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Additional Information
Thermoprocessed starch-polyester bilayer films as affected by the
addition of gellan or xanthan gum

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ABSTRACT

Monolayer films based on cassava starch (CS) or maize starch (MS), with and without 10 % of gellan or xanthan gum, and PLA-PHBV (75:25) blend films, were obtained by melt-blending and compression moulding, using glycerol (for starch blends) and PEG 1000 (for polyester blends) as plasticisers. Bilayer films were obtained by thermo-compression of the different starch based sheets with the polyester sheet. Both mono and bilayers were characterised as to their mechanical and barrier properties, equilibrium moisture, water solubility and microstructure. The incorporation of gellan gum and xanthan gum improved the mechanical properties of starch-based films, especially in the case of MS, although the highest EM and TS values were obtained for CS-gum films. The incorporation of either gellan or xanthan gum decreased the water vapour and oxygen permeability of starch-based films; the CS films with gums being the least permeable to oxygen. The lowest changes in mechanical properties throughout storage were obtained in cassava starch-based films, especially those containing xanthan gum. Starch based-polyester bilayers presented a high oxygen and water vapour barrier capacity, as compared to their individual monolayers. Bilayer films with cassava starch including the gums showed the lowest OP and WVP values and the highest elastic modulus and tensile strength, with extensibility values in the range of the corresponding monolayers and slight changes in their physical properties throughout time. The bilayer formed with cassava starch with gellan gum and a PLA-PHBV appeared as the best option for food packaging purposes taking into account its functional properties and the good layer adhesion of the bilayer.

Keywords: biodegradable bilayer films, PLA, PHBV, starch, gellan, xanthan.
1. INTRODUCTION

Over the last few decades, there has been a growing need to find alternatives to petroleum-based non-biodegradable products due to environmental concerns (Martín et al. 2001). This has increased interest in developing biodegradable food packaging materials based on biopolymers, such as starch. The use of thermoplastic starch (TPS) to develop biodegradable packaging materials has several advantages, such as low cost, renewability, sustainable production, good processability by means of conventional techniques, good oxygen barrier capacity and stretchability, as well as suitable transparency, odour and taste (Muller et al., 2017). However, starch materials are water sensitive and exhibit poor water vapour barrier properties, which are greatly affected by their moisture content (Vieira et al., 2011). Additionally, the phenomenon of retrogradation modifies the mechanical behaviour of starch-based materials throughout time, depending on the amylose/amylopectin ratio and moisture content (Cano et al., 2014; López et al., 2013; Ortega-Toro et al. 2014). Glycerol is usually employed as a plasticiser in starch films in order to facilitate thermo-procesing, by reducing the intermolecular forces and increasing the flexibility of starch-based films (Savadekar & Mhaske, 2012). Different strategies have been applied to improve the functional properties of starch based materials, such as blending it with different additives or other biopolymers (Cano et al., 2017; Ortega-Toro et al., 2017; Samsudin & Hani, 2017).

Sapper et al. (2019) observed that the cassava starch mixture with 10 or 20% of different gums of microbial origin, such as gellan, xanthan or pullulan, permitted the improvement of the properties of starch-based films obtained by casting, while maintaining the competitive cost of the material. In general, the addition of such gums improved the mechanical properties of starch films and their storage stability (Kim et al., 2015; Sapper et al, 2019). Xanthan gum enhanced the tensile properties of the films but led to a less extensible matrix (Arismendi et al., 2013).

Developing bilayer films based on biodegradable monolayers formulated with different biopolymers with complementary properties is an innovative approach to improving the performance of the material (laminate), as compared to the use of monolayers, while also meeting the food packaging requirements better (Slavutsky et al., 2018). In this sense, the combination of starch films with sheets of hydrophobic polyesters represents a good alternative means of accomplishing this purpose. Poly(lactic acid) (PLA) and Poly(3-
hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) are biodegradable polyesters obtained from natural resources which can be used for food packaging purposes, due to their ability to form food contact plastic materials at a relatively competitive cost. PLA is a biodegradable thermoplastic linear aliphatic polyester, of great potential in the packaging industry because of its optical properties, good thermal behaviour and water vapour barrier properties (Bonilla et al., 2013; Chaiwutthinan et al., 2015; Muller et al., 2017). However, PLA shows limited gas barrier capacity and is very brittle, with less than 10% elongation at break (Rasal et al., 2010). Therefore, PLA has been combined with different plasticisers and other polyesters, such as PHBV. Although PHBV has physical properties that are comparable to some synthetic polymers, such as polypropylene and polyethylene, the PHBV materials are more brittle with lower elongation at break (Laycock et al., 2013). PHBV and PLA blends have been studied in order to improve the functional properties of the materials. The polymers exhibited low miscibility, so PHBV-PLA blend films had low transparency, but their mechanical resistance was significantly improved as compared with pure PLA or PHBV films (Liu et al., 2015). Different plasticisers, such as acetyl tributyl citrate, limonene, and PEG have been used to improve the extensibility of mixtures of PLA and PHB (Armentano et al., 2015). The addition of PEG1000 and PEG600 at concentrations lower than 10% improved the thermal properties of PLA-PHBV based materials (Thongpina et al., 2017).

Obtaining bilayer structures consisting of a PLA-PHBV blend film layer and a starch-based film layer could represent a good alternative to obtain the target materials with improved mechanical and barrier properties suitable for food packaging applications. The polyester layer would contribute to the strengthening of the bilayer while reducing water vapour permeability, whereas the starch layer would help to control the oxygen and gas barrier capacity of the bilayer assembly. In previous studies, Requena et al., (2018) combined a PLA-PHBV (75:25) monolayer with glycerol-plasticised starch sheets to develop bilayer food packaging materials. In this study, a good layer adhesion was obtained for starch/PLA-PHBV sheets, but the elastic modulus and mechanical resistance of polyester-cassava starch bilayer films were lower than that of the corresponding polyester monolayers due to the weaker strength of starch monolayers. In this sense, the incorporation of xanthan or gellan gum to a thermo-processed starch layer could improve both the monolayer and bilayer functional properties for food packaging purposes. The origin of the starch may also affect both the film and bilayer properties, since different
starches provide the films with more or less mechanical resistance and stability, mainly depending on their amylose/amylopectin ratio (Cano et al., 2014).

The aim of this study was to analyse the physical and microstructural properties of the melt blended and compression moulded starch films, from maize and cassava, as affected by the xanthan or gellan gum incorporation. Thermo-compression-sealed bilayers of the different starch-based films and PLA-PHBV blend sheets were obtained and analysed in order to select the best combination for food packaging purposes.

2. MATERIALS AND METHODS

2.1 Materials

Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) ENMAT Y1000P with 3% hydroxyvalerate was supplied by Helian Polymers B.V. (Belfeld, Holland). Amorphous PLA 4060D, density of 1.24g/cm³ and average molecular weight of 106,226 D with 40% of low molecular weight fraction (275 D) as reported by Muller et al. (2017), was supplied by Natureworks (U.S.A). Maize starch (MS, 27% amylose) and cassava starch (CS, 9% amylose) were supplied by Roquette (Roquette Laisa, Benifaió, Spain) and Quimidroga S.A. (Barcelona, Spain), respectively. Xanthan gum (X) (high molecular weight, $\sim$10⁶ Da), was supplied by EPSA (Valencia, Spain). Negatively charged, low acyl gellan gum (G) KELGOGEL F (MW 3-5x10⁵ Da), was purchased from Premium Ingredients (Murcia, Spain). The plasticiser, poly(ethylene glycol) with a molecular weight of 1000 Da (PEG1000), was purchased from Sigma-Aldrich (Steinheim, Germany), and the glycerol was obtained from Panreac Química S.L.U. (Castellar del Vallés, Barcelona, Spain). For sample conditioning purposes, phosphorus pentoxide ($P_2O_5$) and magnesium nitrate-6-hydrate ($Mg(NO_3)_2$) were supplied by Panreac Química, S.A. (Castellar del Vallès, Barcelona, Spain).

2.2 Preparation of films

2.2.1 Starch monolayer films

For the preparation of maize (MS) and cassava (CS) starch monolayer films, the starch solutions were mixed in the adequate proportion with which to obtain a starch:gum ratio
of 90:10, using glycerol (0.30 g/g of starch) as a plasticiser, by melt blending and compression moulding. The melt blending process was carried out in an internal mixer (HAAKE™ PolyLab™ QC, Thermo Fisher Scientific, Germany) at 130 °C, rotor speed 50 rpm, for 10 min and 50 g of blend were processed in each batch. After processing, blends were cold ground in a refrigerated batch mill (Model M20, IKA, Germany) and the powder conditioned at 25 °C and 53% relative humidity (RH) for one week. Four g of the conditioned powder was required to obtain each film (160 mm in diameter) that were put onto Teflon sheets and preheated at 160 °C or 150 °C (for CS or MS) for 1 (CS) or 5 (MS) min in a hot-plate press (Model LP20, Labtech Engineering, Thailand). Films were obtained by compressing at 160 °C (CS) or 150 °C (MS) for 2 min at 50 (CS) or 30 (MS) bars, followed by 6 min at 100 (CS) or 130 (MS) bars and a final cooling cycle for 3 min until the temperature reached about 70 °C, according to that described by other authors for CS (Requena et al., 2018) and MS (Silva-Guzmán et al., 2018). The obtained films were conditioned at 25 °C and 53% RH until used to obtain bilayer films.

2.2.2 Polyester monolayer films

PLA-PHBV blend monolayers were obtained by melt blending and compression moulding in a ratio of 75:25, using PEG1000 (15 g/100 g polymer) as a plasticiser. The melt blending process was carried out in an internal mixer (HAAKE™ PolyLab™ QC, Thermo Fisher Scientific, Germany) at 170 °C, rotor speed 50 rpm, for 12 min. After processing, blends were cold ground in a refrigerated batch mill (Model M20, IKA, Germany) and conditioned at 25 °C. Only 3 g of the conditioned powder were required to obtain each film (160 mm in diameter) due to the higher flowability of the polyester blends. The powder was put onto Teflon sheets and preheated at 200 °C for 5 min in a hot plate press (Model LP20, Labtech Engineering, Thailand). Films were obtained by compressing at 200 °C for 4 min at 100 bars, and a final cooling cycle for 3 min until the temperature reached about 70 °C (Requena et al., 2018). The obtained films were conditioned at 25 °C and 53% RH until used to obtain bilayer films.

2.2.3 Starch-polyester bilayer films

Starch monolayers and polyester monolayers were submitted to compression moulding in a hydraulic press (Model LP20, Labtech Engineering, Thailand) at 180 °C and 100 bars
for 2 min and cooled down until 80 °C in 2 min, thus obtaining starch-polyester bilayer films. All bilayer films were stored at 25 °C and 53%+ç RH till their analyses.

2.3 Film characterisation

2.3.1 Tensile properties and thickness

The mechanical behaviour of the films was tested by using a universal testing machine (TA-XT plus, Stable Micro Systems, Surrey, United Kingdom) according to the ASTM D882 standard method (ASTM, 2001). The mechanical parameters, tensile strength (TS), elastic modulus (EM) and elongation at break (E), were obtained from the stress–strain curves of the various samples. Equilibrated samples (1 or 5 weeks at 25 °C and 53% RH) of 2.5 cm wide and 10 cm long were mounted in the film extension grips of the testing machine 5 cm apart and the samples were stretched at 50 mm/min until fracture. Eleven replicates were performed for each film formulation. The film thickness was measured to the nearest 0.0025 mm with a Palmer digital micrometer (Electronic Digital Micrometer, Comecta S.A., Barcelona, Spain) at six random positions around the film.

2.3.2 Water vapour permeability (WVP)

The water vapour permeability (WVP) of the films was determined following the gravimetric method ASTM E96-95 (ASTM, 1995), considering the modification proposed by McHugh et al. (1993). Three round film samples (3.5 mm in diameter) of each formulation were placed on Payne permeability cups (3.5 cm in diameter, Elcometer SPRL, Hermelle/s Argenteau, Belgium). The temperature was 25 °C and the relative humidity gradient was 53-100%, which was obtained using magnesium nitrate-6-hydrate and distilled water, respectively. The cup’s weight loss was controlled every 1 h and 30 min using an analytical balance (±0.00001 g), until the steady state was reached. WVP was calculated from the slope of the curves of weight loss versus time as described by Ortega-Toro et al. (2016), taking into account the film thickness. An apparent value of WVP was also determined for bilayer films, considering their total thickness value.

2.3.3 Oxygen permeability (OP)

The oxygen permeability (OP) of the conditioned films was evaluated by measuring the oxygen permeation rate by means of an OX-TRAN 1/50 system (Mocon, Minneapolis,
USA) at 53% RH and 25 °C (ASTM Standard Method D3985-05, 2002). The transmission values were determined every 20 min until equilibrium was reached. The exposure area during the tests was 50 cm² for each formulation. In order to obtain the oxygen permeability (OP), the film thickness was considered in every case. At least two replicates per formulation were made. An apparent value of OP was also determined for bilayer films, considering their total thickness value.

### 2.3.4 Moisture content

The moisture content of film samples previously conditioned at 53% RH and 25 °C was determined. Four samples of each formulation were dried in a vacuum oven (VaciotermT, JP Selecta S.A., Barcelona, Spain) at 60 °C for 24h, and afterwards, the samples were placed into a desiccator with P₂O₅ at 25 °C for 2 weeks, until constant weight was reached.

### 2.3.5 Film water solubility

The solubility was evaluated by a modification of the method described by Balaguer et al. (2011). Film samples (3 cm x 3 cm), previously conditioned in P₂O₅, were weighed and then the dry films were immersed in glass containers in 10 mL of distilled water and kept at 25 °C for 24 h. Then, the solvent was poured into a filter, retaining the film sample, the remaining surface water was removed and the final wet weight was measured. These wet samples were dried till constant weight to evaluate the mass of residual solids in the film after soaking. Each film formulation was analysed in triplicate.

### 2.3.6 Microstructural analyses

Microstructural analyses of the films were carried out by using a Field Emission Scanning Electron Microscope (FESEM Ultra 55, Zeiss, Oxford Instruments, U.K). Film samples were kept in desiccators with P₂O₅ for two weeks at 25 °C in order to eliminate film moisture. Then, film samples were cryofractured with nitrogen liquid in order to observe the cross-sections and adequately placed on support stubs and coated with platinum. The samples were observed using an accelerating voltage of 2 kV.

### 2.3.7 Statistical analysis

The statistical analysis of the results was performed through an analysis of variance (ANOVA) using Statgraphics Centurion XVII-X64. Both a One-way and multifactor
ANOVA were used to analyse the influence of composition variables and storage time on the properties of the films. Fisher's least significant difference (LSD) procedure was used at the 95% confidence level.
3. RESULTS AND DISCUSSION

3.1. Properties and microstructure of monolayer films

3.1.1. Thickness, equilibrium moisture content and water solubility

Table 1 shows the thickness, moisture content and water solubility of monolayer films. Significant differences were observed as regards thickness, maize starch (MS) films being slightly thicker (about 220 µm) than those made from cassava starch (CS) (about 190 µm), and both are thicker than the polyester film (130 µm). Differences in the film thickness can be attributed to the different flowability of the material during the hot compression, which is dependent on both the viscosity under the temperature and pressure conditions and on the mass of pellets used in each case (4 or 3 g for starch and polyesters, respectively). The equilibrium moisture content of CS films was slightly higher than that of MS films and the incorporation of the gums slightly decreased the water adsorption capacity. This could be attributed to the formation of hydrogen bonds between the chains of starch and the gums, which could reduce the number of active points for water sorption. A similar trend was observed by Sapper et al. (2019) in cassava starch-gellan films obtained by casting. The polyester films showed lower moisture content values, as expected from their hydrophobic nature.

About 90% of the total solids of every starch film were solubilised in water without there being any significant differences between the samples. Thus, the water solubility of maize and cassava starch films was not affected by the incorporation of gum. However, Sapper et al. (2019) observed lower solubility values after the addition of gellan or xanthan gums in cassava starch films obtained by casting, probably due to the establishment of more gum-starch hydrogen bond interactions in the polymer aqueous solution than in the blend melt. Thus, the method of obtaining the films affected polymer chain interactions with water molecules and, therefore, their solubility. The polyester films exhibited very low solubility in water, coherently with their hydrophobic nature. The solubilised solids would probably be made up of 15% PEG1000 (plasticiser) and some small oligomers of PLA or PHBV.
3.1.2. Tensile properties and barrier properties

Table 2 shows tensile parameters (Elastic modulus: EM, tensile strength: TS and percentage deformation E% at break) and barrier properties (water vapour: WVP and oxygen permeability: OP) of monolayer films after 1 week and 5 weeks of storage at 25°C and 53% RH.

EM values were significantly (p<0.05) affected by the type of starch, the type of gum and the storage time, as well as the interactions between these factors. EM values were higher for cassava starch films than maize starch films. In both cases the EM increased over time, this increase being more noticeable in maize starch. Although the incorporation of gums increased EM in both of the films formulated with starches, the incorporation of gellan gum was more effective in cassava starch films while xanthan gum was more effective in films prepared with maize starch. Tensile strength (TS) showed a similar trend to EM, but there was no statistically significant interaction between storage time and the type of gum (p >0.05), which means that the effect of storage time was similar for films with both kinds of gums. TS was higher for cassava starch films than for maize starch and increased to a greater extent during storage in MS films. This was coherent with the higher amylose content (27%) of maize starch than cassava starch (9%), which is more affected by the retrogradation phenomena during storage (Cano et al., 2014). The incorporation of both gums led to higher TS values as compared to those obtained in pure starch films, this increase being more significant in the films containing gellan gum. The films formulated with gums exhibited more stable TS values over time than pure starch films, regardless of the type of gum used. Gellan gum was more effective than xanthan gum at increasing the resistance to break in cassava starch films, while both gums had a similar effect on maize starch-based films.

The percentage of deformation at break (E%) was significantly (p<0.05) affected by the three parameters (type of starch, gum and storage time) and their interactions. Maize starch-based films were more stretchable than cassava starch films, but the extensibility was reduced by more than half in maize starch-based films during storage whereas the ability to stretch slightly decreased over time in cassava starch-based films. The incorporation of gums decreased the extensibility of all the starch-based films, but more significantly in films prepared with xanthan gum. Throughout the 5 weeks of storage, the
stretchability of the films formulated with gellan gum decreased to almost half, while in the films formulated with xanthan gum it remained constant.

The obtained tensile behaviour of the different thermoprocessed films revealed a greater structural toughness in films based on CS, with lower amylose ratio, than in MS based films. This could be related with the higher molecular weight of the highly branched amylopectin that could offer the possibility of a greater chain entanglement in the melt, thus forming a more cohesive, less extensible polymer matrix, with lower retrogradation degree during storage. The incorporation of gums with high molecular weight (10^5-10^6 Da) will contribute to reinforce the starch polymer matrix, creating association domains in the matrix where gums and starch polymers could participate through the aggregation of the helical conformations of the different chains. Gellan gum is an anionic polysaccharide hydrogel-forming polymer that comprises a tetrasaccharide repeat unit of two $\beta$-D-glucoses, one $\beta$-D-glucuronate, and one a-L-rhamnose. It forms a physical gel by undergoing a random coil to double helix transition upon cooling. This molecular characteristic could provide a greater reinforcing effect in the CS matrix, with lower amylose (with potential helical associations) content, whereas its effect was less appreciable in the MS matrix with higher amylose ratio. In contrast, xanthan gum consists of a main chain of D-glucopyranosyl with a $\beta$ 1-4 bond, as in cellulose, with trisaccharide side chains composed of D-mannopyranosyl and D-glucopyranosyluronic acid residues. Different interactions of xanthan gum and starch have been described, depending on the starch source and the amylose/amylopectin ratio (Sikora et al., 2008). Differences in the molecular structure of the gums and the amylose/amylopectin ratio in starch may explain the observed tensile behaviour of the blend films, depending on their composition. The linear structure of gellan chains could better reinforce the CS matrix with a lower ratio of amylose, providing it with more regions with glucose helical associations, while this contribution could be less noticeable in MS matrices, with higher amylose content. Despite the structural differences, both gums enhanced the toughness of the starch matrix structure, giving rise to a better mechanical performance of the starch-based films.

As regards water vapour permeability (WVP), significant effects as a result of the type of starch, the type of gum and storage time were observed, as were interactions between the type of starch and gum. Maize starch films were more permeable to water vapour than cassava starch films. Over time there was a slight increase in WVP, which was more
significant in maize starch films. The incorporation of gums significantly reduced WVP, xanthan gum being more effective in maize starch-based films. Both gums minimised the impact of storage time on the WVP values of the films and, in general, there were no significant changes as far as WVP is concerned throughout storage.

The OP values were significantly affected by the type of starch and gum together with the storage time and the interactions between the type of starch and the type of gum. The incorporation of both gums reduced the OP of starch films, which coincides with the results obtained by Sapper et al. (2019) for cassava starch films prepared by casting. Maize starch films were more permeable to oxygen and were more affected by storage time, with a more noticeable increase in the OP at the end of the 5-week storage.

The observed effects of gums on the starch film barrier properties were coherent with that commented on above as regards the reinforcing effect of gums in the starch matrices. This reinforce implied the formation of a tougher, more cohesive network that limited the mass transfer phenomena to greater extent than pure starch matrix. Different effects of each gum depending on the starch source was also observed, as previously described for its influence in the film tensile behaviour.

The changes in the properties of starch films over time are attributable to the recrystallisation of amylose, or starch retrogradation, (Cano et al. 2017) whose proportion is higher in maize starch as mentioned above. Crystallisation led to more rigid (with greater EM) and less extensible (lower %E) films, while hydration promoted the plasticisation of the amorphous fraction of the films and led to an increased molecular mobility, thus favouring the diffusion-dependent phenomena, such as the mass transport associated with the permeation of water or gas molecules through the amorphous regions of the films. In general, storage time had a more significant effect on tensile properties than on barrier properties. The WVP showed an increase throughout storage in maize starch films without gums, which also presented a slightly higher equilibrium moisture content. As concerns the tensile properties, changes over time led to an increase in the EM and TS and a decrease in the film extensibility, which was more marked in maize starch films, with a higher amylose content and, thus, greater sensitivity to crystallisation. This was mitigated to a greater extent by xanthan gum. The most resistant films were those formulated with cassava starch with gellan gum, which also showed a more limited
increase in strength and toughness during storage time, while exhibiting reduced extensibility of 3-5%.

PLA: PHBV blend films (P) showed high EM and TS values and low extensibility E(%), all of which were in the range of those of cassava starch films with gums, the most rigid, resistant and least extensible starch-based films. The EM values increased slightly over time, which could be attributed to the progressive crystallisation of PHBV (Arismendi et al., 2013).

3.1.3 Microstructural analysis of the monolayer films

Figure 1 shows FSEM micrographs of the cross-section of the monolayer films. Gums were only partially miscible with starch and gum-rich domains appeared dispersed in the starch-rich continuous phase. The different cryofracture behaviour of the starch continuous phase of the films reveals the partial miscibility of the gums in the starch phase, which reinforced the starch matrix, as revealed by the higher structural toughness deduced from the tensile parameters of blend films. The lack of polyester miscibility can also be observed in Figure 1 where different domains of PLA and PHBV can be observed, as previously reported (Gasmi et al., 2019).

3.2 Properties and microstructure of bilayer films

Table 3 shows the thickness values, moisture content and solubility of starch-polyester bilayer films. The bilayer films were not as thick as expected from the values of the monolayers, which indicates creep phenomena (flow of material) during the thermo-compression carried out to adhere the sheets. This creep was higher in the bilayers prepared with maize starch monolayers than in those made with cassava starch monolayers, probably due to the smaller amount of flow provoked in the maize starch monolayers obtained at milder compression and lower temperatures. In fact, a second thermo-compression of the different monolayers under the conditions used for the laminate thermo-sealing revealed a 20-30% reduction in thickness for MS-based films and only a 15-25% reduction for CS-based films. A mere 5% reduction in thickness was observed for the polyester films during the second thermo-compression. However, the thickness values observed for the bilayers were still lower than that predicted from the sum of the corresponding thicknesses of monolayers submitted to an equivalent second thermo-compression. This indicates that the creep phenomenon occurred to a greater
extent when both layers were in contact and could imply different interactions between
the polymers in contact associated with the thermal compression of the bilayers. In all
likelihood, the proximity to the softening point of the polymers and the migration of some
components of the respective monolayer, such as plasticisers, promoted the mobility of
the polymers in the different sheets, thus encouraging flowability during compression.

In Figure 2, the FSEM images of the bilayer cross-section can be observed, where the
polyester sheet can be seen to be much less thick, which indicates that the flow of the
polyester layer during the thermo-sealing of the layers was much more intense and
variable than that undergone by the starch-based layers. The thickness of the polyester
film ranged between 20-30 µm in most of the cases, whereas the starch-based layers
ranged between 160-180 µm for MS sheets and 110-140 µm for CS sheets. Although the
polyester-starch mass ratio in the laminate was 3:4, the thickness ratio of the sheets fell
sharply, thus reflecting the greater flowability of polyester as compared to starch, when
both are in contact during thermo-compression. In fact, the variable, low thickness values
of the polyester layers observed in Figure 2 are remarkable. Likewise, cryo-fracture
provoked a partial detachment of the layers, as shown in Figure 2, except for the laminate
with cassava starch-gellan and polyesters, which exhibited a clear well-adhered interface.
The laminates with cassava starch-xanthan exhibited an irregular interface where a partial
detachment could also be observed. Therefore, from the structural point of view, the
bilayers with cassava starch and gellan with the polyester layer were the best option.

The total thickness values of bilayers estimated from the FSEM micrographs are, in
general, lower than those directly measured with the calliper (Table 3), which can be
explained in terms of the film swelling with water adsorption when conditioned at 53%
RH (values from Table 3); in FSEM analyses, however, the films were completely dried
(P₂O₅ conditioned).

The water solubility of the bilayers was reduced from about 90% in the starch monolayers
to about 75%, without there being any significant differences between bilayers. Although
this implied a notable reduction in water solubility with respect to that of starch
monolayers, this was slightly lower than that expected from the mass ratio and the
respective solubility of starch-based and polyester sheets, which would suppose a
solubility of only 60-65%. Therefore, using thermo-compression to obtain the laminate
could promote the water solubility of the hydrophobic polyester monolayer due to the
interactions between layer components at high temperatures and pressures. Particularly, the water diffusion from the starch layer to the polyester layer could promote the chain hydrolysis, giving rise to water soluble oligomers.

**Figure 3** shows the tensile properties of the starch-polyester bilayer films as compared to the corresponding values of the starch monolayers. The cassava starch-polyester bilayer films with gums, with EM values near those of the polyester sheet, presented values of EM slightly greater than those of their corresponding starch monolayers. However, in the rest of the cases, EM was slightly lower, or in the range of, the corresponding hydrophilic monolayer. This indicates that the thicker starch monolayer mostly determined the stiffness of the bilayer. The cassava starch-polyester bilayer films with gums presented the highest elastic modulus.

The cassava starch-polyester bilayers with gums presented similar TS values to those of corresponding starch monolayers, whereas lower values were observed for the rest of bilayers. In general, the extensibility of the bilayers was similar to or lower than that of the corresponding starch monolayers and in the range of the extensibility of the polyester sheet which limited the stretchability of the bilayers in every case.

The EM of bilayers decreased over time in practically every case, which is contrary to that observed in monolayers (Table 2). This could be associated with the diffusion of compounds, such as water, glycerol, PEG 1000 or oligomers, present or formed during the polymer processing, which could affect the tensile behaviour of each sheet, and so of the assembly. In particular, water migration from the starch based sheet to the polyester sheet could provoke a partial hydrolysis, reducing the toughness of the matrix. The maize starch bilayer films without gums showed EM values that were stable over time. In the same way, the TS of bilayers decreased over time in every case, whereas the extensibility was more stable, but with some fluctuations. The cassava starch-polyester films with gums exhibited the highest values of EM and TS, regardless of the storage time.

**Figure 4** shows the barrier properties (WVP and OP) of the different bilayer films, compared to the corresponding starch monolayer. In every case, a significant decrease in both apparent permeability values (to water vapour and oxygen) was observed for the bilayer assemblies, with respect to the values of the corresponding starch monolayers, as previously observed in other starch-polyester laminates (Ortega-Toro et al., 2015; Muller et al. 2017; Tampau et al. 2018). The values did not significantly change during the 5-
week storage of the films, which reflects the fact that the barrier capacity of the assemblies remained stable during the storage time.

The parallel assembly of the hydrophilic and hydrophobic layers explains the reduction in WVP with respect to the starch films, since the polyester sheet controlled the water transfer through the laminate. In fact, the apparent WVP values of the bilayers were in the range of those of the polyester films for most cases, except for the MS films where an increase (promoted by storage time) was observed. This could be explained by the migration phenomena, previously commented on, that can reduce the water barrier capacity of the hydrophobic layer by plasticisation, coherently with that observed in the case of tensile parameters.

As concerns oxygen permeability, the apparent values of the bilayers were also reduced with respect to those of the corresponding values of starch monolayers which, in turn, are the controlling sheets for the oxygen transfer according to their lower OP values. The migration of compounds from the polyester sheet to the starch matrices, or the greater compactness of the starch matrix provoked by the second compression, implied a still greater reduction in the OP values of the bilayers, reaching values below those of the initial starch layer. This effect was particularly remarkable for the bilayers of cassava starch with gums that exhibited the lowest apparent oxygen permeability.

4. CONCLUSIONS

The incorporation of gellan and xanthan gums into thermo-processed cassava and maize starch slightly reduced the water adsorption capacity of starch-based films and improved their mechanical properties. This improvement was more noticeable in maize starch films, although the highest EM and TS values were obtained for cassava starch-gum films. The incorporation of either gellan or xanthan gum decreased the water vapour and oxygen permeabilities of starch films, the cassava starch films with gums being the least permeable to oxygen. The cassava starch films were more stable in their mechanical properties over time, especially those incorporating xanthan gum.

The starch-based-polyester laminates exhibited improved oxygen and water vapour barrier capacity with respect to both starch and polyester monolayers. The laminates with cassava starch with gums showed the lowest OP and WVP values and the highest elastic
modulus and tensile strength, with extensibility values in the range of the corresponding monolayers and a reasonable degree of stability throughout time. When also taking the layer adhesion into account, the bilayer formed with the cassava starch with gellan gum and the PLA-PHBV sheet appeared as the best option for food packaging purposes.

Acknowledgements

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REFERENCES


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Table 1. Thickness, equilibrium moisture content and water solubility of cassava starch (CS) and maize starch (MS) films containing or not gellan (G) and xanthan (X) gums, and PLA-PHBV blend films (P). Mean values ± standard deviation.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Thickness (µm)</th>
<th>Equilibrium Moisture (g water/100 g dried film)</th>
<th>Water Solubility (g/ 100 g dried film)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>189 ± 14&lt;sup&gt;(d)&lt;/sup&gt;</td>
<td>9.1 ± 0.3&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>89.5 ± 0.1&lt;sup&gt;(x)&lt;/sup&gt;</td>
</tr>
<tr>
<td>CS-G</td>
<td>191 ± 18&lt;sup&gt;(d)&lt;/sup&gt;</td>
<td>8.0 ± 0.4&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>89.2 ± 0.2&lt;sup&gt;(x)&lt;/sup&gt;</td>
</tr>
<tr>
<td>CS-X</td>
<td>200 ± 16&lt;sup&gt;(c)&lt;/sup&gt;</td>
<td>7.7 ± 0.8&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>89.6 ± 0.1&lt;sup&gt;(ab)&lt;/sup&gt;</td>
</tr>
<tr>
<td>MS</td>
<td>221 ± 17&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>8.3 ± 0.3&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>89.0 ± 0.3&lt;sup&gt;(a)&lt;/sup&gt;</td>
</tr>
<tr>
<td>MS-G</td>
<td>219 ± 20&lt;sup&gt;(a)&lt;/sup&gt;</td>
<td>8.1 ± 0.3&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>89.6 ± 0.1&lt;sup&gt;(ab)&lt;/sup&gt;</td>
</tr>
<tr>
<td>MS-X</td>
<td>210 ± 20&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>8.1 ± 0.9&lt;sup&gt;(b)&lt;/sup&gt;</td>
<td>89.7 ± 0.1&lt;sup&gt;(ab)&lt;/sup&gt;</td>
</tr>
<tr>
<td>P</td>
<td>130 ± 0&lt;sup&gt;(c)&lt;/sup&gt;</td>
<td>0.3 ± 0.1&lt;sup&gt;(c)&lt;/sup&gt;</td>
<td>27.0 ± 3.0&lt;sup&gt;(c)&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different superscript letters (a - e) within the same column indicate significant differences among formulations (p < 0.05).
Table 2. Tensile properties (Elastic modulus: EM, tensile strength: TS and deformation at break: %E) and barrier properties (water vapour (WVP) and oxygen (OP) permeability) of cassava starch (CS) and maize starch (MS) films containing or not gellan (G) and xanthan (X) gums, and PLA-PHBV blend films (P). Mean values after 1 and 5 storage weeks ± standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>EM  (MPa)</th>
<th>TS  (MPa)</th>
<th>%E</th>
<th>WVP (g·mm·kPa⁻¹·h⁻¹·m⁻²)</th>
<th>OP×10¹⁴ (cm³·m⁻²·s⁻¹·Pa⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>500 ±70(±2)</td>
<td>600 ±70(±1)</td>
<td>12.0</td>
<td>4.0 ±0.4(±1)</td>
<td>1.2 ±0.7(±1) ±0.1(±1) ±0.1(±1)</td>
</tr>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 5</td>
<td>Week 1</td>
<td>Week 5</td>
<td>Week 1</td>
</tr>
<tr>
<td>CS-G</td>
<td>600 ±70(±2)</td>
<td>900 ±50(±1)</td>
<td>18.0</td>
<td>5.0 ±0.2(±1)</td>
<td>1.6 ±0.7(±1) ±0.3(±1) ±0.1(±1)</td>
</tr>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 5</td>
<td>Week 1</td>
<td>Week 5</td>
<td>Week 1</td>
</tr>
<tr>
<td>CS-X</td>
<td>700 ±40(±2)</td>
<td>700 ±40(±2)</td>
<td>14.0</td>
<td>3.0 ±0.3(±2)</td>
<td>1.1 ±0.1(±1) ±0.1(±1) ±0.3(±1)</td>
</tr>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 5</td>
<td>Week 1</td>
<td>Week 5</td>
<td>Week 1</td>
</tr>
<tr>
<td>MS</td>
<td>140 ±60(±2)</td>
<td>374 ±60(±1)</td>
<td>7.0</td>
<td>30.0 ±8(±1)</td>
<td>0.8 ±1(±1) ±0.7(±1) ±0.4(±1)</td>
</tr>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 5</td>
<td>Week 1</td>
<td>Week 5</td>
<td>Week 1</td>
</tr>
<tr>
<td>MS-G</td>
<td>360 ±70(±1)</td>
<td>600 ±70(±1)</td>
<td>14.0</td>
<td>11.0 ±6(±1)</td>
<td>0.8 ±0.5(±1) ±0.3(±1) ±0.2(±1)</td>
</tr>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 5</td>
<td>Week 1</td>
<td>Week 5</td>
<td>Week 1</td>
</tr>
<tr>
<td>MS-X</td>
<td>500 ±100(±2)</td>
<td>660 ±100(±1)</td>
<td>11.0</td>
<td>40.0 ±3(±1)</td>
<td>1.2 ±1.1 ±0.1(±1) ±0.1(±1)</td>
</tr>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 5</td>
<td>Week 1</td>
<td>Week 5</td>
<td>Week 1</td>
</tr>
<tr>
<td>P</td>
<td>780 ±190(±3)</td>
<td>860 ±190(±3)</td>
<td>15.0</td>
<td>2.4 ±0.2(±1)</td>
<td>0.2 ±0.3(±1) ±0.1(±2) ±0.1(±1) ±0.3(±1) ±3(±2)</td>
</tr>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 5</td>
<td>Week 1</td>
<td>Week 5</td>
<td>Week 1</td>
</tr>
</tbody>
</table>

Different superscript letters (a-e) within the same column indicate significant differences among formulations (p < 0.05). Different superscript numbers (1-2) within the same row for each parameter and sample indicate significant differences between the two storage times (p < 0.05).
Table 3. Thickness (experimental value and sum of the corresponding monolayer thicknesses submitted to the same thermocompression process used to obtain bilayers), equilibrium moisture content and water solubility of bilayer films obtained from cassava starch (CS) and maize starch (MS) sheets, containing or not gellan (G) and xanthan (X) gums, thermo-compressed with PLA-PHBV blend films (P).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Thickness (µm)</th>
<th>Sum of Monolayer Thickness (µm)</th>
<th>Equilibrium Moisture (g water/100 g dried film)</th>
<th>Water Solubility (g/ 100 g dried film)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-P</td>
<td>205 ±15(ab)</td>
<td>280</td>
<td>8.6 ± 0.6(a)</td>
<td>74 ± 10(a)</td>
</tr>
<tr>
<td>CS-G-P</td>
<td>230 ± 20(a)</td>
<td>264</td>
<td>7.7 ± 0.2(b)</td>
<td>75 ± 3(a)</td>
</tr>
<tr>
<td>CS-X-P</td>
<td>222 ± 16(a)</td>
<td>296</td>
<td>7.4 ± 0.7(b)</td>
<td>71 ± 13(a)</td>
</tr>
<tr>
<td>MS-P</td>
<td>190 ± 15(b)</td>
<td>301</td>
<td>7.4 ± 0.7(b)</td>
<td>72 ± 3(a)</td>
</tr>
<tr>
<td>MS-G-P</td>
<td>230 ± 20(a)</td>
<td>275</td>
<td>9.0 ± 0.5(a)</td>
<td>77 ± 11(a)</td>
</tr>
<tr>
<td>MS-X-P</td>
<td>220 ± 20(ab)</td>
<td>286</td>
<td>8.2 ± 0.7(b)</td>
<td>77 ± 8(a)</td>
</tr>
</tbody>
</table>

Different superscript letters (a-b) within the same column indicate significant differences among formulations (p < 0.05).
Figure 1. FSEM micrographs of monolayer films (cross section) from maize starch (MS) and Cassava starch (CS) and films containing or not gellan (G) and xanthan (X) gums, and PLA-PHBV blend films (P).
Figure 3. Tensile properties (Elastic modulus: EM, tensile strength: TS and deformation at break: %E) bilayer films (Black bars) of Cassava starch (CS) and maize starch (MS) films containing or not gellan (G) and xanthan (X) gums, and PLA-PHBV blend films (P), in comparison with the corresponding values of the respective starch monolayers (white bars). Values after 1 (left) and 5 (right) storage weeks. Arrow indicates the values for the polyester monolayer: EM (week 1: 780, week 5: 860), TS (week 1: 15, week 5: 16) and E (week 1: 2.4, week 5: 3).
**Figure 4.** Barrier properties (water vapour: WVP and oxygen (OP) permeability) of bilayer films (Black bars) of Cassava starch (CS) and maize starch (MS) films containing or not gellan (G) and xanthan (X) gums, and PLA-PHBV blend films (P), in comparison with the corresponding values of the respective starch monolayers (white bars). Values after 1 (left) and 5 (right) storage weeks. Arrow indicates the values of the polyester film: WVP (week 1: 0.2, week 5: 0.3), OP (week 1: 410, week 5: 432).
Figure 2. FSEM micrographs of bilayer films (cross section) obtained by the thermocompression of Cassava starch (CS) and maize starch (MS) films containing or not gellan (G) and xanthan (X) gums, and PLA-PHBV blend films (P). Polyester (P) and starch (S) sheets are marked.