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Active bilayer films of thermoplastic starch and polycaprolactone obtained by compression molding.

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ABSTRACT

Bilayer films consisting of one layer of PCL with either one of thermoplastic starch (S) or one of thermoplastic starch with 5 % PCL (S95) were obtained by compression molding. Before compression, aqueous solutions of ascorbic acid or potassium sorbate were sprayed onto the S or S95 layers in order to plasticize them and favour layer adhesion. S95 films formed bilayers with PCL with very good adhesion and good mechanical performance, especially when potassium sorbate was added at the interface. All bilayers showed excellent barrier properties to water vapour and oxygen. Bilayers consisting of PCL and starch containing 5% PCL, with potassium sorbate at the interface, showed the best mechanical and barrier properties and interfacial adhesion while having active properties, associated with the antimicrobial action of potassium sorbate.

Keywords: starch, polycaprolactone, compression molding, interface, bilayer.

1. INTRODUCTION

In the last few years, the need for replacing petroleum-based plastics by biodegradable polymers has led to a great number of studies focused on the design of environmentally friendly materials. In particular, starch and its derivatives have been widely studied since they could offer an inexpensive solution to such problems (Bastioli, 2001). Starch is obtained from renewable resources, is widely available and low cost and it can be used to obtain biodegradable films for food applications, as it has the ability to form films or coatings with very low oxygen permeability (Zhang, Rempel & McLaren, 2014; Jiménez *et al*., 2012). However, starch-based materials show several disadvantages which reduce their applicability as packaging material, such as their highly hydrophilic character, limited mechanical properties and the retrogradation phenomena that occur during ageing. The blending of starch with other, more hydrophobic, polymers is a widely studied strategy used to improve properties of starch films. The aliphatic polyesters, such as polycaprolactone (PCL) or polylactic acid (PLA), are synthetic biodegradable materials of a more hydrophobic nature that can be combined with starch in different ways to modulate the properties of mixed films. Of them, PCL has the advantage of great stretchability and low water vapour permeability (Averous, Moro, Dole & Fringant, 2000). Nevertheless, the starch-PCL materials obtained by simple blending are not adequate due to the low affinity of both polymers, which leads to polymer phase separation with limited adhesion between the polymer interfaces, thus resulting in poor film properties (Avella, Errico, Laurienzo, Martuscelli, Raimo & Rimedio, 2000). To overcome this, several authors have studied the improvement in the starch-PCL compatibility by using different compounds, which can increase the polymer affinity via different mechanisms, such as PCL-co-pyromellitic anhydride (Avella *et al*, 2000) methylenediphenyldiisocyanate (Wang, Sun & Seib,

2001) or dioctyl maleate (Zhang & Sun, 2004). Nevertheless, the use of these kinds of compounds can compromise the use of films for food packaging due to their potential toxicity. Other authors reported that hydrogen bonds can be established to a certain extent between the starch hydroxyls and the PCL carbonyls at the interface region, which could allow the incorporation of small amounts of PCL in starch matrices without notable polymer separation, improving film properties (Matzinos *et al*, 2002; Ortega-Toro *et al*., 2015a, b).

The development of multilayer films using starch and PCL layers could be a good alternative for developing packaging materials for food applications. A multilayer packaging material can be defined as two or more materials with specific properties combined in a single layered structure. The multilayer films with petrochemical-based materials are already widely used in food packaging applications. (Fang, Fowler, Escrig, Gonzalez, Costa & Chamudis, 2005; Mensitieri, Di Maio, Buonocuore, Nedi, Oliviero, Sansone & Iannace, 2011). The PCL-starch multilayers could exhibit some advantages, such as the decrease in the overall moisture sensitivity and the improvement in the mechanical properties (Yu, Dean & Bi, 2006), by combining the properties of each material (Fabra, Busolo, Lopez-Rubio & Lagaron, 2013). Therefore, starch-PCL multilayers could maintain the excellent gas barrier properties of the starch and the high water vapour barrier of the PCL, which would be difficult to achieve with a single biopolymer-based material. Layered structures based on biopolymers containing antimicrobial compounds have been obtained by co-extrusion (Alix, Mahieu, Terrie, Soulestin, Gerault, Feuilloley, Gattin, Edon, Ait-Younes & Leblanc, 2013) or compression-molding (Takala, Salmieri, Boumail, Khan, Dang, Chauve, Bouchard & Lacroix, 2013).

The incorporation of antioxidant and antimicrobial agents in biodegradable films has been widely studied to obtain active packaging materials with controlled release of bioactives (Ayranci & Tunc, 2003; Wook *et al.* 2013) to enhance food stability and shelf life. These agents have often been included in the biopolymer dispersions used for casting films (Gómez-Guillén, Ihl, Bifani, Silva & Montero, 2007; Jiménez, Fabra, Talens & Chiralt, 2013; Cian, Salgado, Drago, González & Mauri, 2014). Nevertheless, the usual thermal processing of bioplastics make their incorporation necessary during the extrusion or other hot melting steps used at industrial level. Some losses of active compounds can occur during this step due their thermosensitive. For instance, Wook *et al* (2013) reported losses of resveratrol and α-tocopherol incorporated in PLA/starch blend films during the polymer melt blending, ranging between 4 and 26 %, depending on the film formulation.

The aim of this work is to analyse the properties of starch-PCL bilayer films obtained by compression molding by incorporating a common food antimicrobial (potassium sorbate: PS) and an antioxidant (ascorbic acid: AA) at the layers' interface. The interactions of these compounds with both phases could improve the layers' adhesion, while conferring active properties to the layered film. Bilayer films formed with starch layers containing 5 % PCL was compared with those formed with pure starch layers in order to discover the potential improvement of starch-PCL interfacial adhesion when a small amount of PCL is present in the starch matrix.

2. MATERIALS AND METHODS

2.1 Materials

Corn starch was purchased from Roquette (Roquette Laisa España, Benifaió, Spain). Its moisture content was 10 % w/w and amylose percentage was 14%. Glycerol was from

Panreac Química, S.A. (Castellar del Vallès, Barcelona, Spain). Polycaprolactone (PCL) was from Aldrich Chemistry (Sigma-Aldrich Co. LLC Madrid, Spain), with a molecular weight of 80.000 daltons. Potassium sorbate, ascorbic acid, magnesium nitrate 6-hydrate, sodium chloride and phosphorus pentoxide were from Panreac Química, S.A. (Castellar del Vallés, Barcelona, España).

2.2 Film preparation

Three kinds of monolayer films were obtained by melt blending and subsequent compression molding: thermoplastic starch (S), thermoplastic starch with 5 % (g/100g of starch) PCL (S95) and pure PCL (PCL). To prepare S films, native starch and glycerol were dispersed in water using a starch:glycerol ratio of 1:0.3 w/w. When starch films contained PCL, this was added to aqueous dispersions in 1:0.05, w/w starch:PCL ratio.

A two-roll mill (Model LRM-M-100, Labtech Engineering, Thailand) was used for the hot-mixing process where polymers were heated at 160 ºC and 8 rpm for 30 min. The resulting sheets were ground and conditioned at 25 ºC and 75% RH using NaCl oversaturated solutions for 48 h. Afterwards, the monolayer films were obtained using a compression molding press (Model LP20, Labtech Engineering, Thailand). The following process conditions were used: a) pre-heating cycle at 160 ºC for 5 min, b) compression-molding at 50 bar for 2 min, c) compression-molding at 150 bar for 6 min, and d) cooling cycle at 150 bars for 3 min.

Subsequently, the bilayer films were obtained by means of a second compression-molding step where the corresponding two layers were compressed together. The process conditions were 80 ºC and 150 bars for 4 min and cooling cycle for 2 min. The obtained bilayer films always had one PCL layer and an S or S95 layer. Before the

second compression, 1 mL of distilled water (H_2O) or aqueous solutions containing ascorbic acid (AA) or potassium sorbate (PS) were sprayed onto the S or S95 layers in order to plasticize starch and promote layer adhesion. The obtained bilayer films were coded by indicating if the starch layer was S or S95 and the kind of solution sprayed onto the interface (H2O, AA or PS); e.g. S-H_2O indicates that the bilayer film contains S plus PCL layers sprayed with pure water. AA and PS aqueous solutions were prepared in such a way that 1 mL of solution contained 0.1 g of compound /g of starch film. The bilayer films were conditioned at 25 ºC and 53% RH for 1 and 5 weeks before their characterization. The thickness of conditioned films was measured at six random positions with a digital electronic micrometer (Palmer-Comecta, Spain, ± 0.001 mm).

2.3 Film characterization

2.3.1 Scanning Electron Microscopy (SEM)

Cross-section images from resulting films were obtained by means of a scanning electronic microscope (JEOL, JSM-5410, Japan). The conditioned samples (1 and 5 weeks) were stored inside P_2O_5 desiccators for one week before the analysis. Afterwards, the samples were cryofractured, fixed on copper stubs, gold coated, and observed using an accelerating voltage of 10 kV.

2.3.2 Fourier Transform Infrared (FTIR) spectroscopy

Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy was used to analyse component interactions in the films. Measurements were performed at 25 ºC using a Tensor 27 mid-FTIR Bruker spectrometer (Bruker, Karlsruhe, Germany) attached to a platinum ATR optical cell and an RT-Dla TGS detector (Bruker,

Karlsruhe, Germany). The diaphragm was set at 4 mm for the analysis, and the spectra were obtained between 4,000 and 800 cm^{-1} using a resolution of 4 cm^{-1}. The analysis was carried out on both the S or S95 and PCL layers. The data were analyzed using OPUS software (Bruker, Karlsruhe, Germany).

2.3.3 Thermal properties

Thermal degradation of the films and their components was analysed using a thermogravimetric TGA 1 Star^{e} System analyser (Mettler-Toledo, Inc., Switzerland), equipped with an ultra-micro weighing scale (± 0.1 μg), under nitrogen atmosphere. The analysis was carried out using the following temperature programme: heating from 25 to 500 ºC at a 10 ºC/min heating rate. Approximately 4 mg of each sample were used in each test, considering at least two replicates for each one. Initial degradation temperature (Onset) and peak temperature (Peak) were registered from the first derivative of the resulting weight loss curves.

2.3.4 Water content (X_w)

Conditioned films were transferred to a natural convection oven (J.P. Selecta, S.A., Barcelona, Spain) at 60 ºC for 24 h. Then, these samples were stored in a P_2O_5 desiccator for 8 days. Moisture content in dry basis was obtained from the initial and final sample weights.

2.3.5 Film solubility in water

Pieces of dry samples were transferred to glass pots, and covered with bidistilled water at a film:water ratio of 1:9 w/w. After 48 h, the samples were transferred to a natural convection oven at 60 ºC for 24 h in order to remove the water contained in bilayer

films. Finally, the samples were stored in a desiccator containing P_2O_5 for 8 days to complete drying. Film water solubility was estimated from its initial and final weights. Three replicates were considered per formulation.

2.3.6 Water Vapour Permeability (WVP)

The Water Vapour Permeability (WVP) of films was determined according to a modification of E96-95 gravimetric method (ASTM, 1995) proposed by McHugh *et al.* (1993). The measures were carried out exposing the S or S95 layer to 100% RH. To this end, 5 mL of bidistilled water were placed in Payne permeability cups (3.5 cm diameter, Elcometer SPRL, Hermelle/s Argenteau, Belgium). These cups were transferred to a desiccator containing oversaturated $Mg(NO_3)_2$ (53% RH) at 25 ºC. The permeability measurements were performed by weighing the cups periodically (every 1.5 h for 24 h). Eq. (1) proposed by McHugh *et al.* (1993) was used to calculate the vapour pressure on the film's inner surface (p_2).

$$WVTR = \frac{P \cdot D \cdot L_n[P - p_2 \backslash P - p_1]}{R \cdot T \cdot \Delta z} \quad (1)$$

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

$$permeance = \frac{WVTR}{p_2 - p_3} \quad (2)$$

In a first approach, the WVP of S-H_2O and S95-H_2O bilayer films was determined by exposing PCL layer to both the 100% and 53% RH atmospheres in order to verify if there were significant differences among WVP values. As none were observed, measurements were carried out by exposing the starch layer (S or S95) to 100% RH in every case. Values were obtained in triplicate for each film.

2.3.7 Oxygen Permeability (OP)

The oxygen permeability (OP) was determined using an Oxtran 1/50 (Mocon, Minneapolis, USA) system considering the Standard Method D3985-95 (ASTM, 2002) at 25 ºC and 53 % RH. Three films were evaluated for each formulation. The assay was performed exposing the PCL layer to the highest oxygen concentration in every case. The transmission values were determined every 20 min until equilibrium was reached. The exposure area during the tests was 50 cm^2 for each sample. To obtain the oxygen permeability, the film thickness was considered.

2.3.8 Tensile properties

A universal test Machine (TA.XTplus model, Stable Micro Systems, Haslemere, England) was used to determine the elastic modulus (EM) and tensile strength (TS) and elongation (E) at break point of the films, following ASTM standard method D882 (ASTM, 2001). Films conditioned at 25 °C and 53% RH for 1 and 5 weeks were evaluated. EM, TS, and E were determined from the stress-strain curves, estimated from force-distance data obtained for the different films (2.5 cm wide and 5 cm long).

239 Samples were mounted in the film-extension grips of the testing machine and stretched
240 at 50 mm min^{-1} until breaking. At least ten replicates were obtained from each sample.
241
242 2.3.9 Optical properties
243 The films' surface gloss was determined by means of a surface gloss meter (Multi Gloss
244 268, Minolta, Germany) at 85º incidence angle, since films exhibit low gloss, according
245 to D523 standard method (ASTM, 1999). Each film was measured in triplicate on both
246 film faces. Results were expressed as gloss units (GU), relative to a highly polished
247 surface of black glass standard with a value near to 100 GU.
248 Film transparency was determined from the surface reflectance spectra (400 – 700 nm)
249 obtained by a Minolta spectro-colorimeter (CM-3600d model, Minolta Co., Tokyo,
250 Japan) on both white and black backgrounds. Kubelka-Munk theory for multiple
251 scattering was applied in order to determine the internal transmittance (Ti). The Ti of
252 the films was determined using eq. (3). In this equation, R_0 is the reflectance of the film
253 on an ideal black background. Parameters *a* and *b* were calculated by means of eqs. (4)
254 and (5), where R is the reflectance of the sample backed by a known reflectance, Rg.
255
256 $$T_i = \sqrt{(a - R_0)^2 - b^2} \quad (3)$$
257
258 $$a = \frac{1}{2}\left(R + \frac{R_0 - R + R_g}{R_0 R_g}\right) \quad (4)$$
259
260 $$b = \sqrt{a^2 - 1} \quad (5)$$
261
262 2.3.10. Statistical analysis

263 Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, MD) was used to
264 carry out statistical analyses of data through an analysis of variance (ANOVA). Fisher's
265 least significant difference (LSD) was used at the 95% confidence level.
266
267 **3. RESULTS**
268 **3.1. Structural properties**
269 Figure 1A shows the micrographs of the cross section of some of the studied bilayer
270 films where the two polymer layers can be clearly distinguished. PCL layer was much
271 thinner than that of starch, despite the fact that films of similar weight were compressed
272 together. Table 1 shows the thickness values of the mono- and bilayers. S and S95
273 bilayers showed values close to those of the initial starch monolayer, which indicated
274 that both starch and PCL layers became thinner during the second compression.
275 However, SEM micrographs show that PCL films flow to a greater extent during the
276 second compression step, giving rise to PCL layers of about 30-50 μm, while the starch
277 layers barely decrease in their original thickness, especially the S95. This agrees with
278 the higher flowability of the more plastic PCL material. At the temperature of the
279 second compression (80ºC), the melting temperature of PCL (63.5 ºC, Ortega-Toro *et*
280 *al*. 2015a) was reached, while starch (Tg: 126 ºC, Ortega-Toro *et al*. 2015a) was only
281 plasticized at surface level by the moisturising effect of the sprayed solution. The S95
282 bilayers were thicker which indicates that the presence of PCL in starch films decreased
283 their ability to flow during compression. This can be attributed to the reduced water
284 affinity of the starch phase when PCL was finely dispersed in the matrix, which inhibits
285 the water plasticization effect of the sprayed solution.
286 The interfacial adhesion of PCL and starch layers can be observed in Figure 2, where
287 the interface of the different bilayer films is clearly observed. Each layer shows its

typical fracture appearance; pure starch exhibits a continuous and homogeneous aspect, whereas fine, well dispersed PCL particles can be observed in the S95 layer. Previous studies (Ortega-Toro *et al*., 2015a, b) demonstrated a small degree of miscibility of PCL in starch, through the depression of the starch glass transition temperature when PCL was added. PCL layers exhibit filamentous formations, associated with the plastic fracture of their amorphous zones, in agreement with the very low PCL Tg (-61.5 ºC, Averous *et al*., 2000). At higher magnification (Figure 1B), the penetration of fine channels of the PCL phase (arrows) into the starch phase can be observed forming union points between layers. This is probably favoured by the plasticization induced in the starch layer surface by the aqueous solutions sprayed before the compression step. When the starch layer contained PCL, the PCL which flows into the starch phase can bond to the dispersed, melted PCL particles, thus contributing to the layer adhesion. In Figure 2, greater layer adhesion can be observed in films containing S95, especially when the surface was sprayed with potassium sorbate aqueous solution, where the interface can barely be observed.

Figure 3 shows the infrared spectra obtained from the starch face and the wavenumber corresponding to the most characteristic peaks of the compounds present in the different formulations. Spectra obtained from the PCL layer did not differ from that of pure PCL and they were not included. This could indicate that no significant diffusion of compounds added at the interface (AA or PS) occurred through the PCL layer, although they could act at interfacial level.

The spectra of the S95 films showed some differences with respect to those of pure starch, showing characteristic peaks of both S and PCL, with some band shifts. The carbonyl group band (C=O stretching) was shifted with respect to pure PCL, which

suggests the formation of hydrogen bonds between PCL carbonyl and starch hydroxyls, as reported by other authors (Cai *et al*., 2014).

Likewise, small shifts in some peaks can be detected for starch bilayers, as compared to the corresponding bands of the S or S95 monolayer films. This suggests that some changes in molecular interactions inside the starch matrix occurred, associated with the diffusion of compounds added at the interface.

Shifts in bands corresponding to C-O-C (1049-1153 cm^{-1}), C=O (1724-1728 cm^{-1}) and –OH (3300-3319 cm^{-1}) can be due to the presence of small amounts of AA or PS, diffused together with water molecules inside the starch phase. The hydroxyl vibration band showed the highest shift with respect to the S or S95 films, which can be due to the higher hydration of the starch layer due to the surface wetting with the corresponding solution.

3.2. Thermal behaviour

Thermogravimetric analysis allows us to obtain information about the effect of compounds added at the interface on the thermal stability of polymers, due to their diffusion into the layers and potential interactions with each macromolecule. Figure 4 shows the DGTA curves of the bilayers, showing the peaks associated with the different weight losses caused by thermal degradation. All the curves showed three separate steps: a) small, broad peak due to evaporation of bonded water between 50 and 100 °C; b) starch thermal degradation between 283 °C and 290 °C and c) PCL thermal degradation at about 374°C. Table 2 shows thermal degradation temperatures (onset and peak) of mono-and bilayer films. The PCL addition to the starch (S95 sample) provoked a significant increase ($p > 0.05$) in the starch degradation temperature (onset and peak) while the onset temperature of PCL phase also rose. This indicates that polymer

interactions improved the thermal stability of both phases. In bilayers, degradation temperatures of both starch and PCL increased (more in the case of starch), except when PS was added. In these cases, the thermal degradation of polymers in bilayers occurred at a lower temperature than in the corresponding monolayer. This indicates that PS diffused from the interface inside the polymer matrices and the induced molecular interactions with both polymers affected their thermal degradation behaviour. The interactions of PS with polymer layers promoted layer adhesion, as deduced by SEM.

As a reference, the TGA of the glycerol, AA and PS was carried out. The onset temperatures for each compound were 212 ºC, 196 ºC and 448 ºC respectively, which guarantees their stability at the temperatures used in the film thermoprocessing (maximum 160ºC). AA and PS were only heated up to 80 ºC during the second compression. So, the antioxidant effect of AA and the antimicrobial activity of PS could be preserved after compression molding. In fact, the pure PS showed the highest thermal stability, although it promoted the thermal degradation of starch and PCL. Flores *et al*. 2010 also reported that the PS concentration in tapioca starch–glycerol based edible films was not affected by the extrusion process and the preservative was available to act as an antimicrobial agent.

3.3. Physical properties

In Table 1, the water content and film solubility in water are also shown for all the samples conditioned at 25°C and 53% RH for 1 and 5 weeks. At the initial time, water content of bilayers was higher than that of S or S95 films due to water absorption of the sprayed solution and their slow equilibration with the external atmosphere (53% RH).

Nevertheless, after 5 storage weeks, every bilayer had a similar water content (6-8 %) in the range of the starch (S or S95) films.

Water solubility of S95 bilayers showed homogenous values which did not change after 5 storage weeks, whereas S bilayers showed higher solubility after 1 one storage week, when they have a high moisture content, but lower solubility at 5 storage weeks when the film moisture content fell. In S95 bilayers, solubility was reduced to about half of the corresponding value of the S layer, which is coherent with the very low water solubility of the PCL layer. During the solubility test, S95 bilayers remained adhered, whereas in S bilayers, separation occurred at the edges of the films.

Figure 5 shows typical Stress–Hencky Strain curves of the monolayer and bilayer films after 1 week conditioned at 53%RH and 25ºC. The S95 monolayer exhibited lower stress values than the S layer, but greater extensibility. The addition of PCL to the starch matrix provoked a twofold effect on the mechanical response of the film: matrix plasticization effect (Tg decrease of starch) caused by the small PCL miscible fraction and matrix discontinuity due to the non-compatible PCL fraction. Both effects caused weakening of the cohesion forces of the starch phase which decreased the elastic modulus and the tensile strength, although the plasticizing effect enhanced the film stretchability. PCL is a ductile polymer with high deformability, whose deformation at break is near 500 % (Averous *et al*., 2000; Matzinos *et al*., 2002), which was not reached during the tensile test.

The bilayer films did not exhibit a net break but they showed successive micro-fractures from a determined deformation level till the total breakdown of the film. This can be associated with the progressive detachment of the film layers during the tensile test till total fracture. The value of the first fracture point was taken and shown in Table 3, together with the elastic modulus and tensile strength at the first failure point.

Bilayer films showed slightly lower values of EM and TS than the S or PCL monolayer but greater than the S95 monolayer, which indicates that the PCL layer reinforced the S95 bilayer films. The incorporation of AA or PS into the interface slightly decreased the EM values of bilayers, but significantly increased TS and deformation at break in the S95 bilayers. The start of layer detachment (micro-fractures) takes place at a deformation level of about 30 % in S95-AA and S95-PS bilayers, which indicates that they were better adhered than in the other bilayer films when micro-fractures occurred at lower deformation levels. In S bilayers, these compounds were not so effective at promoting S-PCL adhesiveness. Therefore, the diffusion of AA and PS molecules to the PCL chains near the interface in both PCL and S95 layers could promote stronger union forces which contribute to the layer adhesion.

After 5 weeks of storage, all the films had higher EM values, especially S monolayers, due to the phenomenon of starch retrogradation, which also reduced the film extensibility. Nevertheless, S95 maintained a deformability level of about 40 % and the S95-PS bilayer did not reduce its extensibility value. This bilayer exhibited the most stable tensile behavior, which indicates that starch chain association was mitigated by the combined effect of both PCL and PS interactions with starch chains. In general, bilayers better maintain tensile properties throughout storage time. In fact, in no case did tensile stress and deformation at break vary while EM increased in every case, except for the S95-PS samples. The increase in EM can be caused by the reduction in film water content shown in Table 1. The observed behavior suggests there were positive interactions between layers inhibiting the changes in the starch phase, at least near the PCL interface.

Table 4 shows the water vapour permeability (WVP) and the oxygen permeability (OP) of the studied films conditioned at 25°C and 53% RH for 1 and 5 weeks. In general, the

411 WVP values of bilayers were very low, as compared with S or S95 monlayers, and
412 similar to the PCL monolayer. This was highly positive because an effective water
413 vapour barrier is very important in food packaging. The greater WVP of S95 than that
414 of the S monolayer can be explained by the plasticization effect of PCL in the starch
415 matrix, as commented on above (Ortega-Toro *et al*., 2015b). After 5 weeks of storage,
416 no notable changes in the WVP values occurred; the bilayers maintained their high
417 water vapour barrier.
418 The oxygen permeability (OP) of starch films and bilayers was very low and in some
419 cases they were below the sensitivity threshold of the equipment. On the contrary, the
420 OP of PCL film reached the sensitivity limit. So, as expected, the barrier properties of
421 bilayers were greatly improved with respect to the starch or PCL films, exhibiting very
422 low OP (as starch) and WVP (as PCL). As regards the influence of adding AA or PS at
423 the interface, the most noticeable change was produced by PS, which reduces the OP in
424 both S and S95 bilayers to values lower than the detection limit of the Ox-Tran® used
425 to perform the test,. This coincides with the particular interactions of PS with S and
426 PCL, as deduced from the structural and thermal analysis.
427 Table 5 shows the gloss values at 85° of both faces of the films conditioned for 1 and 5
428 weeks. The gloss of S and S95 films was very similar and slightly lower than that of the
429 PCL. Likewise, the gloss of the starch and PCL faces in S bilayers was in the same
430 range as that of the respective controls (S and PCL): However, the S95 bilayers
431 presented lower gloss at both faces than the isolated S95 or PCL films. This is coherent
432 with the special role which dispersed PCL plays in S95 layers, that of favoring the layer
433 bonding. The PCL of both S95 and PCL layers melt at 80ºC and this contributes to
434 compound interpenetration during the second compression, unifying itself after cooling.
435 This melting-crystallization process of PCL at both layers could generate both more

union points at the interface and micro-irregularities on each layer surface. This is a key factor in the engineering design of starch-based bilayers, because it promotes the material stability over time inhibiting the starch retrogradation, as deduced from mechanical behavior after 5 storage weeks.

Figure 6 shows the spectral distribution curves of internal transmittance (Ti) of the films conditioned at 25 °C and 53% RH for 1 week. The results obtained, considering both starch or PCL faces as beam incidence surfaces, did not present significant differences. So, the mean values were considered. In general, films with an isotropic structure have high Ti values since no light dispersion occurs (Villalobos *et al*., 2005; Ortega-Toro *et al*., 2014). The S monolayer was the most transparent, whereas PCL showed a greater opacity. The S95 monolayer lost transparency with respect to the pure starch film due to the presence of a PCL dispersed phase which provoked light dispersion. Bilayers showed transparency levels in the order of those of S95, but the S95 bilayers were slightly more opaque due to the effect of dispersed PCL particles and non-isotropic bilayer formation.

4. CONCLUSIONS

Bilayer films of thermoplastic corn starch and PCL could be obtained by compression molding at 80ºC, by moisturising the interface with aqueous solutions containing ascorbic acid or potassium sorbate which, in turn, can confer active properties to the films. Starch layers containing 5 % PCL formed bilayers which were very well adhered with PCL and exhibited good mechanical performance, especially when potassium sorbate was added at the interface. All the bilayers showed excellent water vapour and oxygen permeability due to the association of two layers with very good barrier properties to each one. Bilayers consisting of PCL and starch containing 5% PCL, with

potassium sorbate at the interface, had the best mechanical and barrier properties and interfacial adhesion while also having active properties associated with the antimicrobial action of potassium sorbate. A study of the release of this compound in different food simulants is running in order to evaluate its potential in food preservation.

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Figure captions

Figure 1.- SEM micrographs of the cross section of some bilayer films, at low (A) and high magnification levels (B) showing the layer interface.

Figure 2.- SEM micrographs of the cross section of the different bilayer films, showing the layer interface.

Figure 3.- FT-IR spectra of monolayers (S, S95 and PCL) and starch-PCL bilayers (S-H_2O, S-AA, S-SP, S95-H_2O, S95-AA, S95-SP) obtained from the starch face.

Figure 4. - DTGA curves of bilayer films showing peaks for starch degradation and PCL degradation.

Figure 5. - Typical stress–Hencky strain curves of the different films conditioned at 25 ºC and 53% relative humidity for 1 week.

Figure 6.- Spectral distribution curves of internal transmittance (Ti) of the studied films conditioned at 25 °C and 53% RH for 1 week.

A

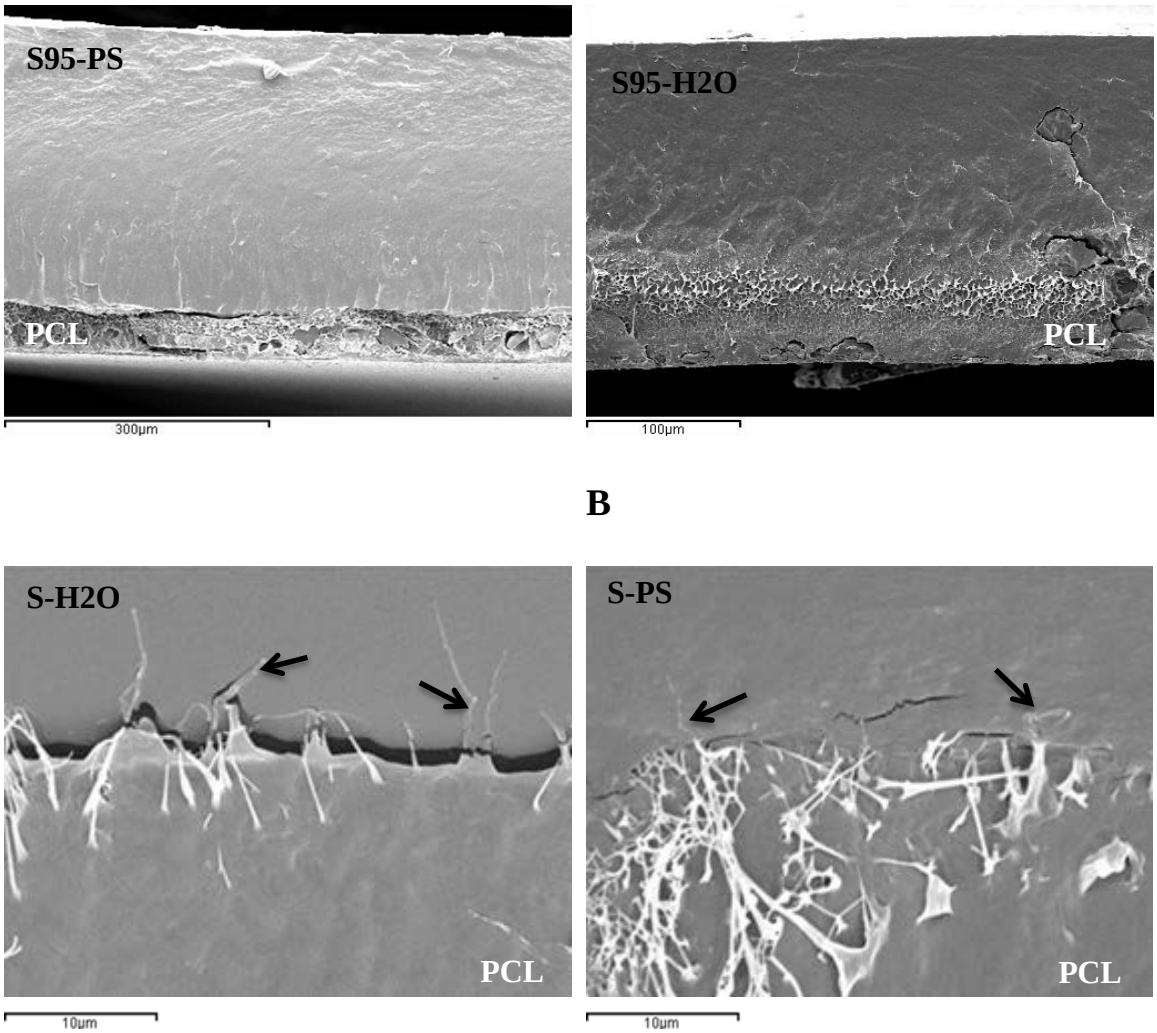


Figure 1

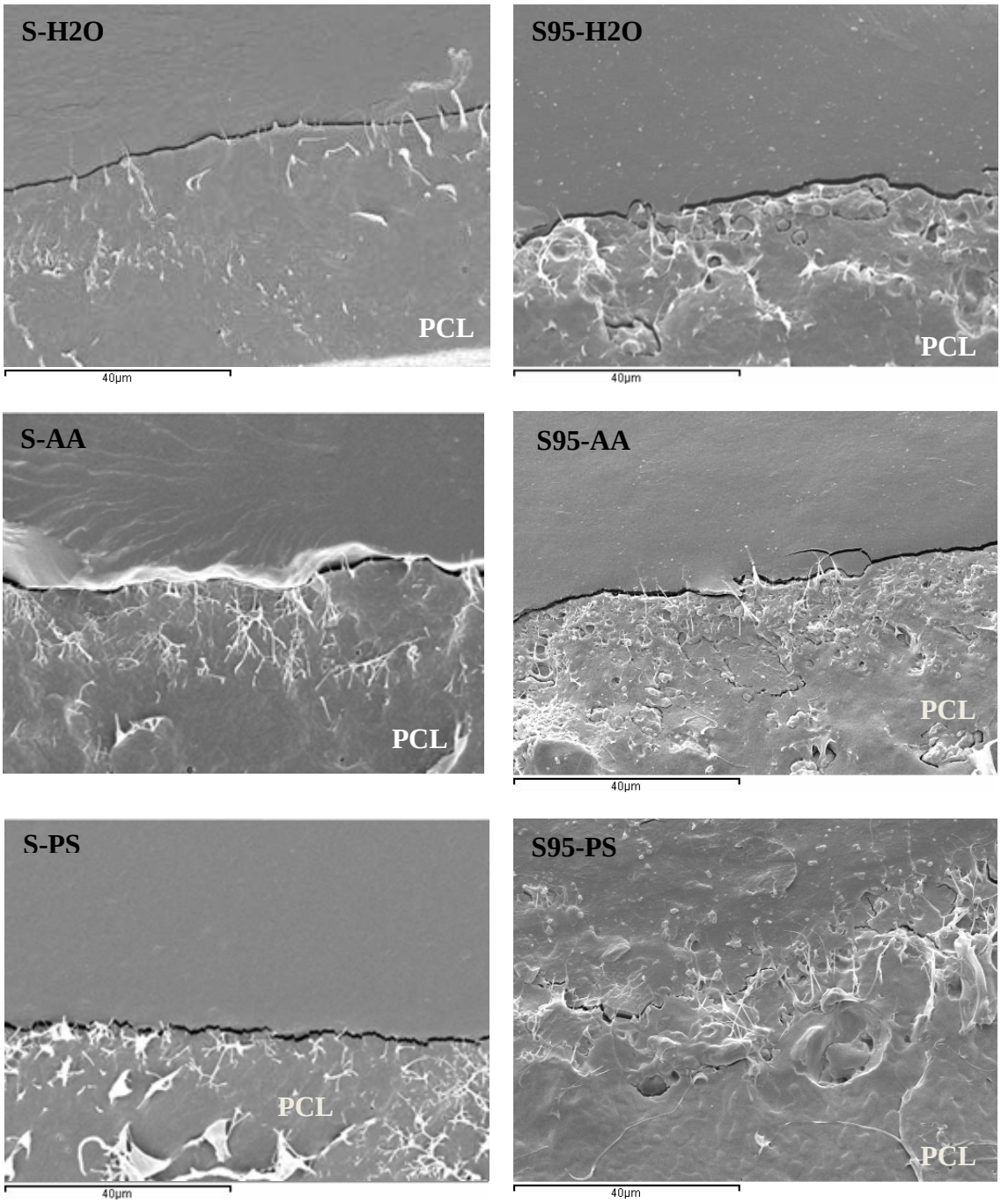

S-H2O
PCL
40µm
S95-H2O
PCL
40µm
S-AA
PCL
S95-AA
PCL
40µm
S-PS
PCL
40µm
S95-PS
PCL
40µm

Figure 2

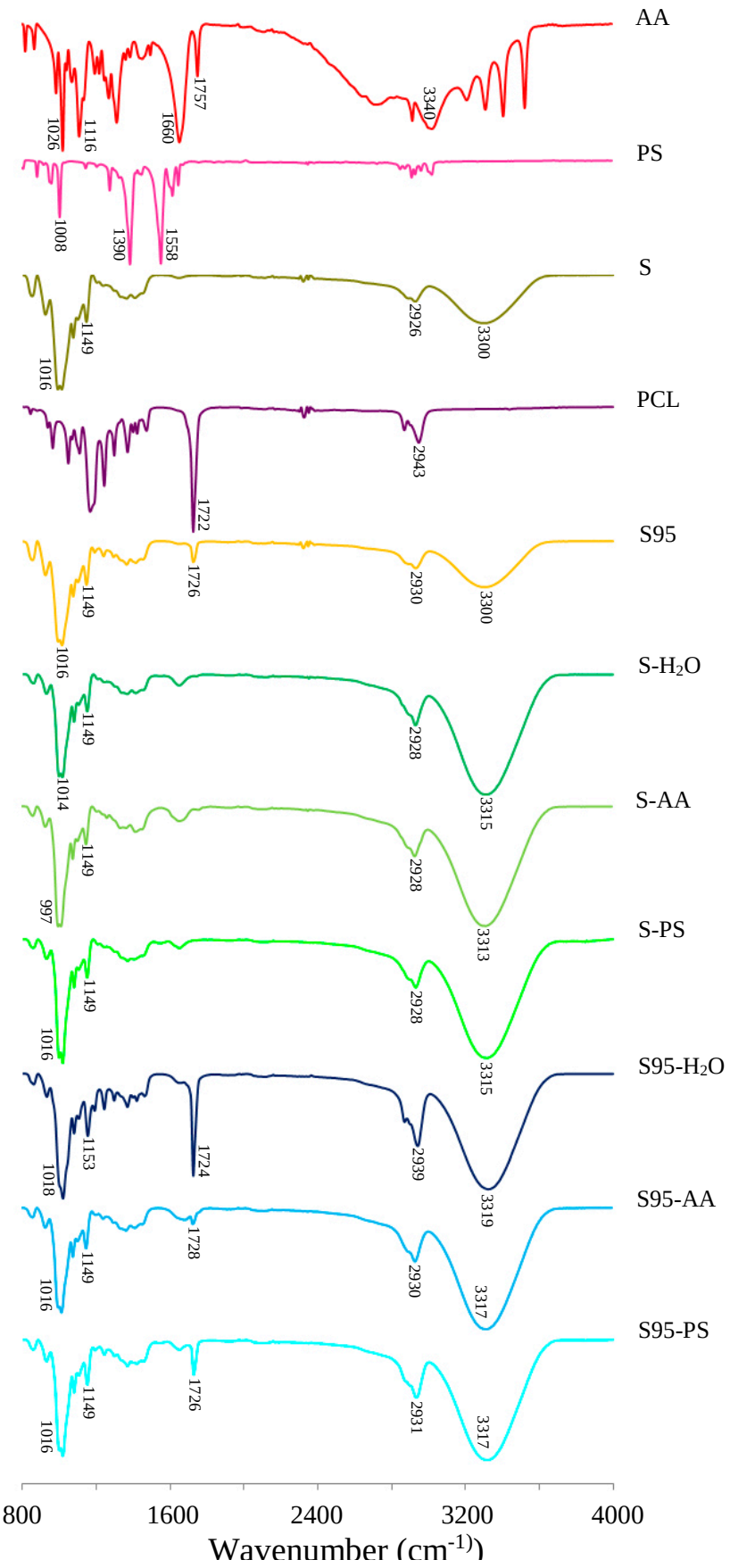

AA
1757
3340
1026
1116
1660
PS
1008
1390
1558
S
2926
3300
1149
1016
PCL
2943
1722
S95
1726
2930
3300
1149
1016
S-H2O
2928
3315
1149
1014
S-AA
2928
3313
1149
997
S-PS
2928
3315
1149
1016
S95-H2O
1724
2939
3319
1153
1018
S95-AA
1728
2930
3317
1149
1016
S95-PS
1726
2931
3317
1149
1016
800
1600
2400
3200
4000
Wavenumber (cm-1))

Figure 3.

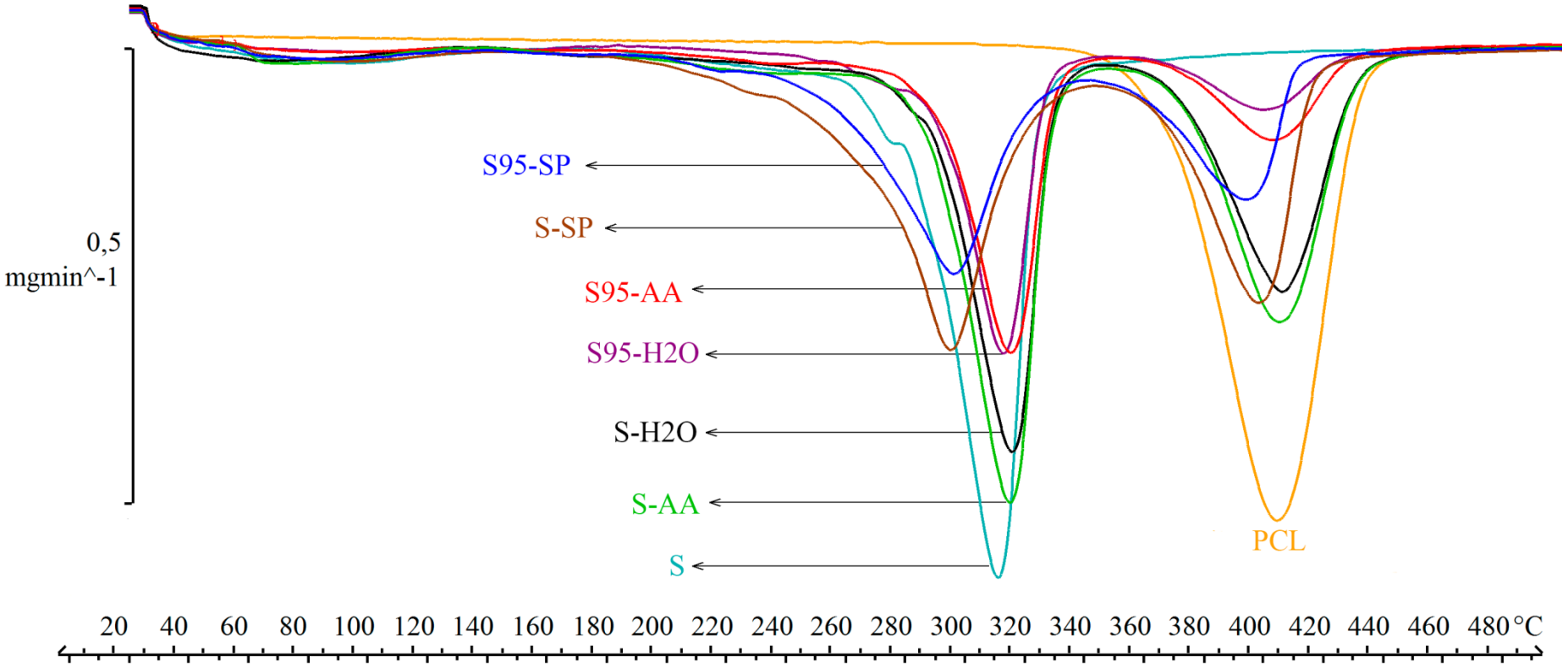
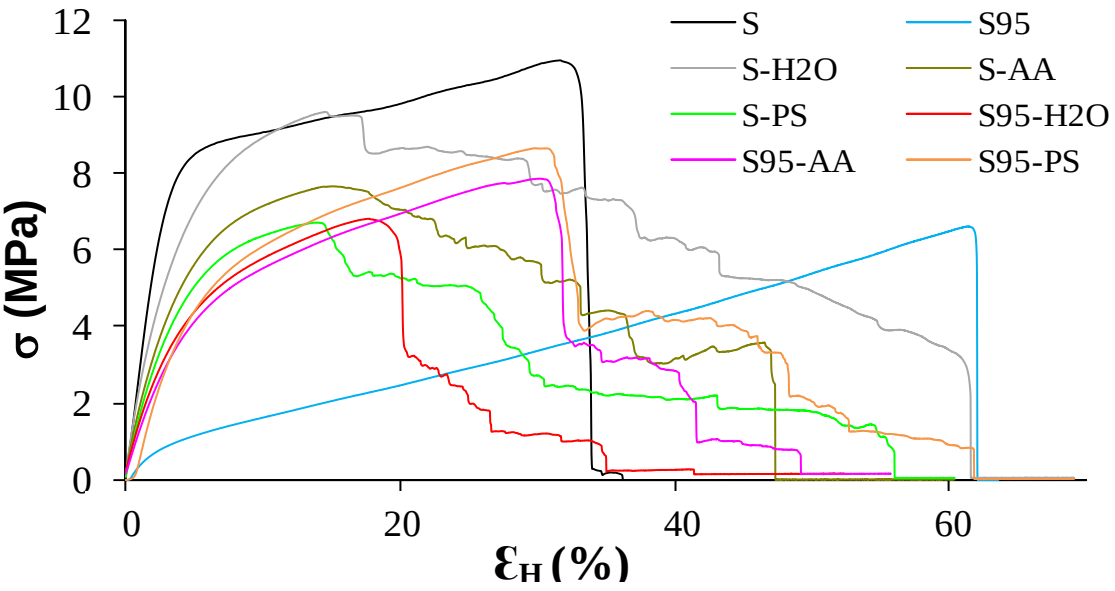

0,5
mgmin^-1
S95-SP
S-SP
S95-AA
S95-H2O
S-H2O
S-AA
S
PCL
20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 °C

Figure 4.



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643 Figure 5.

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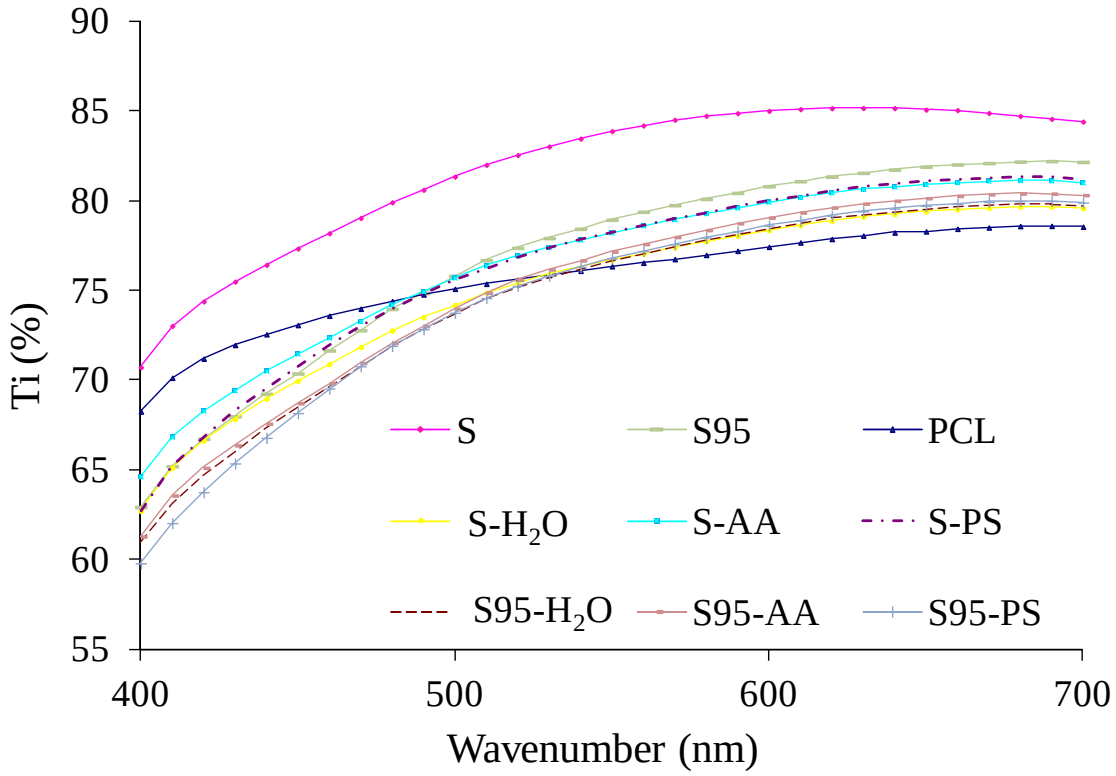

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652 Figure 6.

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Table 1. -Mean values and standard deviation of thickness, water content (g water/g dried film) and water solubility (g solubilised film/g initial dried film) of the different films stored at 53% relative humidity and 25 ºC.

Films	Thickness (μm)	X_w		Film solubility	
		1 week	5 week	1 week	5 week
S	261 ± 22^{bc}	0.061 ± 0.006^{b1}	0.079 ± 0.004^{d2}	0.19 ± 0.07^{d1}	0.24 ± 0.05^{d1}
S95	268 ± 13^{bc}	0.091 ± 0.007^{c1}	0.1146 ± 0.0013^{e2}	0.18 ± 0.05^{cd1}	0.198 ± 0.004^{c1}
PCL	149 ± 17^{a}	0.003 ± 0.002^{a1}	0.0021 ± 0.0005^{a1}	0.00022 ± 0.00011^{a1}	0.0004 ± 0.0002^{a1}
S-H_2O	228 ± 7^{b}	0.372 ± 0.015^{g1}	0.060 ± 0.002^{bc2}	0.30 ± 0.02^{e1}	0.21 ± 0.04^{cd2}
S-AA	236 ± 2^{b}	0.25 ± 0.02^{f1}	0.058 ± 0.008^{b2}	0.20 ± 0.05^{d1}	0.114 ± 0.010^{b2}
S-PS	234 ± 5^{b}	0.46 ± 0.04^{h1}	0.066 ± 0.004^{c2}	0.38 ± 0.05^{f1}	0.297 ± 0.014^{e2}
S95-H_2O	283 ± 30^{c}	0.20 ± 0.02^{d1}	0.067 ± 0.008^{c2}	0.114 ± 0.009^{b1}	0.107 ± 0.014^{b1}
S95-AA	322 ± 25^{d}	0.220 ± 0.012^{de1}	0.085 ± 0.002^{d2}	0.123 ± 0.012^{bc1}	0.142 ± 0.016^{b1}
S95-PS	325 ± 14^{d}	0.237 ± 0.011^{ef1}	0.0804 ± 0.0015^{d2}	0.123 ± 0.017^{bc1}	0.120 ± 0.013^{b1}

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

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

Table 2.- Mean values and standard deviation of thermal degradation of starch and PCL of the studied bilayers equilibrated at 53% relative humidity and 25 ºC

Films	Starch		PCL	
	Onset (ºC)	Peak (ºC)	Onset (ºC)	Peak (ºC)
S	283 ± 7^{c}	313.8 ± 0.4^{b}	---	---
S95	290 ± 2^{d}	317.3 ± 1.3^{c}	383.5 ± 1.4^{e}	402.4 ± 1.1^{b}
PCL	---	---	374.1 ± 0.2^{c}	408.7 ± 0.2^{c}
S-H_2O	293.1 ± 0.8^{de}	320.92 ± 0.12^{d}	378 ± 2^{d}	410.8 ± 0.8^{d}
S-AA	293.0 ± 0.6^{de}	320 ± 0.2^{d}	378.0 ± 0.6^{d}	410.40 ± 0.12^{d}
S-PS	272.3 ± 0.7^{b}	299.9 ± 0.4^{a}	370.6 ± 0.6^{b}	403.4 ± 0.4^{b}
S95-H_2O	298.03 ± 0.13^{e}	319.4 ± 0.6^{d}	377.51 ± 0.11^{d}	409.90 ± 0.12^{cd}
S95-AA	295 ± 2^{de}	319.5 ± 1.2^{d}	376.5 ± 1.3^{cd}	408.9 ± 0.6^{c}
S95-PS	262 ± 2^{a}	300.9 ± 0.4^{a}	364 ± 2^{a}	399.75 ± 0.11^{a}

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

677 Table 3.- Mean values and standard deviation of mechanical properties of films equilibrated at 53% RH and 25 ºC for 1 week (initial) and 5 weeks (final).

Films	EM (MPa)		TS (MPa)		E (%)	
	Initial	Final	Initial	Final	Initial	Final
S	324 ± 48^{e1}	587 ± 65^{e2}	10 ± 2^{d1}	15.7 ± 1.2^{e2}	28 ± 10^{c1}	4.1 ± 0.4^{a2}
S95	48 ± 10^{a1}	103 ± 10^{a2}	5.4 ± 1.5^{a1}	7.8 ± 0.7^{b2}	53 ± 5^{e1}	39 ± 5^{f2}
PCL	304 ± 11^{e1}	314 ± 51^{d1}	---	---	---	---
S-H_2O	215 ± 17^{d1}	284 ± 35^{d2}	9.1 ± 0.7^{c1}	11.9 ± 1.2^{d2}	15 ± 2^{ab1}	15 ± 5^{cd1}
S-AA	181 ± 16^{c1}	205 ± 33^{c1}	8.7 ± 1.0^{c1}	9.9 ± 0.6^{c2}	17 ± 4^{ab1}	17 ± 5^{d1}
S-PS	170 ± 16^{c1}	202 ± 18^{c2}	6.7 ± 0.6^{b1}	7.7 ± 1.0^{b2}	11 ± 5^{a1}	9 ± 4^{b1}
S95-H_2O	129 ± 17^{b1}	311 ± 14^{d2}	6.7 ± 0.6^{b1}	6.7 ± 1.0^{a1}	18 ± 5^{b1}	6.1 ± 0.6^{a2}
S95-AA	114 ± 14^{b1}	213 ± 5^{c2}	8.1 ± 0.9^{c1}	7.5 ± 0.6^{ab2}	35 ± 6^{d1}	12 ± 5^{bc2}
S95-PS	126 ± 27^{b1}	153 ± 30^{b1}	8.5 ± 1.0^{c1}	9 ± 2^{c1}	32 ± 8^{cd1}	27 ± 6^{e1}

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

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

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Table 4.- Mean values and standard deviation of water vapour permeability (WVP) and oxygen permeability (OP) of the different films at 1 (Initial time) and 5 (Final time) weeks of storage at 53% relative humidity and 25 ºC.

Films	WVP ($g \cdot mm \cdot kPa^{-1} \cdot h^{-1} \cdot m^{-2}$)		OP · 10^{14} ($cm^3 \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$)	
	Initial	Final	Inictal	Final
S	18.1 ± 1.4^{c1}	16 ± 2^{d1}	< D.L.	< D.L
S95	20.41 ± 0.02^{d1}	18 ± 2^{e1}	12.2 ± 0.8^{a1}	19 ± 3^{ab2}
PCL	0.120 ± 0.04^{a1}	0.117 ± 0.011^{a1}	> D.L.	> D.L
S-H_2O	0.8 ± 0.3^{ab1}	0.43 ± 0.07^{a1}	12 ± 2^{a1}	11.2 ± 1.5^{a1}
S-AA	0.71 ± 0.07^{ab1}	0.57 ± 0.07^{a1}	15 ± 2^{a1}	9 ± 3^{a1}
S-PS	0.7 ± 0.2^{ab1}	0.63 ± 0.07^{a1}	< D.L.	< D.L.
S95-H_2O	1.4 ± 0.3^{b1}	6.0 ± 0.5^{c2}	28 ± 5^{b1}	15.6 ± 1.0^{b2}
S95-AA	1.5 ± 0.6^{b1}	2.4 ± 0.9^{b1}	14 ± 3^{a1}	10 ± 2^{a1}
S95-PS	0.96 ± 0.04^{ab1}	1.24 ± 0.15^{ab2}	< D.L.	< D.L.

D.L.: 0.1-200 cc/(m^2.day)

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

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

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

Films	Gloss (85°). Starch face		Gloss (85°). PCL face	
	Inicial	Final	Inicial	Final
S	40 ± 5^{c1}	37 ± 2^{c1}	---	---
S95	47 ± 11^{cd1}	25 ± 8^{b2}	---	---
PCL	---	---	59 ± 16^{e1}	57 ± 9^{c1}
S-H_2O	41 ± 5^{c1}	38 ± 9^{c1}	52 ± 9^{cde1}	39 ± 11^{b2}
S-AA	46 ± 7^{cd1}	39 ± 6^{c2}	52 ± 7^{cde1}	38 ± 13^{b2}
S-PS	51 ± 16^{de1}	43 ± 13^{c1}	54 ± 10^{de1}	39 ± 16^{b2}
S95-H_2O	16 ± 2^{ab1}	13 ± 2^{a2}	29 ± 5^{a1}	26 ± 8^{a1}
S95-AA	13 ± 4^{a1}	15 ± 8^{a1}	24 ± 5^{a1}	19 ± 10^{a1}
S95-PS	23 ± 2^{b1}	20 ± 6^{ab1}	45 ± 7^{bc1}	41 ± 9^{b1}

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

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