapor permeability is dependent on the solubility coefficient and the diffusion rate of water in the film (Rogers, 1965) lower WVP of $S\text{-LP}0.75_C$ and $S\text{-LP}1.5_C$ with respect to S_C were consequence of two different contributions. On a one hand, the hydrophobic action of the amino acids that mostly make up the protein fraction had to lead to a decrease in WVP (Bamdad et al., 2006). On the other hand, the decrease of MC of $S\text{-LP}0.75_C$ and $S\text{-LP}1.5_C$ led to less concentration of plasticizer producing decrease in the effective diffusion coefficient of water vapor in the films, and then, a decrease in WVP (Balqis et al., 2017; Guo et al., 2012).

3.3.2. Contact angle (θ)

Films hydrophobicity was investigated through the evaluation of the contact angle (θ) between a drop of water and the surface of each film (Fig. 5).

In the case of casting films, the contact angle increased around 9% when protein was incorporated, regardless of the concentration used. All extruded systems presented higher values of θ than that of casting and did not show significant differences between themselves, indicating that the surface hydrophobicity increased when extrusion/thermo-compression process was implemented. This behavior is consistent with the susceptibility to water results. Casting let to films with higher MC than extrusion/thermo-compression. The presence of more availability OH groups led to greater affinity to the aqueous medium, then to more hydrophilic material. In this context, proteins incorporation led to lower MC in the films prepared by casting, then, to less hydrophilic material compared with S_C (González Seligra et al., 2016).

3.4. Mechanical properties

Fig. 6 shows nominal stress-strain curves of composites obtained by both techniques, casting and extrusion/thermo-compression. All evaluated films were found to be homogeneous and showed thickness at $\sim 0.29 \mu\text{m}$ (Table 1). All curves presented the typical behavior of thermoplastic starch based films, independently of the process methodology and protein concentration: an elastic-linear zone followed by a non-linear behavior until failure, which occurred with a precipitous drop of load at the maximum stress with no necking before fracture (Gutiérrez et al., 2016; Ma, Yu, & Kennedy, 2005).

It is noted that higher Young's modulus (E') and strength at break (σ_b) and a lower strain at break (ϵ_b) values resulted from the extrusion/thermo-compression processing compared to those of casting (Table 3). This behavior can be attributed to the decrease in water content of the films (less plasticization) when extrusion technique was implemented. As it was previously discussed, higher hydroxyl groups, which interacted with the OH of starch chains, were formed in S_C sample compared to S_{ET} , favoring the space between the polymer chains. Thus, a decrease in the intramolecular forces between the starch chains occurred, increasing their mobility and film flexibility (Maran, Sivakumar, Sridhar, & Thirugnanasambandham, 2013). Decreases in E' and σ_b , as well as increases in ϵ_b of starch films developed by casting compared to those prepared by extrusion was reported in the literature (Chen, Zhang, Ishikawa, & Maekawa, 2002; Fakhouri et al., 2013). Finally, no significant differences were observed between fracture toughness values of S_C and S_{ET} (Table 3). This behavior resulted from the combination of the lower σ_b value and greater ϵ_b of the film prepared by casting compared to that fabricated by extrusion/thermo-compression.

When proteins were incorporated increases in Young's modulus and strength at break and decreases in the strain at break were observed, regardless of the processing technique and LP concentration. Higher E' and σ_b values and less ϵ_b when the proteins were used is attributed to the crosslinking between proteins and starch (Chevalier et al., 2018; Zheng, Ai, Chang, Huang, & Dufresne, 2009), discussed in FTIR section. It is known that crosslinking reaction limits chain movement and strengthened starch chain due to the strong attraction between the polymer and the crosslinking agent that favors the mechanical resistance of materials and made them less flexible (Detduangchan, Sridach, & Wittaya, 2014; Javed, Ullsten, Järnström, & Ernstsson, 2016; Shi et al., 2008; Yin, Li, Liu, & Li, 2005).

Finally, starch cross-linked by proteins led to less movement of polymer chains, causing a higher stiffness and less flexibility on the resulting material (Azevedo et al., 2017; Cao, Fu, & He, 2007; Moreno et al., 2015). Moreno et al. (2015) reported similar results for potato starch films added with lactoferrin and lysozyme proteins. Cao et al. (2007) related this behavior to the protein's structure as it confers a higher resistance when added to bioplastics. The films with LP made by casting showed a trend to decrease their fracture toughness while in the case of the extrusion process the trend was reversed. This result is related to the smaller difference of ϵ_b of $S\text{-LP}0.75_C$ and $S\text{-LP}1.5_C$ with respect to that of $S\text{-LP}0.75_{ET}$ and $S\text{-LP}1.5_{ET}$ versus the greater difference of σ_b . The slightly higher difference in the decrease in ϵ_b of $S\text{-LP}0.75_C$ and $S\text{-LP}1.5_C$ with respect to S_C than that of $S\text{-LP}0.75_{ET}$ and $S\text{-LP}1.5_{ET}$ compared to S_{ET} was consequence of the decrease of MC in casting films when proteins was incorporated that it did not happened in the case of $S\text{-LP}0.75_{ET}$ and $S\text{-LP}1.5_{ET}$.

3.5. Biodegradability

Biodegradability in soil was qualitatively evaluated by registering the appearance of the films over time after being buried in vegetable compost. As the experimental part described, every week, one sample of each system was unburied and photographed to examine its aspect (Fig. 7). Samples weight measurement resulted imprecise due to the difficulty to completely separate the compost adhered to the cells containing the films.

All samples showed coloration changes becoming brown and opacity appearance during the biodegradability assays, due to the dust of the soil (compost) that remains attached in the samples and water loss. In addition, they showed pores and breaks, signs of the beginning of degradation. From the photographic images of the systems, the influence of proteins incorporation or preparation technique on the degradation rate of the samples studied cannot be easily determined. All films almost completely degraded on the fifth week of buried on vegetal compost.

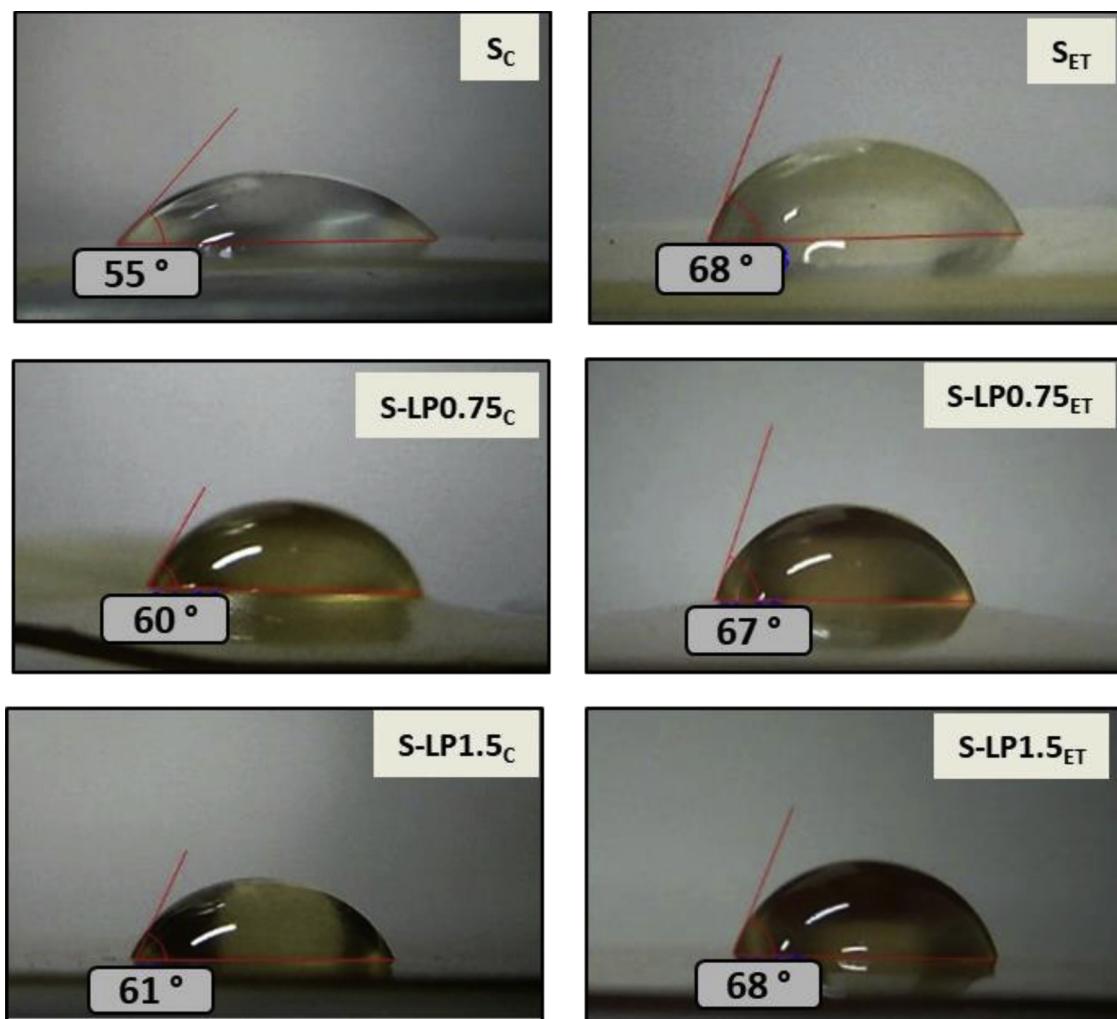


Fig. 5. Image of a drop of water deposited on the surface of all developed films.

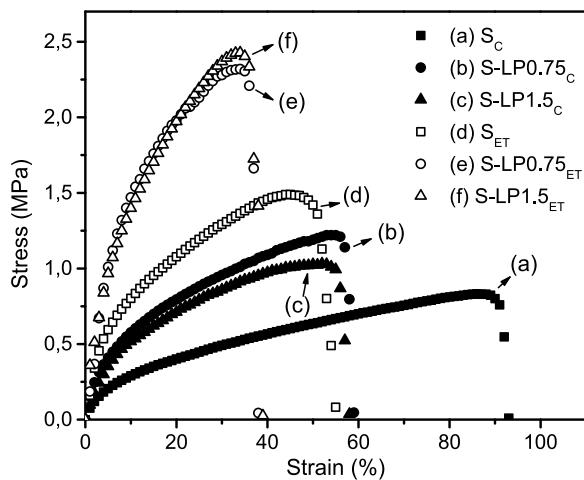


Fig. 6. Representative nominal stress-strain curves of: (a) S_C , (b) $S-LP0.75_C$, (c) $S-LP1.5_C$, (d) S_{ET} , (e) $S-LP0.75_{ET}$ and (f) $S-LP1.5_{ET}$.

4. Conclusions

The effect of processing technique and lentil protein concentration on physicochemical properties and biodegradability of starch films were investigated. Starch and glycerol films with different concentration of proteins (0 wt.%, 0.75 wt.% and 1.5 wt.%, on dry basis) were

Table 3

Thickness, Young's modulus (E'), strength at break (σ_b), strain at break (ϵ_b) and tenacity (T) of the studied films.

Film	Thickness (μm) [± 0.02]	E' (MPa)	σ_b (MPa)	ϵ_b (%) [± 3]	T (J/m^3) \times 10^6 [± 0.5]
S_C	0.29 ^a	4.1 ± 0.8^a	0.8 ± 0.1^a	93 ^a	5.3 ^{a,b}
$S-LP0.75_C$	0.29 ^a	$12 \pm 2^{b,c}$	$1.2 \pm 0.2^{b,c}$	59 ^b	5.1 ^{a,b}
$S-LP1.5_C$	0.30 ^a	10 ± 1^b	1.0 ± 0.1^b	58 ^b	4.4 ^a
S_{ET}	0.28 ^a	15 ± 2^a	1.5 ± 0.2^a	55 ^b	5.6 ^{b,c}
$S-LP0.75_{ET}$	0.28 ^a	21 ± 2^d	2.4 ± 0.2^d	38 ^c	6.4 ^c
$S-LP1.5_{ET}$	0.29 ^a	22 ± 2^d	2.4 ± 0.2^d	39 ^c	6.6 ^c

a,b,c,d Different superscript letters mean significant differences ($P < 0.05$).

prepared by casting and extrusion/thermo-compression processes. Greater stiffness and toughness in the extruded materials were obtained while more flexibility revealed the films obtained by casting. In addition, films with lower moisture content and water vapor permeability with hydrophobic behavior resulted by the extrusion/thermo-compression process. FTIR showed crosslinking effect of proteins. The starch-proteins samples prepared by casting presented few holes and broken starch grains while homogeneous films were obtained after extrusion/thermo-compression, regardless the protein addition. In casting films, the proteins cross-linked the starch by replacing the natural intermolecular hydrogen bond, improving water resistibility. Films with the protein increased the modulus and the strength at break

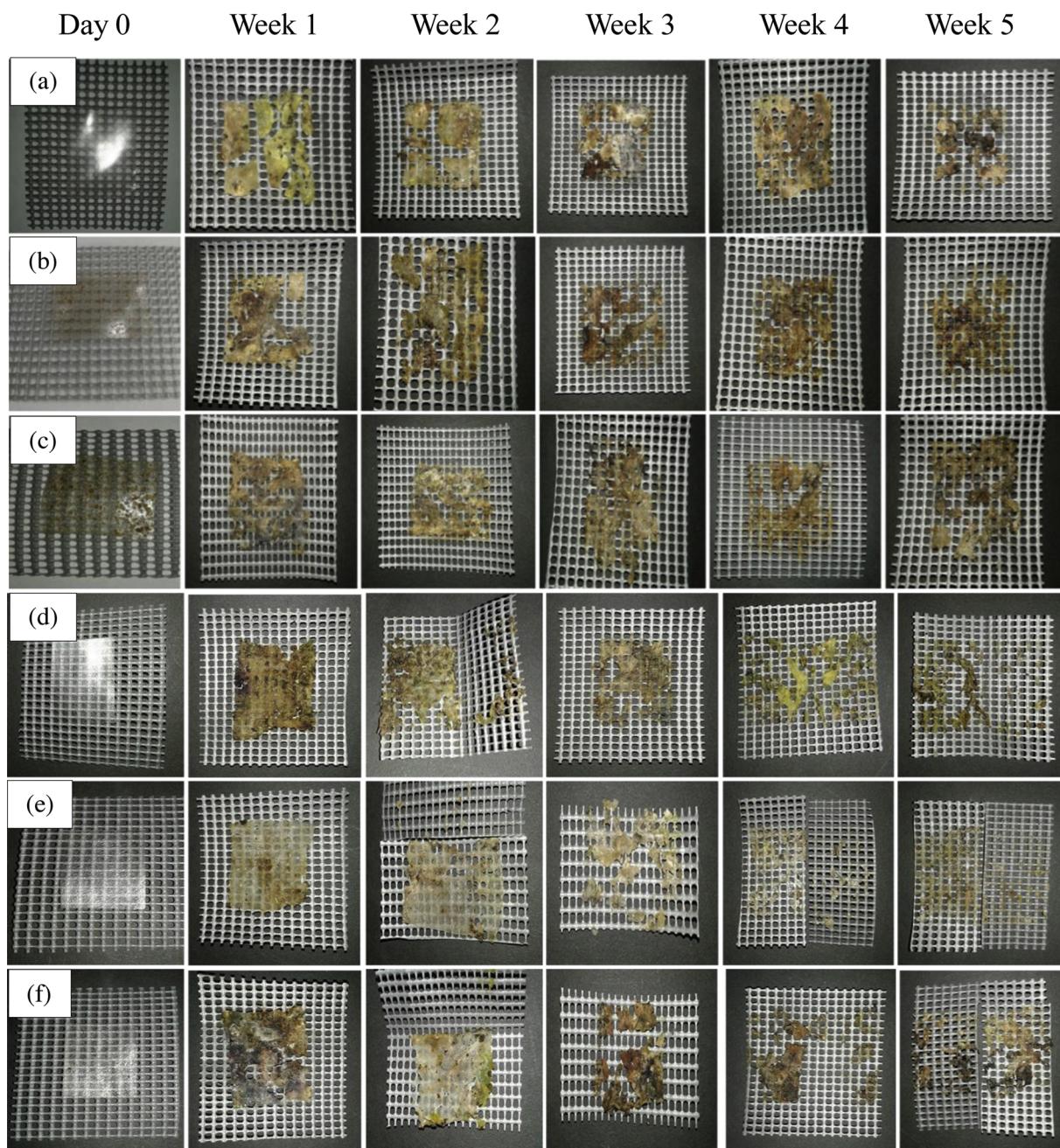


Fig. 7. Photographs of the films after being buried in vegetable compost: (a) S_C , (b) $S\text{-LP}0.75_C$, (c) $S\text{-LP}1.5_C$, (d) S_{ET} , (e) $S\text{-LP}0.75_{ET}$ and (f) $S\text{-LP}1.5_{ET}$.

and decreased the strain at break of the films, regardless the processing methodology, due to the crosslinking reaction. All films resulted thermal stable until 240 °C and biodegraded in 5 weeks.

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