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Hernandez-Garcia, E.; Vargas, M.; Chiralt Boix, MA. (2021). Thermoprocessed starch-polyester bilayer films as affected by the addition of gellar or xantham gum. Food Hydrocolloids. 113:1-9. <https://doi.org/10.1016/j.foodhyd.2020.106509>



The final publication is available at

<https://doi.org/10.1016/j.foodhyd.2020.106509>

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Additional Information

1 **Thermoprocessed starch-polyester bilayer films as affected by the**
2 **addition of gellan or xanthan gum**

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12 **ABSTRACT**

13 Monolayer films based on cassava starch (CS) or maize starch (MS), with and without 10
14 % of gellan or xanthan gum, and PLA-PHBV (75:25) blend films, were obtained by melt-
15 blending and compression moulding, using glycerol (for starch blends) and PEG 1000
16 (for polyester blends) as plasticisers. Bilayer films were obtained by thermo-compression
17 of the different starch based sheets with the polyester sheet. Both mono and bilayers were
18 characterised as to their mechanical and barrier properties, equilibrium moisture, water
19 solubility and microstructure. The incorporation of gellan gum and xanthan gum
20 improved the mechanical properties of starch-based films, especially in the case of MS,
21 although the highest EM and TS values were obtained for CS-gum films. The
22 incorporation of either gellan or xanthan gum decreased the water vapour and oxygen
23 permeability of starch-based films; the CS films with gums being the least permeable to
24 oxygen. The lowest changes in mechanical properties throughout storage were obtained
25 in cassava starch-based films, especially those containing xanthan gum. Starch based-
26 polyester bilayers presented a high oxygen and water vapour barrier capacity, as
27 compared to their individual monolayers. Bilayer films with cassava starch including the
28 gums showed the lowest OP and WVP values and the highest elastic modulus and tensile
29 strength, with extensibility values in the range of the corresponding monolayers and slight
30 changes in their physical properties throughout time. The bilayer formed with cassava
31 starch with gellan gum and a PLA-PHBV appeared as the best option for food packaging
32 purposes taking into account its functional properties and the good layer adhesion of the
33 bilayer.

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35 **Keywords:** biodegradable bilayer films, PLA, PHBV, starch, gellan, xanthan.

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43 **1. INTRODUCTION**

44 Over the last few decades, there has been a growing need to find alternatives to petroleum-
45 based non-biodegradable products due to environmental concerns (Martín et al. 2001).
46 This has increased interest in developing biodegradable food packaging materials based
47 on biopolymers, such as starch. The use of thermoplastic starch (TPS) to develop
48 biodegradable packaging materials has several advantages, such as low cost,
49 renewability, sustainable production, good processability by means of conventional
50 techniques, good oxygen barrier capacity and stretchability, as well as suitable
51 transparency, odour and taste (Muller et al., 2017). However, starch materials are water
52 sensitive and exhibit poor water vapour barrier properties, which are greatly affected by
53 their moisture content (Vieira et al., 2011). Additionally, the phenomenon of
54 retrogradation modifies the mechanical behaviour of starch-based materials throughout
55 time, depending on the amylose/amylopectin ratio and moisture content (Cano et al.,
56 2014; López et al., 2013; Ortega-Toro et al. 2014). Glycerol is usually employed as a
57 plasticiser in starch films in order to facilitate thermo-processing, by reducing the
58 intermolecular forces and increasing the flexibility of starch-based films (Savadekar &
59 Mhaske, 2012). Different strategies have been applied to improve the functional
60 properties of starch based materials, such as blending it with different additives or other
61 biopolymers (Cano et al., 2017; Ortega-Toro et al., 2017; Samsudin & Hani, 2017).
62 Sapper et al. (2019) observed that the cassava starch mixture with 10 or 20% of different
63 gums of microbial origin, such as gellan, xanthan or pullulan, permitted the improvement
64 of the properties of starch-based films obtained by casting, while maintaining the
65 competitive cost of the material. In general, the addition of such gums improved the
66 mechanical properties of starch films and their storage stability (Kim et al., 2015; Sapper
67 et al, 2019). Xanthan gum enhanced the tensile properties of the films but led to a less
68 extensible matrix (Arismendi et al., 2013).

69 Developing bilayer films based on biodegradable monolayers formulated with different
70 biopolymers with complementary properties is an innovative approach to improving the
71 performance of the material (laminate), as compared to the use of monolayers, while also
72 meeting the food packaging requirements better (Slavutsky et al., 2018). In this sense, the
73 combination of starch films with sheets of hydrophobic polyesters represents a good
74 alternative means of accomplishing this purpose. Poly(lactic acid) (PLA) and Poly(3-

75 hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) are biodegradable polyesters obtained
76 from natural resources which can be used for food packaging purposes, due to their ability
77 to form food contact plastic materials at a relatively competitive cost. PLA is a
78 biodegradable thermoplastic linear aliphatic polyester, of great potential in the packaging
79 industry because of its optical properties, good thermal behaviour and water vapour
80 barrier properties (Bonilla et al., 2013; Chaiwutthinan et al., 2015; Muller et al., 2017).
81 However, PLA shows limited gas barrier capacity and is very brittle, with less than 10%
82 elongation at break (Rasal et al., 2010). Therefore, PLA has been combined with different
83 plasticisers and other polyesters, such as PHBV. Although PHBV has physical properties
84 that are comparable to some synthetic polymers, such as polypropylene and polyethylene,
85 the PHBV materials are more brittle with lower elongation at break (Laycock et al., 2013).
86 PHBV and PLA blends have been studied in order to improve the functional properties
87 of the materials. The polymers exhibited low miscibility, so PHBV-PLA blend films had
88 low transparency, but their mechanical resistance was significantly improved as
89 compared with pure PLA or PHBV films (Liu et al., 2015). Different plasticisers, such as
90 acetyl tributyl citrate, limonene, and PEG have been used to improve the extensibility of
91 mixtures of PLA and PHB (Armentano et al., 2015). The addition of PEG1000 and
92 PEG600 at concentrations lower than 10% improved the thermal properties of PLA-
93 PHBV based materials (Thongpina et al., 2017).

94 Obtaining bilayer structures consisting of a PLA-PHBV blend film layer and a starch-
95 based film layer could represent a good alternative to obtain the target materials with
96 improved mechanical and barrier properties suitable for food packaging applications. The
97 polyester layer would contribute to the strengthening of the bilayer while reducing water
98 vapour permeability, whereas the starch layer would help to control the oxygen and gas
99 barrier capacity of the bilayer assembly. In previous studies, Requena et al., (2018)
100 combined a PLA-PHBV (75:25) monolayer with glycerol-plasticised starch sheets to
101 develop bilayer food packaging materials. In this study, a good layer adhesion was
102 obtained for starch/PLA-PHBV sheets, but the elastic modulus and mechanical resistance
103 of polyester-cassava starch bilayer films were lower than that of the corresponding
104 polyester monolayers due to the weaker strength of starch monolayers. In this sense, the
105 incorporation of xanthan or gellan gum to a thermo-processed starch layer could improve
106 both the monolayer and bilayer functional properties for food packaging purposes. The
107 origin of the starch may also affect both the film and bilayer properties, since different

108 starches provide the films with more or less mechanical resistance and stability, mainly
109 depending on their amylose/amylopectin ratio (Cano et al., 2014).

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

115

116 **2. MATERIALS AND METHODS**

117 *2.1 Materials*

118 Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) ENMAT Y1000P with 3%
119 hydroxyvalerate was supplied by Helian Polymers B.V. (Belfeld, Holland). Amorphous
120 PLA 4060D, density of 1.24g/cm³ and average molecular weight of 106,226 D with 40%
121 of low molecular weight fraction (275 D) as reported by Muller et al. (2017), was supplied
122 by Natureworks (U.S.A). Maize starch (MS, 27% amylose) and cassava starch (CS, 9%
123 amylose) were supplied by Roquette (Roquette Laisa, Benifaió, Spain) and Quimidroga
124 S.A. (Barcelona, Spain), respectively. Xanthan gum (X) (high molecular weight, ~10⁶ Da),
125 was supplied by EPSA (Valencia, Spain). Negatively charged, low acyl gellan gum (G)
126 KELGOGEL F (MW 3-5x10⁵ Da), was purchased from Premium Ingredients (Murcia,
127 Spain). The plasticiser, poly(ethylene glycol) with a molecular weight of 1000 Da
128 (PEG1000), was purchased from Sigma-Aldrich (Steinheim, Germany), and the glycerol
129 was obtained from Panreac Química S.L.U. (Castellar del Vallés, Barcelona, Spain). For
130 sample conditioning purposes, phosphorus pentoxide (P₂O₅) and magnesium nitrate-6-
131 hydrate (Mg(NO₃)₂) were supplied by Panreac Química, S.A. (Castellar del Vallès,
132 Barcelona, Spain).

133 *2.2 Preparation of films*

134 *2.2.1 Starch monolayer films*

135 For the preparation of maize (MS) and cassava (CS) starch monolayer films, the starch
136 solutions were mixed in the adequate proportion with which to obtain a starch:gum ratio

137 of 90:10, using glycerol (0.30 g/g of starch) as a plasticiser, by melt blending and
138 compression moulding. The melt blending process was carried out in an internal mixer
139 (HAAKE™ PolyLab™ QC, Thermo Fisher Scientific, Germany) at 130 °C, rotor speed
140 50 rpm, for 10 min and 50 g of blend were processed in each batch. After processing,
141 blends were cold ground in a refrigerated batch mill (Model M20, IKA, Germany) and
142 the powder conditioned at 25 °C and 53% relative humidity (RH) for one week. Four g
143 of the conditioned powder was required to obtain each film (160 mm in diameter) that
144 were put onto Teflon sheets and preheated at 160 °C or 150 °C (for CS or MS) for 1 (CS)
145 or 5 (MS) min in a hot-plate press (Model LP20, Labtech Engineering, Thailand). Films
146 were obtained by compressing at 160 °C (CS) or 150 °C (MS) for 2 min at 50 (CS) or 30
147 (MS) bars, followed by 6 min at 100 (CS) or 130 (MS) bars and a final cooling cycle for
148 3 min until the temperature reached about 70 °C, according to that described by other
149 authors for CS (Requena et al., 2018) and MS (Silva-Guzmán et al., 2018). The obtained
150 films were conditioned at 25 °C and 53% RH until used to obtain bilayer films.

151 2.2.2 Polyester monolayer films

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

164 2.2.3 Starch-polyester bilayer films

165 Starch monolayers and polyester monolayers were submitted to compression moulding
166 in a hydraulic press (Model LP20, Labtech Engineering, Thailand) at 180 °C and 100 bars

167 for 2 min and cooled down until 80 °C in 2 min, thus obtaining starch-polyester bilayer
168 films. All bilayer films were stored at 25 °C and 53%+ç RH till their analyses.

169 2.3 Film characterisation

170

171 2.3.1 Tensile properties and thickness

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

182 2.3.2 Water vapour permeability (WVP)

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

194 2.3.3 Oxygen permeability (OP)

195 The oxygen permeability (OP) of the conditioned films was evaluated by measuring the
196 oxygen permeation rate by means of an OX-TRAN 1/50 system (Mocon, Minneapolis,

197 USA) at 53% RH and 25 °C (ASTM Standard Method D3985-05, 2002). The transmission
198 values were determined every 20 min until equilibrium was reached. The exposure area
199 during the tests was 50 cm² for each formulation. In order to obtain the oxygen
200 permeability (OP), the film thickness was considered in every case. At least two replicates
201 per formulation were made. An apparent value of OP was also determined for bilayer
202 films, considering their total thickness value.

203 2.3.4 *Moisture content*

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

208 2.3.5 *Film water solubility*

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

216 2.3.6 *Microstructural analyses*

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

223 2.3.7 *Statistical analysis*

224 The statistical analysis of the results was performed through an analysis of variance
225 (ANOVA) using Statgraphics Centurion XVII-X64. Both a One-way and multifactor

226 ANOVA were used to analyse the influence of composition variables and storage time on
227 the properties of the films. Fisher's least significant difference (LSD) procedure was used
228 at the 95% confidence level.

229 3. RESULTS AND DISCUSSION

230 3.1. Properties and microstructure of monolayer films

231 3.1.1. Thickness, equilibrium moisture content and water solubility

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

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

257

258

259 3.1.2. *Tensile properties and barrier properties*

260 **Table 2** shows tensile parameters (Elastic modulus: EM, tensile strength: TS and
261 percentage deformation E% at break) and barrier properties (water vapour: WVP and
262 oxygen permeability: OP) of monolayer films after 1 week and 5 weeks of storage at 25
263 °C and 53% RH.

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

283 The percentage of deformation at break (E%) was significantly ($p < 0.05$) affected by the
284 three parameters (type of starch, gum and storage time) and their interactions. Maize
285 starch- based films were more stretchable than cassava starch films, but the extensibility
286 was reduced by more than half in maize starch-based films during storage whereas the
287 ability to stretch slightly decreased over time in cassava starch-based films. The
288 incorporation of gums decreased the extensibility of all the starch-based films, but more
289 significantly in films prepared with xanthan gum. Throughout the 5 weeks of storage, the

290 stretchability of the films formulated with gellan gum decreased to almost half, while in
291 the films formulated with xanthan gum it remained constant.

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

318 As regards water vapour permeability (WVP), significant effects as a result of the type of
319 starch, the type of gum and storage time were observed, as were interactions between the
320 type of starch and gum. Maize starch films were more permeable to water vapour than
321 cassava starch films. Over time there was a slight increase in WVP, which was more

322 significant in maize starch films. The incorporation of gums significantly reduced WVP,
323 xanthan gum being more effective in maize starch-based films. Both gums minimised the
324 impact of storage time on the WVP values of the films and, in general, there were no
325 significant changes as far as WVP is concerned throughout storage.

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

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

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

353 increase in strength and toughness during storage time, while exhibiting reduced
354 extensibility of 3-5%.

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

360 *3.1.3 Microstructural analysis of the monolayer films*

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

369 *3.2 Properties and microstructure of bilayer films*

370 **Table 3** shows the thickness values, moisture content and solubility of starch-polyester
371 bilayer films. The bilayer films were not as thick as expected from the values of the
372 monolayers, which indicates creep phenomena (flow of material) during the thermo-
373 compression carried out to adhere the sheets. This creep was higher in the bilayers
374 prepared with maize starch monolayers than in those made with cassava starch
375 monolayers, probably due to the smaller amount of flow provoked in the maize starch
376 monolayers obtained at milder compression and lower temperatures. In fact, a second
377 thermo-compression of the different monolayers under the conditions used for the
378 laminate thermo-sealing revealed a 20-30% reduction in thickness for MS-based films
379 and only a 15-25% reduction for CS-based films. A mere 5% reduction in thickness was
380 observed for the polyester films during the second thermo-compression. However, the
381 thickness values observed for the bilayers were still lower than that predicted from the
382 sum of the corresponding thicknesses of monolayers submitted to an equivalent second
383 thermo-compression. This indicates that the creep phenomenon occurred to a greater

384 extent when both layers were in contact and could imply different interactions between
385 the polymers in contact associated with the thermal compression of the bilayers. In all
386 likelihood, the proximity to the softening point of the polymers and the migration of some
387 components of the respective monolayer, such as plasticisers, promoted the mobility of
388 the polymers in the different sheets, thus encouraging flowability during compression.

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

404 The total thickness values of bilayers estimated from the FSEM micrographs are, in
405 general, lower than those directly measured with the calliper (Table 3), which can be
406 explained in terms of the film swelling with water adsorption when conditioned at 53%
407 RH (values from Table 3); in FSEM analyses, however, the films were completely dried
408 (P_2O_5 conditioned).

409 The water solubility of the bilayers was reduced from about 90% in the starch monolayers
410 to about 75%, without there being any significant differences between bilayers. Although
411 this implied a notable reduction in water solubility with respect to that of starch
412 monolayers, this was slightly lower than that expected from the mass ratio and the
413 respective solubility of starch-based and polyester sheets, which would suppose a
414 solubility of only 60-65%. Therefore, using thermo-compression to obtain the laminate
415 could promote the water solubility of the hydrophobic polyester monolayer due to the

416 interactions between layer components at high temperatures and pressures. Particularly,
417 the water diffusion from the starch layer to the polyester layer could promote the chain
418 hydrolysis, giving rise to water soluble oligomers.

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

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

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

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

448 week storage of the films, which reflects the fact that the barrier capacity of the assemblies
449 remained stable during the storage time.

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

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

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467 **4. CONCLUSIONS**

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

476 The starch based-polyester laminates exhibited improved oxygen and water vapour
477 barrier capacity with respect to both starch and polyester monolayers. The laminates with
478 cassava starch with gums showed the lowest OP and WVP values and the highest elastic

479 modulus and tensile strength, with extensibility values in the range of the corresponding
480 monolayers and a reasonable degree of stability throughout time. When also taking the
481 layer adhesion into account, the bilayer formed with the cassava starch with gellan gum
482 and the PLA-PHBV sheet appeared as the best option for food packaging purposes.

483 **Acknowledgements**

484 The authors would like to thank the Ministerio de Ciencia e Innovación of Spain, for
485 funding this study through the Project AGL2016-76699-R and PID2019-105207RB-I00,
486 and the predoctoral research grant # BES-2017-082040.

487 **REFERENCES**

- 488 Arismendi, C., Chillo, S., Conte, A., Del Nobile, M. A., Flores, S., & Gerschenson, L. N.
489 (2013). Optimization of physical properties of xanthan gum/tapioca starch edible
490 matrices containing potassium sorbate and evaluation of its antimicrobial
491 effectiveness. *LWT - Food Science and Technology*, 53(1), 290–296.
492 <https://doi.org/10.1016/j.lwt.2013.01.022>
- 493 Armentano, I., Fortunati, E., Burgos, N., Dominici, F., Luzi, F., Fiori, S., Jiménez, A.,
494 Yoon, K., Ahn, J., Kang, S., & Kenny, J. M. (2015). Bio-based PLA_PHB
495 plasticized blend films: Processing and structural characterization. *LWT - Food
496 Science and Technology*, 64(2), 980–988. <https://doi.org/10.1016/j.lwt.2015.06.032>
- 497 ASTM. (1995). Standard test methods for water vapor transmission of materials. Standard
498 designations: E96-95. In ASTM, *Annual book of ASTM standards* (pp. 406-413).
499 Philadelphia, PA: American Society for Testing and Materials.
- 500 ASTM. (2001). Standard test method for tensile properties of thin plastic sheeting.
501 Standard designations: D882. In *Annual book of ASTM standards* (pp. 162-170).
502 Philadelphia, PA: American Society for Testing and Materials.
- 503 ASTM. (2002). Standard test method for oxygen gas transmission rate through plastic
504 film and sheeting using a coulometric sensor. Standard designations: 3985-95. In
505 *Annual book of ASTM standards* (pp. 472-477). Philadelphia, PA: American
506 Society for Testing and Materials.
- 507 Balaguer, M. P., Gómez-Estaca, J., Gavara, R., & Hernandez-Munoz, P. (2011).
508 Biochemical properties of bioplastics made from wheat gliadins cross-linked with
509 cinnamaldehyde. *Journal of Agricultural and Food Chemistry*, 59(24), 13212–
510 13220. <https://doi.org/10.1021/jf203055s>
- 511 Bonilla, J., Fortunati, E., Vargas, M., Chiralt, A., & Kenny, J. M. (2013). Effects of
512 chitosan on the physicochemical and antimicrobial properties of PLA films. *Journal
513 of Food Engineering*, 119(2), 236–243.
514 <https://doi.org/10.1016/j.jfoodeng.2013.05.026>
- 515 Cano, A., Chafer, M., Chiralt, A., & Gonzalez-Martinez, C. (2017). Strategies to improve
516 the functionality of starch-based films. *Handbook of Composites from Renewable
517 Materials*, 1–8, 311–337. <https://doi.org/10.1002/9781119441632.ch74>

- 518 Cano, Amalia, Jiménez, A., Cháfer, M., González, C., & Chiralt, A. (2014). Effect of
519 amylose:amylopectin ratio and rice bran addition on starch films properties.
520 *Carbohydrate Polymers*, *111*, 543–555.
521 <https://doi.org/10.1016/j.carbpol.2014.04.075>
- 522 Chaiwutthinan, P., Pimpan, V., Chuayjuljit, S., & Leejarkpai, T. (2015). Biodegradable
523 Plastics Prepared from Poly(lactic acid), Poly(butylene succinate) and
524 Microcrystalline Cellulose Extracted from Waste-Cotton Fabric with a Chain
525 Extender. *Journal of Polymers and the Environment*, *23*(1), 114–125.
526 <https://doi.org/10.1007/s10924-014-0689-0>
- 527 Gasmi, S., Hassan, M. K., & Luyt, A. S. (2019). Crystallization and dielectric behavior
528 of PLA and PHBV in PLA/PHBV blends and PLA/PHBV/TIO 2 nanocomposites.
529 *Express Polymer Letters*, *13*(2), 199–212.
530 <https://doi.org/10.3144/expresspolymlett.2019.16>
- 531 Kim, S. R. B., Choi, Y. G., Kim, J. Y., & Lim, S. T. (2015). Improvement of water
532 solubility and humidity stability of tapioca starch film by incorporating various
533 gums. *LWT - Food Science and Technology*, *64*(1), 475–482.
534 <https://doi.org/10.1016/j.lwt.2015.05.009>
- 535 Laycock, B., Halley, P., Pratt, S., Werker, A., & Lant, P. (2013). The chemomechanical
536 properties of microbial polyhydroxyalkanoates. *Progress in Polymer Science*, *38*(3–
537 4), 536–583. <https://doi.org/10.1016/j.progpolymsci.2012.06.003>
- 538 Liu, Q., Wu, C., Zhang, H., & Deng, B. (2015). Blends of polylactide and poly(3-
539 hydroxybutyrate-co-3-hydroxyvalerate) with low content of hydroxyvalerate unit:
540 Morphology, structure, and property. *Journal of Applied Polymer Science*, *132*(42),
541 1–9. <https://doi.org/10.1002/app.42689>
- 542 López, O. V., Zaritzky, N. E., Grossmann, M. V. E., & García, M. A. (2013). Acetylated
543 and native corn starch blend films produced by blown extrusion. *Journal of Food
544 Engineering*, *116*(2), 286–297. <https://doi.org/10.1016/j.jfoodeng.2012.12.032>
- 545 Hugh, T. H., Avena-Bustillos, R., & Krochta, J. M. (1993). Hydrophilic Edible Films:
546 Modified Procedure for Water Vapor Permeability and Explanation of Thickness
547 Effects. *Journal of Food Science*, *58*(4), 899–903. <https://doi.org/10.1111/j.1365-2621.1993.tb09387.x>
- 549 Martin, O., Schwach, E., Avérous, L., & Couturier, Y. (2001). Properties of
550 biodegradable multilayer films based on plasticized wheat starch. *Starch/Staerke*,
551 *53*(8), 372–380. [https://doi.org/10.1002/1521-379X\(200108\)53:8<372::AID-STAR372>3.0.CO;2-F](https://doi.org/10.1002/1521-379X(200108)53:8<372::AID-STAR372>3.0.CO;2-F)
- 553 Muller, J., González-Martínez, C., & Chiralt, A. (2017). Poly(lactic) acid (PLA) and
554 starch bilayer films, containing cinnamaldehyde, obtained by compression
555 moulding. *European Polymer Journal*, *95*(July), 56–70.
556 <https://doi.org/10.1016/j.eurpolymj.2017.07.019>
- 557 Ortega-Toro, R., Bonilla, J., Talens, P., & Chiralt, A. (2017). Future of Starch-Based
558 Materials in Food Packaging. In *Starch-Based Materials in Food Packaging:
559 Processing, Characterization and Applications*. Elsevier Inc.
560 <https://doi.org/10.1016/B978-0-12-809439-6.00009-1>

- 561 Ortega-Toro, R., Jiménez, A., Talens, P., & Chiralt, A. (2014). Properties of starch-
562 hydroxypropyl methylcellulose based films obtained by compression molding.
563 *Carbohydrate Polymers*, 109, 155–165.
564 <https://doi.org/10.1016/j.carbpol.2014.03.059>
- 565 Ortega-Toro, R., Morey, I., Talens, P., & Chiralt, A. (2015). Active bilayer films of
566 thermoplastic starch and polycaprolactone obtained by compression molding.
567 *Carbohydrate Polymers*, 127, 282–290.
568 <https://doi.org/10.1016/j.carbpol.2015.03.080>
- 569
- 570 Rasal, R. M., Janorkar, A. V., & Hirt, D. E. (2010). Poly(lactic acid) modifications.
571 *Progress in Polymer Science (Oxford)*, 35(3), 338–356.
572 <https://doi.org/10.1016/j.progpolymsci.2009.12.003>
- 573 Requena, R., Vargas, M., & Chiralt, A. (2018). Obtaining antimicrobial bilayer starch and
574 polyester-blend films with carvacrol. *Food Hydrocolloids*, 83, 118–133.
575 <https://doi.org/10.1016/j.foodhyd.2018.04.045>
- 576 Samsudin, H., & Hani, N. M. (2017). Use of Starch in Food Packaging. In *Starch-Based*
577 *Materials in Food Packaging: Processing, Characterization and Applications* (Vol.
578 2019). Elsevier Inc. <https://doi.org/10.1016/B978-0-12-809439-6.00008-X>
- 579 Sapper, M., Talens, P., & Chiralt, A. (2019). Improving functional properties of cassava
580 starch-based films by incorporating xanthan, gellan, or pullulan gums. *International*
581 *Journal of Polymer Science*, 2019(6), 1–9. <https://doi.org/10.1155/2019/5367164>
- 582 Savadekar, N. R., & Mhaske, S. T. (2012). Synthesis of nano cellulose fibers and effect
583 on thermoplastics starch based films. *Carbohydrate Polymers*, 89(1), 146–151.
584 <https://doi.org/10.1016/j.carbpol.2012.02.063>
- 585 Sikora, M., Kowalski, S., Piotr Tomasiak, P. (2008). Binary hydrocolloids from starches
586 and xanthan gum. *Food Hydrocolloids*, 22(5), 943–952.
587 <https://doi.org/10.1016/j.foodhyd.2007.05.007>
- 588 Silva-Guzmán, J. A., Anda, R. R., Fuentes-Talavera, F. J., Manríquez-González, R., &
589 Lomeli-Ramírez, M. G. (2018). Properties of Thermoplastic Corn Starch Based
590 Green Composites Reinforced with Barley (*Hordeum vulgare* L.) Straw Particles
591 Obtained by Thermal Compression. *Fibers and Polymers*, 19(9), 1970–1979.
592 <https://doi.org/10.1007/s12221-018-8023-4>
- 593 Slavutsky, A. M., Gamboni, J. E., & Bertuzzi, M. A. (2018). Formulation and
594 characterization of bilayer films based on Brea gum and Pectin. *Brazilian Journal*
595 *of Food Technology*, 21(December). <https://doi.org/10.1590/1981-6723.21317>
- 596 Tampau, A., González-Martínez, C., & Chiralt, A. (2018). Release kinetics and
597 antimicrobial properties of carvacrol encapsulated in electrospun poly-(ε-
598 caprolactone) nanofibres. Application in starch multilayer films. *Food*
599 *Hydrocolloids*, 79, 158–169. <https://doi.org/10.1016/j.foodhyd.2017.12.021>
- 600 Thongpina, C., Tippuwanan, C., Buaksuntear, K., & Chuawittayawuta, T. (2017).
601 Mechanical and thermal properties of PLA melt blended with high molecular weight
602 PEG modified with peroxide and organo-clay. *Key Engineering Materials*, 751, 337-
603 343. <https://doi.10.4028/www.scientific.net/KEM751.337>

604 Vieira, M. G. A., Da Silva, M. A., Dos Santos, L. O., & Beppu, M. M. (2011). Natural-
605 based plasticizers and biopolymer films: A review. *European Polymer Journal*,
606 47(3), 254–263. <https://doi.org/10.1016/j.eurpolymj.2010.12.011>

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

Formulation	Thickness (μm)	Equilibrium Moisture (g water/100 g dried film)	Water Solubility (g/ 100 g dried film)
CS	189 \pm 14 ^(d)	9.1 \pm 0.3 ^(a)	89.5 \pm 0.1 ^(a)
CS-G	191 \pm 18 ^(d)	8.0 \pm 0.4 ^(b)	89.2 \pm 0.2 ^(a)
CS-X	200 \pm 16 ^(c)	7.7 \pm 0.8 ^(b)	89.6 \pm 0.1 ^(ab)
MS	221 \pm 17 ^(a)	8.3 \pm 0.3 ^(b)	89.0 \pm 0.3 ^(a)
MS-G	219 \pm 20 ^(a)	8.1 \pm 0.3 ^(b)	89.6 \pm 0.1 ^(ab)
MS-X	210 \pm 20 ^(b)	8.1 \pm 0.9 ^(b)	89.7 \pm 0.1 ^(ab)
P	130 \pm 0 ^(e)	0.3 \pm 0.1 ^(c)	27.0 \pm 3.0 ^(c)

612 Different superscript letters (a - e) within the same column indicate significant differences among
 613 formulations ($p < 0.05$).

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634 **Table 2.** Tensile properties (Elastic modulus: EM, tensile strength: TS and deformation at break: %E) and
635 barrier properties (water vapour (WVP) and oxygen (OP) permeability) of cassava starch (CS) and maize
636 starch (MS) films containing or not gellan (G) and xanthan (X) gums, and PLA-PHBV blend films (P).
637 Mean values after 1 and 5 storage weeks \pm standard deviation.

	EM		TS		E		WVP		OPx1014	
	(MPa)		(MPa)		(%)		(g·mm·kPa ⁻¹ ·h ⁻¹ ·m ⁻²)		(cm ³ ·m ⁻¹ ·s ⁻¹ ·Pa ⁻¹)	
	Week 1	Week 5	Week 1	Week 5	Week 1	Week 5	Week 1	Week 5	Week 1	Week 5
CS	500 $\pm 70^{(c,2)}$	600 $\pm 70^{(c,1)}$	12.0 $\pm 1^{(bc,2)}$	13.0 $\pm 2^{(d,1)}$	4.0 $\pm 0.4^{(c,1)}$	3.0 $\pm 0.4^{(b,2)}$	13.4 $\pm 1.2^{(a,2)}$	13.5 $\pm 0.7^{(b,1)}$	3.7 $\pm 0.1^{(c,1)}$	3.9 $\pm 0.1^{(d,1)}$
CS-G	600 $\pm 70^{(b,2)}$	900 $\pm 30^{(a,1)}$	18.0 $\pm 2^{(a,2)}$	23.0 $\pm 2^{(a,1)}$	5.0 $\pm 0.2^{(c,1)}$	3.0 $\pm 0.4^{(b,2)}$	11.0 $\pm 1.6^{(b,2)}$	11.0 $\pm 0.7^{(c,1)}$	2.6 $\pm 0.3^{(d,1)}$	2.7 $\pm 0.1^{(e,1)}$
CS-X	700 $\pm 40^{(a,2)}$	700 $\pm 80^{(b,1)}$	14.0 $\pm 4^{(b,2)}$	17.0 $\pm 1^{(b,1)}$	3.0 $\pm 1^{(c,1)}$	3.0 $\pm 0.3^{(b,2)}$	11.4 $\pm 0.9^{(b,2)}$	13.4 $\pm 1.1^{(b,1)}$	2.4 $\pm 0.1^{(d,1)}$	2.6 $\pm 0.3^{(e,1)}$
MS	140 $\pm 60^{(e,2)}$	374 $\pm 20^{(d,1)}$	7.0 $\pm 1^{(e,2)}$	13.0 $\pm 1^{(cd,1)}$	30.0 $\pm 8^{(a,1)}$	13.0 $\pm 5^{(a,2)}$	15.0 $\pm 0.8^{(a,2)}$	19.8 $\pm 1^{(a,1)}$	10.1 $\pm 0.7^{(a,1)}$	11.2 $\pm 0.4^{(a,1)}$
MS-G	360 $\pm 70^{(d,1)}$	600 $\pm 70^{(c,1)}$	9.0 $\pm 1^{(d,2)}$	14.0 $\pm 1^{(c,1)}$	11.0 $\pm 6^{(b,1)}$	4.0 $\pm 0.4^{(b,2)}$	13.4 $\pm 0.8^{(ab,2)}$	12.9 $\pm 2^{(b,1)}$	5.3 $\pm 0.3^{(b,1)}$	5.8 $\pm 0.2^{(b,1)}$
MS-X	500 $\pm 100^{(c,2)}$	660 $\pm 30^{(bc,1)}$	11.0 $\pm 2^{(c,2)}$	15.0 $\pm 1^{(c,1)}$	40.0 $\pm 0.6^{(c,1)}$	3.0 $\pm 0.3^{(b,2)}$	11.8 $\pm 0.6^{(b,2)}$	12.0 $\pm 1.2^{(b,1)}$	4.6 $\pm 0.1^{(b,1)}$	4.9 $\pm 0.1^{(c,1)}$
P	780 $\pm 190^{(a,1)}$	860 $\pm 80^{(a,2)}$	15.0 $\pm 2^{(b,1)}$	16.0 $\pm 1^{(bc,1)}$	2.4 $\pm 0.2^{(d,1)}$	3.0 $\pm 0.2^{(b,1)}$	0.20 $\pm 0.03^{(c,1)}$	0.3 $\pm 0.1^{(c,2)}$	410 $\pm 7^{(c,1)}$	432 $\pm 3^{(e,2)}$

638 Different superscript letters (a-e) within the same column indicate significant differences among
639 formulations ($p < 0.05$). Different superscript numbers (1-2) within the same row for each parameter and
640 sample indicate significant differences between the two storage times ($p < 0.05$).

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653 **Table 3.** Thickness (experimental value and sum of the corresponding monolayer thicknesses submitted to
 654 the same thermocompression process used to obtain bilayers), equilibrium moisture content and water
 655 solubility of bilayer films obtained from cassava starch (CS) and maize starch (MS) sheets, containing or
 656 not gellan (G) and xanthan (X) gums, thermo-compressed with PLA-PHBV blend films (P).

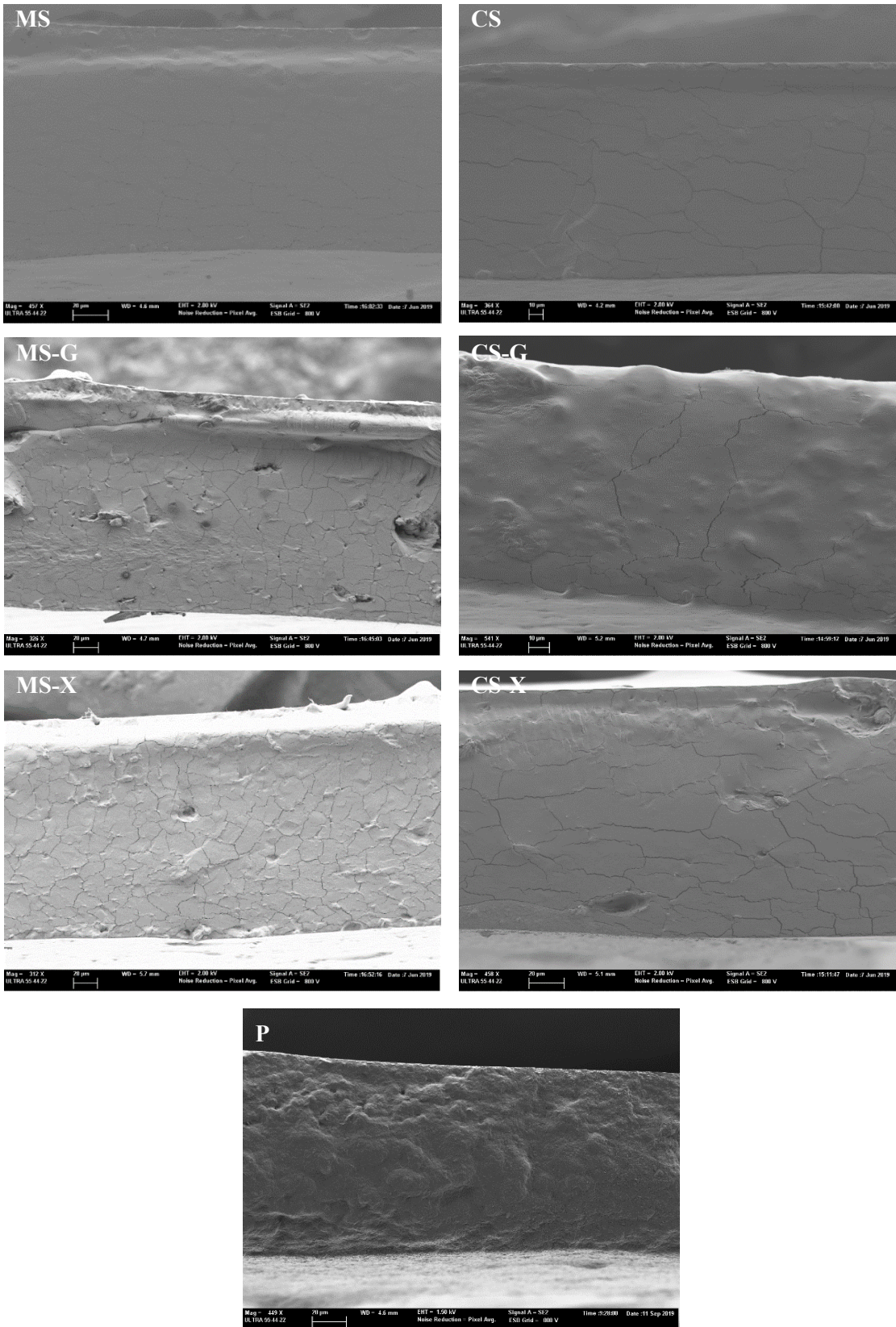
Formulation	Thickness (μm)	Sum of Monolayer Thickness (μm)	Equilibrium Moisture (g water/100 g dried film)	Water Solubility (g/ 100 g dried film)
CS-P	205 \pm 15 ^(ab)	280	8.6 \pm 0.6 ^(a)	74 \pm 10 ^(a)
CS-G-P	230 \pm 20 ^(a)	264	7.7 \pm 0.2 ^(b)	75 \pm 3 ^(a)
CS-X-P	222 \pm 16 ^(a)	296	7.4 \pm 0.7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

657 Different superscript letters (a-b) within the same column indicate significant differences among
 658 formulations ($p < 0.05$).

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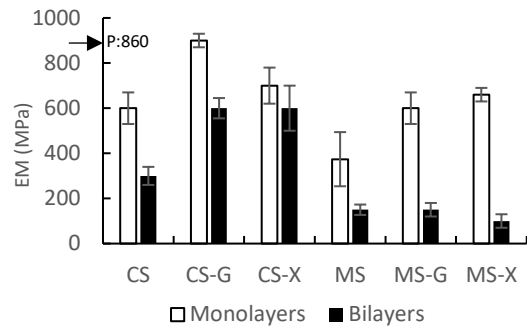
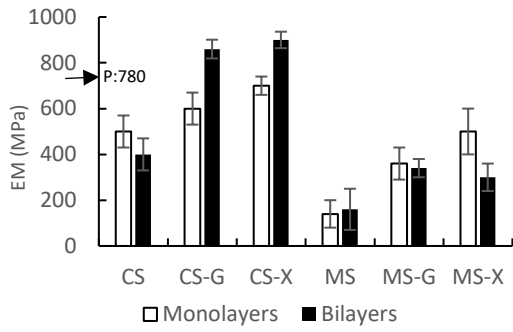
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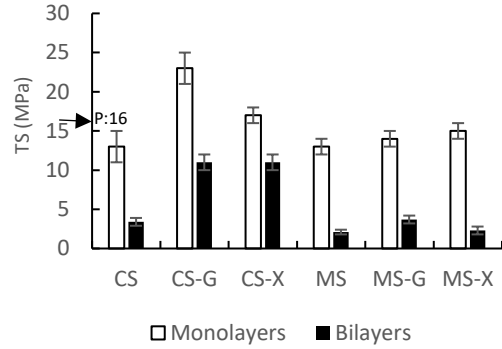
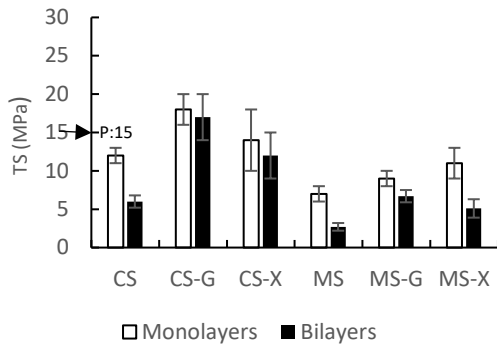


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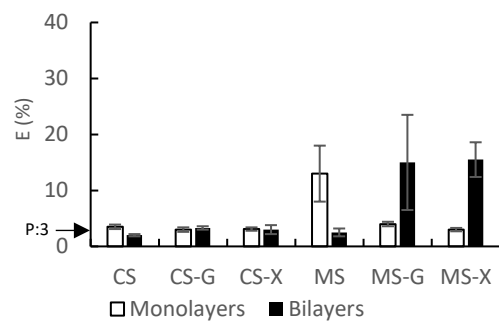
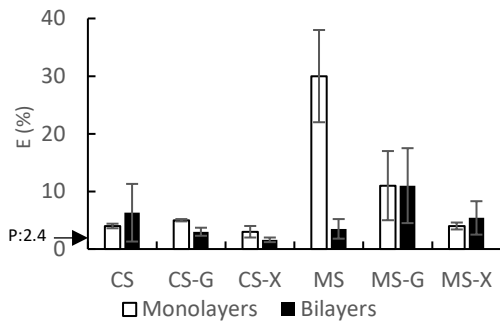
Figure 1. FSEM micrographs of monolayer films (cross section) from maize starch (MS) and Cassava starch (CS) and films containing or not gellan (G) and xanthan (X) gums, and PLA-PHBV blend films (P).



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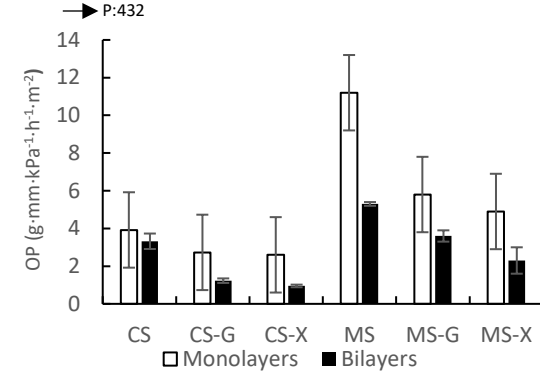
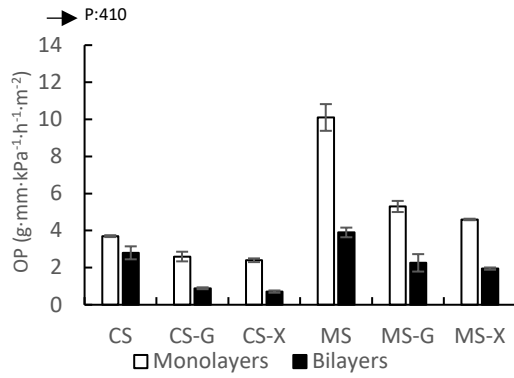
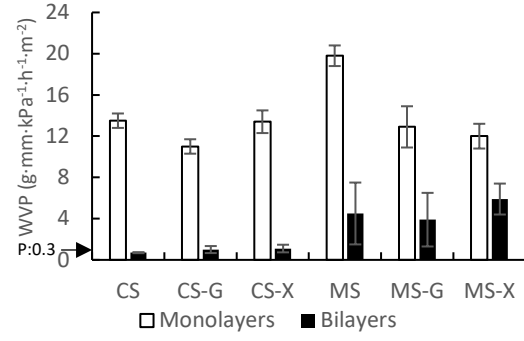
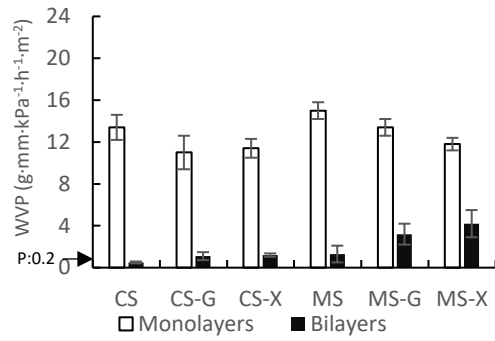
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676 **Figure 3.** Tensile properties (Elastic modulus: EM, tensile strength: TS and deformation at break: %E)
 677 bilayer films (Black bars) of Cassava starch (CS) and maize starch (MS) films containing or not gellan (G)
 678 and xanthan (X) gums, and PLA-PHBV blend films (P), in comparison with the corresponding values of
 679 the respective starch monolayers (white bars). Values after 1 (left) and 5 (right) storage weeks. Arrow
 680 indicates the values for the polyester monolayer: EM (week 1: 780, week 5: 860), TS (week 1: 15, week 5:
 681 16) and E (week 1: 2.4, week 5: 3).

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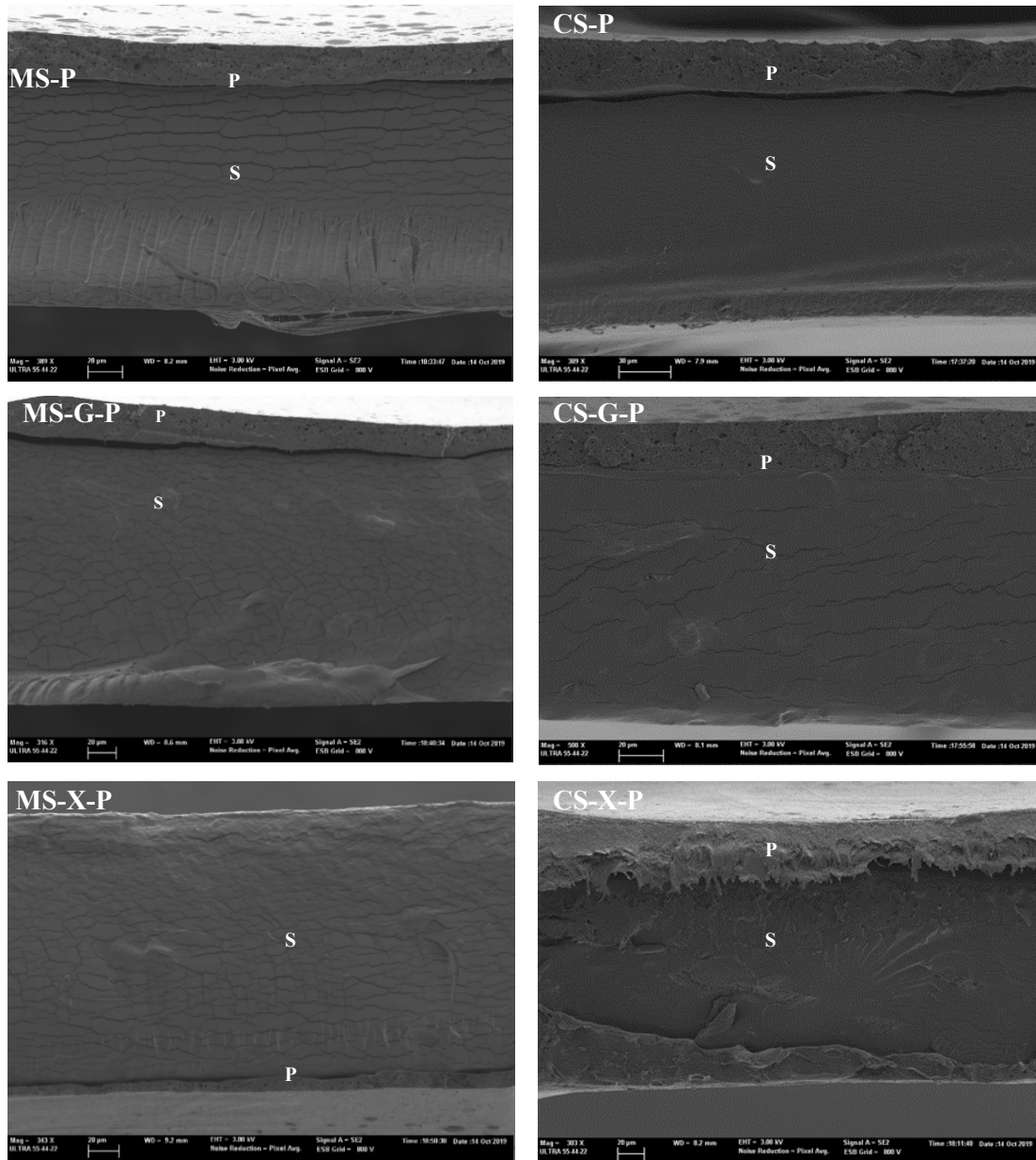


683

685 **Figure 4.** Barrier properties (water vapour: WVP and oxygen (OP) permeability) of bilayer films (Black
 686 bars) of Cassava starch (CS) and maize starch (MS) films containing or not gellan (G) and xanthan (X)
 687 gums, and PLA-PHBV blend films (P), in comparison with the corresponding values of the respective
 688 starch monolayers (white bars). Values after 1(left) and 5 (right) storage weeks. Arrow indicates the values
 689 of the polyester film: WVP (week 1: 0.2, week 5: 0.3), OP (week 1: 410, week 5: 432).

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691



692 **Figure 2.** FSEM micrographs of bilayer films (cross section) obtained by the thermocompression of
 693 Cassava starch (CS) and maize starch (MS) films containing or not gellan (G) and xanthan (X) gums, and
 694 PLA-PHBV blend films (P). Polyester (P) and starch (S) sheets are marked.