

Document downloaded from:

<http://hdl.handle.net/10251/115834>

This paper must be cited as:

Valencia-Sullca, CE.; Atarés Huerta, LM.; Vargas, M.; Chiralt, A. (2018). Physical and Antimicrobial Properties of Compression-Molded Cassava Starch-Chitosan Films for Meat Preservation. *Food and Bioprocess Technology*. 11(7):1339-1349. doi:10.1007/s11947-018-2094-5



The final publication is available at

<http://doi.org/10.1007/s11947-018-2094-5>

Copyright Springer-Verlag

Additional Information

1 **Physical and antimicrobial properties of compression-moulded cassava starch-**
2 **chitosan films for meat preservation.**

3 Cristina Valencia-Sullca, Lorena Atarés, Maria Vargas*, Amparo Chiralt

4 *Instituto Universitario de Ingeniería de Alimentos para el Desarrollo, Universitat

5 Politècnica de València, Spain. *mavarco@tal.upv.es

6 <https://orcid.org/0000-0001-6024-0952>

7

8 **Abstract**

9 Cassava starch-chitosan films were obtained by melt bending and compression
10 moulding, using glycerol and polyethylene glycol as plasticizers. Both the
11 starch:chitosan and the polymer:plasticizer ratios were varied in order to analyse their
12 effect on the physical properties of the films. Additionally, the antimicrobial activity of
13 70:30 polymer:plasticizer films was tested in cold-stored pork meat slices as affected
14 by chitosan content. All film components were thermally stable up to 200 °C, which
15 guaranteed their thermostability during film processing. Starch and chitosan had limited
16 miscibility by melt blending, which resulted in heterogeneous film microstructure.
17 Polyethylene glycol partially crystallized in the films, to a greater extent as the chitosan
18 ratio increased, which limited its plasticizing effect. The films with the highest plasticizer
19 ratio were more permeable to water vapour, less rigid and less resistant to break. The
20 variation in the chitosan content did not have a significant effect on water vapour
21 permeability. As the chitosan proportion increased, the films became less stretchable,
22 more rigid and more resistant to break, with a more saturated yellowish colour. The
23 incorporation of the highest amount of chitosan in the films led to the reduction in
24 coliforms and total aerobic counts of cold-stored pork meat slices, thus extending their
25 shelf-life.

26

27 **Keywords:** thermoplastic starch, microstructure, thermal analysis, mechanical
28 properties, antimicrobial.

30 **1. Introduction**

31 In recent years, increasing interest in biodegradable materials has developed mainly
32 due to concerns over the disposal of conventional synthetic plastic materials derived
33 from petroleum, which take a long time to be degraded (Fang et al., 2005).

34 Starch has been widely used for the preparation of biodegradable films because of its
35 abundance, low cost, renewable nature, biocompatibility and non-toxicity (Carvalho,
36 2008). Native starch can be converted into thermoplastic starch by the disruption of the
37 polymeric chains interactions, under specific conditions of temperature and /or
38 mechanical energy, in the presence of a plasticizer (Tomé et al., 2012). In order to
39 obtain thermoplastic starch, scaling-up processing methods using equipment designed
40 for synthetic polymers is indispensable (Thunwall et al., 2006). For example, extrusion,
41 blowing, injection and thermocompression are viable alternatives due to their energy-
42 efficiency and high productivity (Pelissari et al., 2012). As opposed to casting, these
43 techniques are suitable for film production at industrial scale (Tomé et al., 2012).

44 Starch-based films exhibit low oxygen permeability (Jiménez et al., 2012a). However,
45 they show several disadvantages, which reduce their applicability as packaging
46 material, such as their highly hydrophilic character, limited mechanical properties and
47 the retrogradation phenomena that occur during aging (Ortega-Toro et al., 2015). The
48 use of plasticizers reduces the interchain forces associated to hydrogen bonds,
49 enhancing molecular mobility, which helps to overcome film brittleness, making the film
50 more extensible.

51 Aiming to improving its film-forming performance, starch has been combined with both
52 synthetic and natural polymers. Amongst them, chitosan has led to positive results in
53 films obtained by casting (Chillo et al., 2008; Bonilla et al., 2013) and by thermal
54 processing (López et al., 2014; Mendes et al., 2016). Incorporating chitosan into
55 starch-based films has been reported to reduce their water affinity and improve their
56 mechanical properties, due to the formation of intermolecular hydrogen bonds between

57 the amino and hydroxyl groups of chitosan and the hydroxyl groups of starch (Xu et al.,
58 2005). Moreover, chitosan provided the starch-chitosan blend films obtained by casting
59 with antibacterial properties (Bonilla et al., 2013). However, to the best of our
60 knowledge, the studies on the antimicrobial performance of thermoplastic starch-
61 chitosan films for food preservation are still scarce.

62 The aim of this work was to characterize the microstructural, tensile, barrier and optical
63 properties, as well as the thermal behaviour of starch-chitosan films obtained by
64 compression-moulding as affected by the polymer:plasticizer and the starch:chitosan
65 ratios. The effect of chitosan content on the antimicrobial activity of the films was also
66 evaluated in cold-stored pork-meat slices.

67 .

68 **2. Materials and methods**

69 2.1. Materials

70 Cassava starch (CS) with 9.28 % amylose and an amylose:amylopectin ratio of 1:9.8,
71 was produced by Asia CO, LDT (Kalasin, Thailand) and purchased by Quimidroga SA
72 (Barcelona, Spain). Chitosan (CH) of high molecular weight (practical grade, >75%
73 deacetylation degree, Batch MKBP1333V) and polyethylene glycol 4000 (PEG) were
74 purchased from Sigma-Aldrich (Madrid, Spain). Glycerol (Gly) and $Mg(NO_3)_2$ were
75 provided by Panreac Química, S.A. (Castellar del Vallés, Barcelona, Spain). Pork meat
76 was purchased in a local supermarket and processed at the laboratory. Buffered
77 peptone water, Violet Red Bile Agar and Plate Count Agar were provided by Scharlau
78 (Barcelona, Spain).

79

80 2.2. Film preparation

81 Eight formulations based on CS, CH, with Gly and PEG as plasticizer (P), were
82 prepared. In all cases, plasticizers were mixed in a constant Gly: PEG mass ratio
83 75:25. Two series of formulations were prepared with polymer: plasticizer mass ratios
84 70:30 and 60:40. In each series, CS:CH mass ratios were 100:0, 90:10, 80:20 and

85 70:30. After weighing, CS, CH and plasticizers were dispersed in distilled water. The
86 formulations were melt-blended on a two roll mill (Model LRM-M-100, Labtech
87 Engineering, Thailand) at 160 °C and 10 rpm for 30 minutes until a homogeneous paste
88 was obtained. Before compression moulding, the paste was conditioned at 25 °C and
89 53% relative humidity (RH) using $Mg(NO_3)_2$ oversaturated solutions for 72 h. The films
90 were obtained by compression moulding (Model LP20, Labtech Engineering, Thailand).
91 Four grams of the paste were put onto steel sheets and preheated on the heating unit
92 for 5 min. The films were performed at 160 °C for 2 min at 5 MPa (50 bar), followed by
93 6 min at 12 MPa (120 bar); thereafter, a cooling cycle (40 °C/min) was applied for 3
94 min. The films obtained were conditioned at 25 °C and 53% RH for 1 or 5 weeks before
95 characterization.

96

97 2.3. Characterization of the films

98 2.3.1. Microstructural analysis

99 The microstructural analysis of the cross-sections of the films was carried out by
100 means of scanning electron microscope (JEOL JSM-5410, Japan, model JSM-5410).
101 The film samples were maintained in desiccators with P_2O_5 in order to eliminate film
102 moisture. Film pieces (5x5 mm approximately) were cryofractured and mounted on
103 copper stubs. After gold coating, the samples were observed using an accelerating
104 voltage of 10 kV.

105 2.3.2. Thermogravimetric analysis

106 The thermal stability of the films and their components was analysed using a
107 thermogravimetric analyser (TGA/SDTA 851e, Mettler Toledo, Schwerzenbach,
108 Switzerland), equipped with an ultra-micro weighing scale ($\pm 0.1 \mu g$), under nitrogen
109 flow (50 mL/min). The analysis was carried out during heating from 25 to 600 °C at 10
110 °C /min. Approximately 3 mg of sample were used in each test, considering at least two
111 replicates per formulation. Initial degradation temperature (T_0), i.e. the temperature at

112 which 10% mass loss is registered, and maximum degradation rate temperature
113 (T_{max}) were registered.

114 2.3.3. Differential scanning calorimetry

115 The thermal properties were analysed using a differential scanning calorimeter (DSC 1
116 Star System, Mettler- Toledo, Inc., Switzerland) with a 20 mL/min nitrogen flow. Film
117 samples were desiccated with P_2O_5 and crushed with a mortar. Two samples
118 (approximately 10 mg) per formulation were placed into aluminium pans and sealed.
119 An empty sample pan was taken as a reference. A first heating step was done from 0
120 °C to 160 °C at 50 °C/min to remove any residual water, followed by a cooling step to 0
121 °C at the same speed, and a second heating to 200 °C at 10 °C /min.

122 2.3.4. Moisture content and water vapour permeability

123 The moisture content of film samples previously conditioned at 53% RH was
124 determined by the gravimetric method. Five samples per formulation were considered.
125 Water was eliminated from them using a two-step method: desiccation in a vacuum
126 oven (60 °C - 24 h), and storage in desiccators with P_2O_5 until constant weight was
127 reached. The results were expressed as g of water per 100 g of dry film.

128 The water vapour permeability (WVP) of the film samples was determined by means of
129 the ASTM E96-95 gravimetric method (ASTM, 1995), as described by Ortega-Toro et
130 al. (2014).

131 2.3.5. Mechanical properties

132 A universal test machine (TA-XT plus, Stable Micro Systems, Surrey, United Kingdom)
133 was used to determine the elastic modulus (EM), tensile strength at break (TS) and
134 percentage of elongation at break (%E) of the film samples. These parameters were
135 obtained from stress-Hencky strain curves, following ASTM standard method D882
136 (ASTM, 2001). Ten film stripes (25 mm wide and 100 mm long) per formulation were
137 tested. Film thickness was measured in four positions along the stripe to the nearest
138 0.0025 mm with a hand-held digital micrometer (Electronic Digital Micrometer,
139 Comecta S.A., Barcelona, Spain). Equilibrated samples were mounted in the film-

140 extension grips of the testing machine and stretched at 50 mm min^{-1} until breaking.
141 The relative humidity of the environment was held constant, at approximately 53%
142 during the tests, which were performed at $25 \text{ }^\circ\text{C}$.

143 2.3.6. Optical properties

144 The surface reflectance spectra of the films were obtained from 400 to 700 nm with
145 both a white and a black background by using a spectrophotometer (CM-3600d,
146 Minolta CO., Tokyo, Japan). The Kubelka-Munk theory for multiple scattering was
147 applied to the reflection spectra to determine internal transmittance (T_i) (Hutchings,
148 1999). Lightness (L^*), chroma (C^*_{ab}) and hue (h^*_{ab}) values of the films were obtained
149 from the surface reflectance spectra, taking into account illuminant D65 and observer
150 10° . Whiteness index (WI) was calculated according to Atarés et al. (2010). The gloss
151 was determined at a 60° incidence angle by means of a flat surface gloss meter (Multi
152 Gloss 268, Minolta, Germany), following the ASTM standard D523 method (ASTM,
153 1999). The measurements of each sample were taken in triplicate and four films were
154 measured per formulation. The results were expressed as gloss units (GU), relative to
155 a highly polished surface of black glass standard with a value near to 100 GU.

156 2.3.7. Antimicrobial properties

157 Films were tested for their antibacterial properties using sliced pork meat and following
158 a methodology adapted from Bonilla et al. (2014). Each test sample was obtained by
159 placing 10 g of meat in a petri dish (5 cm diameter), and coating with the films. Non-
160 coated samples (control) and samples coated were stored in duplicate at $10 \text{ }^\circ\text{C}$ for 7
161 days. To perform the microbiological analyses, each sample was homogenized in a
162 Stomacher (Bag Mixer 400, Interscience) with 90 mL of sterile buffered peptone water
163 for 2 min. Then, serial dilutions were made and plated out. Total viable and coliform
164 microorganism counts were determined at 0, 1, 4 and 7 days. Total aerobic counts
165 were determined in Plate Count Agar incubated at $37 \text{ }^\circ\text{C}$ for 48 h, while coliforms were
166 determined in Violet Red Bile Agar incubated at $37 \text{ }^\circ\text{C}$ for 48 h. All tests were made in
167 triplicate.

168

169 2.4 Statistical analysis

170 Results were statistically analysed through the analysis of variance (ANOVA) with a
171 95% significance level. The analyses were performed by using Statgraphics
172 Centurion® version XVI. II. Multiple comparisons were performed by means of 95%
173 Fisher's least significant difference (LSD) intervals.

174

175 **3. Results and discussion**

176 3.1. Microstructural analysis

177 Figure 1 shows the micrographs of the cross-section of the films after a 1-week storage
178 period at 25 °C and 53% RH. Films showed a quite homogenous structure where no
179 starch granules were observed, which indicates that starch was gelatinized during the
180 roller mill step. Nevertheless, all samples exhibited micro-cracks, which reflects the
181 sample fragility after desiccation with P₂O₅. The increase in the plasticizer content did
182 not change this behaviour.

183 Incorporation of chitosan into the film matrix was not homogenous due to the lack of
184 miscibility of polymers by melt blending. Although a homogeneous matrix was obtained
185 when starch-chitosan blend films were obtained by casting the aqueous solutions of
186 both macromolecules at different ratios (Bonilla et al., 2013), no total compatibility of
187 polymers was obtained in dry conditions. This may be due to difficulties in the melt
188 blending, where the much higher viscosity of the medium and the difficulties for chain
189 extension inhibit the establishment of adequate interactions between the polymer
190 segments. Therefore, some flakes with different sizes of chitosan in the starch matrix
191 can be observed in samples regardless the ratio of chitosan. These were observed in
192 different zones of the films with a random distribution. Even at the lowest chitosan ratio,
193 immiscibility of melt polymers was evident.

194

195 3.2 Thermogravimetric analysis

196 The mass loss curves of the films showed three separate steps (Figure 2). The first
197 step corresponded to the initial weight loss attributed to the loss of water, being the
198 mass reduction (about 10-15%) coherent with the water content of the films. The
199 second step at around 200 °C was attributed to the degradation of glycerol (Dou,
200 Dupont, Williams, Chen and Ding, 2009). The third highest mass loss step appeared at
201 about 320 °C and corresponded to the degradation of the polymers.

202 Table 1 shows the values of the initial degradation temperature (T_0) and maximum
203 degradation rate temperature (T_{max}) of the films components and films, as well as the
204 percentage mass loss of all samples at the end of the TGA test (600°C). The T_{max}
205 values of the pure compounds (native CS, CH, Gly and PEG) were 321°C, 302°C,
206 251°C and 407°C, respectively. Similar results have been reported previously (Dou et
207 al., 2009; Pelissari et al., 2009). Therefore, the T_0 values of all film components were
208 higher than 200 °C, which guarantees their stability in the elaboration of the films by
209 compression moulding.

210 The addition of chitosan up to the mass ratio 70:30 (CS:CH) did not affect the thermal
211 stability of the films. Both pure polymers showed similar degradation pattern and their
212 blend degradation were mainly affected by the presence of plasticizers. Degradation
213 process in the blend films was more extended, starting at lower temperature due to the
214 early glycerol degradation. Pelissari et al. (2009) also reported similar T_{max} values for
215 cassava starch-chitosan films produced by extrusion. The reported values of T_0 and
216 T_{max} of the starch films, regardless the plasticizer proportion, were similar to those
217 reported for corn starch and cassava starch films obtained by compression moulding
218 and extrusion (Ortega-Toro et al., 2014; Dang, & Yoksan, 2015). Additionally, it was
219 observed that, as chitosan proportion was increased in the film formulation, the
220 percentage mass loss at 600 °C was reduced, in agreement with the greater mass
221 residue of CH. This is coherent with previous studies on cassava starch-chitosan films
222 (Dang & Yoksan, 2015).

223

224 3.3. Differential scanning calorimetry

225 Table 2 shows the thermal properties (T_m , ΔH_m and % crystallinity of PEG) of the
226 studied films conditioned for 1 week at 25 °C and 53% RH, along with those of pure
227 PEG. No glass transition or melting of polymers were observed in the temperature
228 range analysed, but both in the first and the second heating steps, a melting endotherm
229 attributable to PEG was observed at the temperatures (T_m) reported in Table 2, with the
230 corresponding ΔH_m values. Pure PEG sample shows an endothermic peak at 62.5 °C,
231 with a melting enthalpy of 194 J/g. Similar results were obtained by Song, Xue, He, Liu,
232 & Xiao (2008). Over the first heating step of the film samples, this endotherm was
233 observed at slightly lower temperatures, the T_m values ranging between 56 °C and 61
234 °C. T_m reduction was also observed over the second heating step when the thermal
235 history of the sample had been deleted. This reduction in the melting temperature, with
236 respect to that of pure PEG, suggests that some film components are partially miscible
237 with PEG, depressing its melting point, coherently with previous reports (Song et al.,
238 2008). In a compatible blend, the melting temperature of one component is often
239 reduced owing to the increasing of lattice defects resulted from the partial miscibility of
240 the noncrystalline phase. The crystallization degree of PEG in the films was estimated
241 from the enthalpy values of pure compound assuming complete crystallization in this
242 case. In general, crystallization degree increased when the CH ratio rose in the film,
243 which could be related with the interactions between these components. Nevertheless,
244 sample with the highest CH ratio and 40% plasticizers exhibited the lowest
245 crystallization degree. Higher plasticization promoted molecular mobility and so the
246 polymer crystallization. The PEG crystallization in the films limited its plasticizing effect
247 since the crystallized fraction was in a separated phase without interactions with the
248 polymer chains. Then, the reduction of PEG ratio in the films should be considered.

249

250 3.4. Thickness, moisture content and water vapour permeability

251 Table 3 shows the values of thickness along with water vapour permeability (WVP) and
252 moisture content data for 1 and 5-week storage period at 53% RH-25 °C.

253 As can be observed, the film thickness increased when CH was incorporated into the
254 film (from 170 µm to 260 µm). Similar results were reported by Pelissari et al. (2009)
255 and López et al. (2014) studying starch and chitosan films obtained by extrusion. As
256 observed in Figure 1, the partial incompatibility of both polymers led to more
257 heterogeneous structures while blend films were also thicker. The increase in the film
258 thickness, associated with the rise in CH ratio, may be attributed to the lower
259 flowability of the chitosan melt (with higher viscosity) as compared to the starch melt,
260 which limits the blend extension during compression moulding.

261 The moisture content values ranged between 10 and 15 g water / 100 g dry film. It was
262 significantly increased after 5 storage weeks ($p < 0.05$), indicating that films equilibrate
263 slowly with the ambient relative humidity, even though storage time did not affect WVP
264 significantly. Similar results have been reported previously by Ortega-Toro et al. (2014)
265 for CS-HPMC films obtained by compression moulding, and by Jiménez et al. (2012b)
266 in CS- fatty acids films obtained by casting. In these studies, storing the films for 5
267 weeks resulted in a significant increase ($p < 0.05$) in the film moisture content with no
268 effect on the WVP values, regardless of the film composition. Very small differences in
269 the moisture content of the films were observed for the different CS:CH ratios, although
270 the increase in the plasticizer content promoted moisture uptake.

271 The polymer: plasticizer ratio had a significant effect ($P^* < 0.05$) on both the moisture
272 content and WVP values. Regardless the storage time, as the plasticizer proportion
273 was increased, both properties rose. Previous works have reported this effect of
274 plasticizer on the moisture content on the films (Mali et al., 2006) and WVP (Alves et
275 al., 2007; Chillo et al., 2008). The addition of plasticizer modifies the molecular
276 organization making the structure less compact and therefore more permeable. An
277 increase in inter-chain spacing due to inclusion of plasticizer molecules may promote
278 water vapour diffusivity through the film, hence enabling water vapour transmission

279 (Yang & Paulson, 2000). Contrarily, the CS:CH mass ratio did not have a significant
280 effect on WVP.

281

282 3.5. Mechanical properties

283 Table 4 shows these parameters for each film formulation after 1 and 5 weeks of
284 storage at 25 °C and 53% RH, the corresponding stress-strain curves of the films are
285 shown in Figure 3.

286 The polymer: plasticizer ratio had a significant effect on both EM and TS (*P <0.05),
287 whereas no effect on %E was found. After one week or five weeks of storage, the films
288 with higher plasticizer ratio (60:40) were significantly less stiff and less resistant to
289 break (lower EM and TS) than those with a lower ratio (70:30). This is in accordance
290 with previous reports. Da Róz et al. (2006) observed that the quantity and type of
291 plasticizer influenced the mechanical properties of thermoplastic starch obtained by
292 melt processing, and a softening effect caused by the plasticizing of the amorphous
293 phase was observed. Mali et al. (2006) observed a significant reduction of EM and TS
294 as the glycerol ratio increased in compression moulded cassava starch films. The
295 molecular size, configuration and total number of functional hydroxide groups of the
296 plasticizer as well as its compatibility with the polymer could affect the interactions
297 between the plasticizer and the polymer (Yang & Paulson, 2000). Being a small
298 molecule, glycerol can get into the polymer chains and weaken the interactions
299 between them (Su et al., 2010).

300 The CS:CH ratio also affected the mechanical properties significantly, and as the CH
301 ratio was increased, the films became stiffer, more resistant and less stretchable (*P
302 <0.05), regardless of the storage time (Table 4). In fact, no plastic deformation was
303 observed for CH containing films (Figure 3).

304 The improvement of the tensile parameters in starch films caused by chitosan addition
305 had previously been observed both in films obtained by casting (Chillo et al., 2008;
306 Bonilla et al., 2013) and by extrusion (Bourtoom & Chinnan, 2008; Pelissari et al.,

2012; López et al., 2014). This effect could be attributed to the intermolecular hydrogen bonding between starch -OH groups with chitosan – NH₂ groups (López et al., 2014). As the chitosan proportion increased in the film formulation, more -NH₂ groups are available to form hydrogen bonds with starch, hence improving the film resistance (Pelissari et al., 2012). The stretchability reduction in starch blend films caused by chitosan addition has been previously reported by several authors (Xu et al., 2005; Bourtoom & Chinnan, 2008; Pelissari et al., 2012; López et al., 2014). Pelissari et al. (2012) justified this reduction by pointing out the potential increase of the starch crystallinity caused by chitosan, although no starch melting was observed in the studied formulations at the usual temperature (150-200 °C) according to López et al (2014). The mixture effect increasing the structural heterogeneity could also result in some film shortening and an additional effect of the reduced plasticizing efficiency of the PEG, due to its greater crystallization in CH containing films, could also be pointed out. Storage time did not modify the tensile parameters following a clear pattern. In fact, the effect of plasticizer content and CH was more coherent at 5 storage weeks probably by the complete film equilibration, which avoided variability associated to differences in the moisture content. The small changes observed in tensile behaviour must be attributed to the moisture content equilibration more than to relevant changes in the polymer matrix during storage.

326

327 3.6. Optical properties: transparency, colour and gloss

328 Figure 4 shows typical internal transmittance (T_i) spectra of films, from 400 to 700 nm, 329 as a transparency indicator. Higher values of T_i are related with higher structural 330 homogeneity and more transparent films (Villalobos et al., 2005). Of the films tested, 331 those with no CH were the most transparent, regardless the plasticizer ratio and the 332 storage time (Figure 4). Cassava starch has previously shown high transparency in a 333 comparative study reported by Cano et al. (2014). On the other hand, the incorporation 334 of CH brought about a decrease in T_i , closely related to the CH proportion

335 incorporated. This is in accordance to the increased heterogeneity of the films caused
336 by CH incorporation, as previously described. The heterogeneous structure implies the
337 occurrence of changes in the refractive index and higher light dispersion resulting in
338 transparency loss, which is especially remarkable at low wavelength. This transparency
339 reduction of starch films caused by chitosan addition was previously observed
340 (Pelissari et al., 2012; López et al., 2014; Dang & Yoksan, 2015). The selective
341 reduction of T_i at low wavelengths was coherent with the above-mentioned yellowness
342 development in the films during thermoprocessing.

343 Lightness, chroma, hue, whiteness index and gloss values of the films after 1 and 5
344 weeks storage at 25 °C and 53% RH are shown in Table 5. Regardless of the
345 plasticizer proportion and the storage time, the increase in the chitosan content
346 resulted in a decrease in lightness and hue values and a significant increase in terms
347 of chroma values (*P <0.05). In fact, the films with higher CH proportion showed a
348 more saturated yellowish colour (lower whiteness index). Similar results were obtained
349 when CH was incorporated in extruded cassava starch films (Dang & Yoksan, 2015;
350 Khanh & Rangrong, 2015) or in corn starch films prepared by thermo-compression
351 (López et al., 2014). This colour change was attributed to the occurrence of Maillard
352 reactions between amino groups of chitosan and the carbonyl groups of the starch
353 fraction (López et al., 2014). This reaction is promoted by the thermoprocessing
354 temperature and, in the early stage, involves the formation of conjugates between the
355 carbonyl and amine groups of chitosan, producing Schiff bases, Amadori compounds
356 and insoluble polymeric compounds (melanoidins). In this sense, it is noteworthy that
357 films prepared with 40% plasticizer exhibited less browning just after processing, but
358 the Maillard reaction progressed during film storage.

359 In this study, all films showed low gloss values at both 1 and 5 storage weeks. This is
360 in accordance with previous results on starch-HPMC thermo-compressed films
361 (Ortega-Toro et al., 2014). The limited compatibility of both polymers, as observed in

362 section 3.1., may be responsible for the films surface roughness and the consequent
363 reduced gloss.

364

365 3.7. Antimicrobial properties

366 Table 6 shows the progress over storage time (up to 7 days) of the total aerobic and
367 coliform counts of control sample (sliced pork meat without film) and samples coated
368 with the four films with the proportion of polymer: plasticizer (70:30), since they show
369 better tensile and barrier properties. In all cases, chitosan proportion in the films had an
370 effect on the progression of the counts and the final values. Cassava starch films with
371 no chitosan had no antimicrobial effect, and the bacterial growth in this case was even
372 slightly higher than that found for control samples (*P <0.05). After 7 days of storage,
373 the lowest populations corresponded to the films with the highest chitosan proportion
374 (*P <0.05), although only a 1 Log reduction was achieved. The compression moulding
375 method that was used for film preparation could lead to strong interactions between the
376 hydroxyl groups in starch and amino groups in chitosan, thus affecting the diffusion
377 phenomenon of chitosan from the film matrix into the food system (López et al., 2014).
378 Likewise, Pelissari et al. (2009) reported that chitosan incorporation into cassava starch
379 films produced by extrusion did not result in antimicrobial action. The significant
380 antimicrobial activity of chitosan observed by Bonilla et al. (2013) in previous studies
381 carried out with starch-chitosan films produced by casting method can be explained by
382 the previous solution of chitosan in acetic acid, which protonates the amino groups of
383 chitosan and enhances its solubility. Whenever chitosan dissolution is unnecessary
384 (i.e. extrusion and compression moulding method) the amino groups are not
385 protonated, which could explain the observed reduced antimicrobial activity of the
386 obtained films. Likewise the loss of free amine groups during thermal processing could
387 also contribute to reduce the antibacterial action of chitosan. Nevertheless, despite this
388 reduction, films containing 70:30 starch:chitosan ratio extended the shelf-life of meat by

389 3 days, as compared to the control samples, and considering the commission limits on
390 aerobic colony counts for meat (Commission Regulation 2073/2005).

391

392 **4. Conclusions**

393 The physical properties of thermoplastic cassava starch films were significantly
394 affected by the incorporation of chitosan and the polymer:plasticizer proportion. Starch
395 and chitosan exhibited lack of miscibility by melt blending. Chitosan incorporation
396 induced Maillard reaction that occurred during the films thermoprocessing, causing
397 films yellowing. As the chitosan ratio increased, the films became stiffer and more
398 resistant to break but less stretchable. Polyethylene glycol was crystallized in the films,
399 which limited its plasticizing effect, being the films with the highest plasticizer ratio the
400 ones that showed the less convenient barrier and mechanical properties for food
401 packaging purposes. The addition of the highest amount of chitosan yielded
402 antimicrobial films that extended the shelf-life of cold-stored pork meat slices. Taking
403 into account the barrier and tensile properties of the films and their antibacterial activity,
404 films with 70:30 starch chitosan ratio and 30 % total plasticiser could be recommended
405 to extend the shelf-life of pork meat.

406

407 **Acknowledgements**

408 The authors acknowledge the financial support provided by the Spanish Ministerio de
409 Economía y Competividad (Projects AGL2013-42989-R and AGL2016-76699-R).
410 Author Cristina Valencia-Sullca thanks the Peruvian Grant National Program
411 (PRONABEC Grant).

412

413 **References**

414 Alves V.D., Mali S., Beleia A., & Grossmann M.V. (2007). Effect of glycerol and
415 amylose enrichment on cassava starch film properties. *Journal of Food*
416 *Engineering*, 78, 941-946.

417 ASTM (1995). Standard test methods for water vapour transmission of materials. In
418 Standards designations: E96-95. Annual book of ASTM standards (pp. 406-413).
419 Philadelphia, PA: American Society for Testing and Materials.

420 ASTM (1999). Standard test method for specular gloss. In Designation (D523). Annual
421 book of ASTM standards, Vol. 06.01. Philadelphia, PA: American Society for
422 Testing and Materials.

423 ASTM (2001). Standard test method for tensile properties of thin plastic sheeting. In
424 Standard D882 Annual book of American standard testing methods. Philadelphia,
425 PA: American Society for Testing and Materials.

426 Atarés, L., Bonilla, J., & Chiralt, A. (2010). Characterization of sodium caseinate-based
427 edible films incorporated with cinnamon or ginger essential oils. *Journal of Food*
428 *Engineering*, 100, 678–687.

429 Bonilla, J., Atarés, L., Vargas, M., & Chiralt, A. (2013). Properties of wheat starch film-
430 forming dispersions and films as affected by chitosan addition. *Journal of Food*
431 *Engineering*, 114, 303 – 312.

432 Bonilla, J., Fortunati, E., Atarés, L., Chiralt A., & Kenny, J. (2014). Physical, structural
433 and antimicrobial properties of poly vinyl alcohol-chitosan biodegradable films.
434 *Food Hydrocolloids*, 35, 463-470.

435 Bourtoom, T., & Chinnan, M.S. (2008). Preparation and properties of rice starch–
436 chitosan blend biodegradable film. *LWT-Food Science and Technology*, 41,
437 1633–1641.

438 Cano, A., Jiménez, A., Cháfer, M., González-Martínez, C., & Chiralt, A. (2014). Effect
439 of amylose:amylopectin ratio and rice bran addition on starch films properties.
440 *Carbohydrate Polymers*, 111 (0), 543–555.

441 Carvalho, A.J.F. (2008). Starch: Major sources, properties and applications as
442 thermoplastic materials. In: *Monomers, Polymers and Composites from*
443 *Renewable Resources*, M.N. Belgacem, and A. Gandini (eds.), Elsevier,
444 Amsterdam.

445 Chillo, S., Flores, S., Mastromatteo, M., Conte, A., Gerschenson, L., & Del Nobile, M.A.
446 (2008). Influence of glycerol and chitosan on tapioca starch-based edible film
447 properties. *Journal of Food Engineering*, 88, 159–168.

448 Commission Regulation, 2005 (EC) No 2073/2005 of 15 November 2005 on
449 microbiological criteria for foodstuffs. In Official Journal of the European Union pp
450 338/1- 338/26.

451 Da Róz, A., Carvalho, A., Gandini, A., & Curvelo, A. (2006). The effect of plasticizers
452 on thermoplastic starch compositions obtained by melt processing. *Carbohydrate*
453 *Polymers*, 63, 417–424.

454 Dang, K., & Yoksan, R. (2015). Development of thermoplastic starch blown film by
455 incorporating plasticized chitosan. *Carbohydrate Polymers*, 115, 575–581.

456 Dou, B., Dupont, V., Williams, P. T., Chen, H., & Ding, Y. (2009). Thermogravimetric
457 kinetics of crude glycerol. *Bioresource technology*, 100(9), 2613-2620.

458 Fang, J., Fawler, P., Eserig, C., González, R., Costa, J., & Chamudis, L. (2005).
459 Development of biodegradable laminate films derived from naturally occurring
460 carbohydrate polymers. *Carbohydrate Polymers*, 60, 39-42.

461 Hutchings, J. B. (1999). Food color and appearance (2nd Ed.). Gaithersburg, Maryland,
462 USA: Aspen Publishers, Inc.

463 Jiménez, A., Fabra, M. J., Talens, P., & Chiralt, A. (2012a). Edible and biodegradable
464 starch films: A review. *Food Bioprocessing Technology*, 5, 2058–2076.

465 Jiménez, A., Fabra, M. J., Talens, P., & Chiralt, A. (2012b). Effect of re-crystallization
466 on tensile, optical and water vapour barrier properties of corn starch films
467 containing fatty acids. *Food Hydrocolloids*, 26(1), 302–310.

468 Khanh, M., & Rangrong, Y. (2015). Development of thermoplastic starch blown film by
469 incorporating plasticized chitosan. *Carbohydrate Polymers*, 115, 575–581.

470 López, O., Garcia, A., Villar, M., Gentili, A., Rodriguez, M., & Albertengo, L. (2014).
471 Thermo-compression of biodegradable thermoplastic corn starch films containing
472 chitin and chitosan. *LWT - Food Science and Technology*, 57, 106 106 -1515.

473 Mali, S., Grossmann, M. V. E., García, M. A., Martino, M. N., & Zaritsky, N. E. (2006).
474 Effects of controlled storage on thermal, mechanical and barrier properties of
475 plasticized films from different starch sources. *Journal of Food Engineering*, 75,
476 453 - 460.

477 Mendes, J.F., Paschoalin, R.T., Carmona, V.B., Sena Neto, Alfredo R A., Marques,
478 C.P., Marconcini, J.M., Mattoso, L.H.C., Medeiros, E.S., Oliveira, J.E. (2016).
479 Biodegradable polymer blends based on corn starch and thermoplastic chitosan
480 processed by extrusion. *Carbohydrate Polymers*, 137, 452-458.

481 Ortega-Toro, R., Jiménez, A., Talens, P. & Chiralt, A. (2014). Properties of starch–
482 hydroxypropyl methylcellulose based films obtained by compression molding.
483 *Carbohydrate Polymers*, 109, 155–165.

484 Ortega-Toro, R., Morey, I., Talens, P. & Chiralt, A. (2015). Active bilayer films of
485 thermoplastic starch and polycaprolactone obtained by compression molding.
486 *Carbohydrate Polymers*, 127, 282–290.

487 Pelissari F., Grossmann M., Yamashita F., & Pineda E. (2009). Antimicrobial,
488 Mechanical and Barrier Properties of cassava starch-chitosan films incorporated
489 with oregano essential oil. *Journal of Agricultural and Food Chemistry*, 57, 7499-
490 7504.

491 Pelissari, F. M., Yamashita, F., García, M. A., Martino, M. N., Zaritzky, N. E., &
492 Grossmann, M. V. E. (2012). Constrained mixture design applied to the
493 development of cassava starch-chitosan blown films. *Journal of Food*
494 *Engineering*, 108, 262 - 267.

495 Song, R., Xue, R., He, L. H., Liu, Y., & Xiao, Q. L. (2008). The structure and properties
496 of chitosan/polyethylene glycol/silica ternary hybrid organic-inorganic films.
497 *Chinese Journal of Polymer Science*, 26(05), 621-630.v

498 Su J.F., Huang Z., Yuan X.Y., Wang X.Y., & Lim, M. (2010). Structure and properties of
499 carboxymethyl cellulose/soy protein isolate blend edible films crosslinked by
500 Maillard reactions. *Carbohydrate Polymers*, 79(1), 145-153.

501 Thunwall, M., Boldizar, A., & Rigdahl, M. (2006). Compression molding and tensile
502 properties of thermoplastic potato starch materials. *Biomacromolecules*, 7, 981 -
503 986.

504 Tomé, L., Fernandes, S., Sadocco, P., Causio, J., Silvertre, A., Neto, P., & Freire, C.
505 (2012). Antibacterial thermoplastic starch- chitosan based materials prepared by
506 melt-mixing. *BioResources*, 7 (3), 3398 – 3409.

507 Villalobos, R., Chanona, J., Hernández, P., Gutiérrez, G., & Chiralt, A. (2005). Gloss
508 and transparency of hydroxypropyl methylcellulose films containing surfactants
509 as affected by their microstructure. *Food Hydrocolloids*, 19(53 - 61).

510 Xu, Y.X., Kim, K.M., Hanna, M.A., & Nag, D. (2005). Chitosan–starch composite film:
511 preparation and characterization. *Industrial Crops and Products*, 21, 185–192.

512 Yang, L., & Paulson, A. T. (2000). Mechanical and water vapour barrier properties of
513 edible gellan. *Food Research International*, 33, 563-570.

514

515 **Table 1.** Thermal properties of the films analysed by TGA (T_0 , T_{max} , % Mass loss over
 516 degradation). Mean values and standard deviation in brackets.

Samples	T_0 (°C)	T_{max} (°C)	% Mass loss
Native CS	294.5 (0.4)	321.3 (0.4)	90.5 (0.3)
CH	278.9 (0.1)	302.5 (0.5)	76.1 (0.1)
Gly	220.7 (0.3)	250.8 (0.4)	96.9 (0.1)
PEG	372.8 (0.3)	406.9 (0.1)	98.3 (0.1)
(CS₁₀₀-CH₀)₇₀-P₃₀	299.5 (0.3) ^e	319.3 (0.5) ^{ab}	92.4 (0.1) ^f
(CS₉₀-CH₁₀)₇₀-P₃₀	291.1 (1.4) ^{cd}	318.8 (0.1) ^{ab}	84.2 (0.1) ^{cd}
(CS₈₀-CH₂₀)₇₀-P₃₀	281.0 (4.0) ^b	319.8 (0.6) ^{bc}	82.2 (0.2) ^a
(CS₇₀-CH₃₀)₇₀-P₃₀	268.7 (1.4) ^a	319.9 (0.1) ^{bc}	81.7 (0.6) ^a
(CS₁₀₀-CH₀)₆₀-P₄₀	296.2 (1.4) ^{de}	318.1 (1.5) ^a	86.6 (0.3) ^e
(CS₉₀-CH₁₀)₆₀-P₄₀	273.0 (6.0) ^a	319.1 (0.7) ^{ab}	83.3 (0.5) ^b
(CS₈₀-CH₂₀)₆₀-P₄₀	286.3 (0.5) ^{bc}	322.9 (0.1) ^d	84.8 (0.1) ^d
(CS₇₀-CH₃₀)₆₀-P₄₀	293.1 (1.2) ^{de}	321.1 (0.7) ^c	83.6 (0.2) ^{bc}

517 Different superscripts (a-f) within the same column indicate significant differences among films (*P < 0.05).

518

519 **Table 2.** Thermal properties of films analysed by DSC. Mean values and standard
 520 deviation in brackets.

Samples	T_m (°C)		ΔH_m (J/g PEG)		% Crystallinity* (PEG)	
	1 st heating	2 nd heating	1 st heating	2 nd heating	1 st heating	2 nd heating
(CS ₁₀₀ -CH ₀) ₇₀ -P ₃₀	56.4 (0.6) ^{ax}	50.4 (1.1) ^{bcy}	68.6 (0.4) ^{fx}	28.9 (0.9) ^{ey}	35.4 (0.7) ^{bx}	8.8 (0.2) ^{by}
(CS ₉₀ -CH ₁₀) ₇₀ -P ₃₀	58.1 (0.6) ^{bcx}	52.4 (0.3) ^{dy}	73.4 (0.9) ^{ex}	47.3 (0.5) ^{cy}	37.9 (0.6) ^{cx}	24.9 (0.0) ^{gy}
(CS ₈₀ -CH ₂₀) ₇₀ -P ₃₀	56.8 (0.0) ^{abx}	50.8 (0.2) ^{bcy}	81.2 (1.4) ^{dx}	38.9 (1.4) ^{dy}	42.0 (0.7) ^{dx}	20.1 (0.4) ^{ey}
(CS ₇₀ -CH ₃₀) ₇₀ -P ₃₀	58.6 (1.2) ^{cx}	51.0 (0.7) ^{cdy}	104.0 (4.0) ^{bx}	57.0 (2.0) ^{by}	53.4 (0.5) ^{fx}	29.7 (0.5) ^{hy}
(CS ₁₀₀ -CH ₀) ₆₀ -P ₄₀	58.5 (0.0) ^{cx}	48.8 (0.4) ^{ay}	73.8 (1.4) ^{ex}	14.6 (0.1) ^{gy}	38.2 (0.7) ^{cx}	6.4 (0.1) ^{ay}
(CS ₉₀ -CH ₁₀) ₆₀ -P ₄₀	60.6 (1.7) ^{dx}	50.5 (0.4) ^{bcy}	91.0 (2.0) ^{cx}	41.0 (0.6) ^{dy}	47.2 (0.9) ^{ex}	21.8 (0.2) ^{fy}
(CS ₈₀ -CH ₂₀) ₆₀ -P ₄₀	60.2 (1.2) ^{dx}	50.8 (0.1) ^{bcy}	95.0 (2.0) ^{cx}	30.1 (0.9) ^{ey}	48.9 (1.1) ^{ex}	17.6 (0.1) ^{dy}
(CS ₇₀ -CH ₃₀) ₆₀ -P ₄₀	57.3 (0.6) ^{abcx}	49.4 (0.5) ^{aby}	62.0 (4.0) ^{gx}	21.3 (1.4) ^{fy}	32.2 (0.5) ^{ax}	11.3 (0.2) ^{cy}
PEG	62.5 (0.3) ^{ex}	56.4 (0.5) ^{ey}	194.0 (3.0) ^{ax}	174.0 (3.0) ^{ay}		

521 Different superscripts indicate significant differences among films (a-h) or between the first and the second
 522 heating step (x,y) (P < 0.05). *Estimated from the crystallization enthalpy values of pure completely
 523 crystallized PEG (194 J/g).

524

525 **Table 3.** Thickness, moisture content and water vapour permeability (WVP) of films
 526 equilibrated at 53% RH. Mean values and standard deviation in brackets.

Film	Thickness (μm)	WVP (g mm kPa ⁻¹ h ⁻¹ m ⁻²)		Moisture content (g water/ 100g dry film)	
		Week 1	Week 5	Week 1	Week 5
(CS ₁₀₀ -CH ₀) ₇₀ -P ₃₀	199 (12) ^b	16.0 (3.0) ^{abc1}	15.1 (0.9) ^{bc1}	10.4 (0.4) ^{a1}	11.8 (0.4) ^{b2}
(CS ₉₀ -CH ₁₀) ₇₀ -P ₃₀	216 (5) ^c	15.3 (0.8) ^{ab1}	15.5 (0.4) ^{bc1}	9.8 (0.5) ^{a1}	11.8 (0.8) ^{b2}
(CS ₈₀ -CH ₂₀) ₇₀ -P ₃₀	237 (6) ^d	14.1 (0.1) ^{a1}	13.2 (0.6) ^{a1}	9.7 (1.1) ^{a1}	10.2 (0.5) ^{a1}
(CS ₇₀ -CH ₃₀) ₇₀ -P ₃₀	260 (12) ^f	14.7 (1.5) ^{a1}	14.5 (0.9) ^{ab1}	9.9 (0.8) ^{a1}	10.7 (0.2) ^{a2}
(CS ₁₀₀ -CH ₀) ₆₀ -P ₄₀	170 (6) ^a	21.0 (3.0) ^{e1}	16.9 (1.2) ^{de2}	12.4 (0.5) ^{b1}	13.7 (1.2) ^{c2}
(CS ₉₀ -CH ₁₀) ₆₀ -P ₄₀	193 (8) ^b	19.0 (2.0) ^{cd1}	17.1 (1.1) ^{de1}	12.8 (0.4) ^{b1}	15.0 (1.1) ^{d2}
(CS ₈₀ -CH ₂₀) ₆₀ -P ₄₀	229 (4) ^d	20.0 (2.0) ^{de1}	16.3 (1.1) ^{cd1}	12.2 (0.4) ^{b1}	14.6 (0.2) ^{cd2}
(CS ₇₀ -CH ₃₀) ₆₀ -P ₄₀	247 (10) ^e	18.0 (2.0) ^{bcd1}	18.0 (2.0) ^{e1}	12.4 (0.5) ^{b1}	14.4 (0.7) ^{cd2}

527

528

529 Different superscript (a-e) within the same column indicate significant differences among films (*P < 0.05).

530 Different superscript (1,2) within the same row indicate significant differences due to storage time (*P <

531 0.05).

532

533 **Table 4.** Tensile properties (elastic modulus: EM, tensile strength: TS and deformation:
534 E %, at break) of all films equilibrated at 53% RH after 1 and 5 weeks of storage. Mean
535 values and standard deviation in brackets.

Films	EM (MPa)		TS (MPa)		E (%)	
	Week 1	Week 5	Week 1	Week 5	Week 1	Week 5
(CS ₁₀₀ -CH ₀) ₇₀ -P ₃₀	321 (19) ^{b1)}	542 (28) ^{e2}	7.4 (0.5) ^{a1}	13.3 (0.8) ^{b2}	7.9 (0.6) ^{h1}	5.2 (0.3) ^{e2}
(CS ₉₀ -CH ₁₀) ₇₀ -P ₃₀	422 (12) ^{d1}	761 (49) ^{f2}	13.7 (0.7) ^{e1}	14.5 (0.4) ^{c2}	4.7 (0.3) ^{f1}	2.0 (0.2) ^{a2}
(CS ₈₀ -CH ₂₀) ₇₀ -P ₃₀	596 (22) ^{g1}	930 (20) ^{g2}	16.8 (0.3) ^{f1}	18.1 (0.5) ^{d2}	3.3 (0.2) ^{e1}	1.9 (0.1) ^{a2}
(CS ₇₀ -CH ₃₀) ₇₀ -P ₃₀	732 (16) ^{h1}	1118 (36) ^{h2}	20.0 (0.9) ^{g1}	22.2 (0.8) ^{e2}	2.8 (0.2) ^{d1}	1.9 (0.1) ^{a2}
(CS ₁₀₀ -CH ₀) ₆₀ -P ₄₀	245 (6) ^{a1}	145 (11) ^{a2}	9.3 (0.3) ^{b1}	8.6 (0.2) ^{a2}	6.4 (0.5) ^{g1}	8.5 (0.3) ^{g2}
(CS ₉₀ -CH ₁₀) ₆₀ -P ₄₀	344 (15) ^{c1}	281 (16) ^{b2}	9.8 (0.4) ^{bc1}	8.3 (0.5) ^{a2}	2.3 (0.1) ^{bc1}	5.6 (0.4) ^{f2}
(CS ₈₀ -CH ₂₀) ₆₀ -P ₄₀	463 (14) ^{e1}	350 (20) ^{c2}	10.1 (0.6) ^{c1}	8.1 (0.7) ^{a2}	2.1 (0.2) ^{ab1}	4.4 (0.3) ^{d2}
(CS ₇₀ -CH ₃₀) ₆₀ -P ₄₀	546 (32) ^{f1}	459 (35) ^{d2}	10.8 (0.8) ^{d1}	8.5 (0.3) ^{a2}	1.93 (0.13) ^{a1}	2.6 (0.2) ^{bc2}

536 Different superscripts (a-h) within the same column indicate significant differences among formulations (*P
537 < 0.05). Different superscripts numbers (1, 2) within the same row indicate significant differences for the
538 same formulation with different storage time (*P < 0.05).

539

540

541

542

543 **Table 5.** Lightness (L^*), chroma (C_{ab}^*), hue (h_{ab}^*), whiteness index ($WI = 100 - (100 - L^{*2} +$
544 $a^{*2} + b^{*2})^{0.5}$) and gloss at 60° after 1 and 5 week storage. Mean values and standard
545 deviation in brackets.

	Films	L^*	C_{ab}^*	h_{ab}^*	WI	Gloss (60°)
WEEK 1	(CS ₁₀₀ -CH ₀) ₇₀ -P ₃₀	74.4 (0.3) ^{e1}	8.1 (0.3) ^{a1}	84.4 (0.1) ^{f1}	73.1 (0.4) ^{g1}	6.4 (1.1) ^{a1}
	(CS ₉₀ -CH ₁₀) ₇₀ -P ₃₀	62.7 (0.5) ^{d1}	24.5 (0.6) ^{b1}	73.9 (0.4) ^{d1}	55.3 (0.7) ^{e1}	7.1 (0.6) ^{b1}
	(CS ₈₀ -CH ₂₀) ₇₀ -P ₃₀	58.8 (0.7) ^{b1}	29.8 (0.6) ^{e1}	69.9 (0.4) ^{b1}	49.2 (0.9) ^{b1}	9.0 (0.9) ^{d1}
	(CS ₇₀ -CH ₃₀) ₇₀ -P ₃₀	52.8 (0.2) ^{a1}	32.2 (0.7) ^{f1}	66.7 (0.4) ^{a1}	43.2 (0.0) ^{a1}	9.3 (1.2) ^{d1}
	(CS ₁₀₀ -CH ₀) ₆₀ -P ₄₀	73.3 (0.3) ^{f1}	8.3 (0.2) ^{a1}	85.4 (0.4) ^{g1}	72.0 (0.3) ^{f1}	5.9 (0.7) ^{a1}
	(CS ₉₀ -CH ₁₀) ₆₀ -P ₄₀	59.1 (0.4) ^{b1}	28.8 (0.6) ^{d1}	71.7 (0.5) ^{c1}	50.0 (0.7) ^{b1}	7.3 (0.8) ^{b1}
	(CS ₈₀ -CH ₂₀) ₆₀ -P ₄₀	63.0 (0.3) ^{d1}	27.5 (0.5) ^{c1}	74.4 (0.3) ^{e1}	54.0 (0.5) ^{d1}	7.4 (0.3) ^{b1}
	(CS ₇₀ -CH ₃₀) ₆₀ -P ₄₀	62.1 (1.1) ^{c1}	29.5 (0.1) ^{e1}	71.5 (0.4) ^{c1}	51.7 (0.9) ^{c1}	8.0 (0.8) ^{c1}
WEEK 5	(CS ₁₀₀ -CH ₀) ₇₀ -P ₃₀	73.6 (0.4) ^{f1}	8.6 (0.6) ^{a1}	83.8 (1.2) ^{f1}	72.3 (0.3) ^{g2}	6.6 (0.6) ^{bc1}
	(CS ₉₀ -CH ₁₀) ₇₀ -P ₃₀	65.5 (1.1) ^{e2}	26.4 (0.4) ^{b2}	76.6 (1.1) ^{e2}	56.8 (0.4) ^{e2}	6.9 (0.4) ^{d1}
	(CS ₈₀ -CH ₂₀) ₇₀ -P ₃₀	61.6 (0.3) ^{d2}	30.7 (0.5) ^{d2}	73.2 (0.3) ^{c2}	50.8 (0.5) ^{c2}	8.6 (0.5) ^{e1}
	(CS ₇₀ -CH ₃₀) ₇₀ -P ₃₀	54.5 (0.7) ^{b2}	35.3 (0.6) ^{g2}	68.5 (0.6) ^{a2}	42.6 (0.9) ^{a1}	8.7 (0.5) ^{e2}
	(CS ₁₀₀ -CH ₀) ₆₀ -P ₄₀	72.8 (0.2) ^{g1}	8.7 (0.7) ^{a1}	83.7 (0.9) ^{f2}	71.4 (0.2) ^{f1}	6.5 (0.6) ^{b2}
	(CS ₉₀ -CH ₁₀) ₆₀ -P ₄₀	61.8 (0.4) ^{d2}	27.8 (0.7) ^{c2}	74.3 (0.5) ^{d2}	52.7 (0.7) ^{d2}	6.0 (0.4) ^{a2}
	(CS ₈₀ -CH ₂₀) ₆₀ -P ₄₀	53.4 (0.4) ^{a2}	33.4 (0.2) ^{f2}	68.3 (0.2) ^{a2}	42.8 (0.2) ^{a2}	6.2 (0.4) ^{a2}
	(CS ₇₀ -CH ₃₀) ₆₀ -P ₄₀	58.6 (1.3) ^{c2}	32.7 (0.5) ^{e2}	70.6 (0.7) ^{b2}	47.2 (1.2) ^{b2}	6.9 (0.5) ^{cd2}

546 Different superscript (a-f) within the same column indicate significant differences among films (*P < 0.05).

547 Different superscript (1,2) for the same film indicate significant differences due to storage time (*P < 0.05).

548

549

550 **Table 6.** Antimicrobial activity of the films on sliced pork meat during storage at 10 °C.

551 Mean values and standard deviation in brackets.

Microbial counts (log CFU/g)	Sample	Time (days)			
		0	1	4	7
Coliforms	Control	0.27 (0.02) ^{a1}	1.07 (0.04) ^{(c)(2)}	3.50 (0.02) ^{d3}	5.36 (0.03) ^{c4}
	(CS ₁₀₀ -CH ₀) ₇₀ -P ₃₀	0.64 (0.03) ^{c1}	1.30 (0.04) ^{(e)(2)}	3.67 (0.02) ^{e3}	5.59 (0.03) ^{d4}
	(CS ₉₀ -CH ₁₀) ₇₀ -P ₃₀	0.67 (0.03) ^{c1}	1.17 (0.05) ^{d2}	3.42 (0.04) ^{c3}	5.37 (0.04) ^{c4}
	(CS ₈₀ -CH ₂₀) ₇₀ -P ₃₀	0.57 (0.03) ^{b1}	0.99 (0.04) ^{b2}	3.10 (0.03) ^{b3}	5.14 (0.03) ^{b4}
	(CS ₇₀ -CH ₃₀) ₇₀ -P ₃₀	0.28 (0.02) ^{a1}	0.69 (0.05) ^{a2}	2.96 (0.09) ^{a3}	4.53 (0.11) ^{a4}
Total aerobic	Control	1.28 (0.07) ^{b1}	2.68 (0.08) ^{c2}	4.74 (0.07) ^{c3}	6.43 (0.04) ^{d4}
	(CS ₁₀₀ -CH ₀) ₇₀ -P ₃₀	1.45 (0.04) ^{c1}	2.86 (0.13) ^{d2}	5.12 (0.03) ^{e3}	6.55 (0.04) ^{e4}
	(CS ₉₀ -CH ₁₀) ₇₀ -P ₃₀	1.88 (0.03) ^{e1}	3.21 (0.14) ^{e2}	4.53 (0.04) ^{d3}	5.47 (0.02) ^{c4}
	(CS ₈₀ -CH ₂₀) ₇₀ -P ₃₀	1.75 (0.03) ^{d1}	2.11 (0.04) ^{b2}	4.15 (0.04) ^{b3}	5.26 (0.09) ^{b4}
	(CS ₇₀ -CH ₃₀) ₇₀ -P ₃₀	1.13 (0.06) ^{a1}	1.71 (0.08) ^{a2}	3.79 (0.09) ^{a3}	5.01 (0.05) ^{a4}

552 Different superscripts (a-e) within the same column indicate significant differences among formulations for
 553 the same microbiological analysis (*P < 0.05). Different superscripts (1-4) within the same row indicate
 554 significant differences due to storage time (*P < 0.05).

555

556

557 **Figure captions**

558 **Figure 1.** Scanning electron microscopy micrographs of the cross-sections of the films.

559 (a) Polymer: plasticizer proportion 70:30. (b) Polymer: plasticizer proportion 60:40.

560 **Figure 2.** Typical thermogravimetric curves (mass loss vs. temperature) and first

561 derivative (mg/s vs temperature) for a) Polymer: plasticizer proportion 70:30 and b)

562 Polymer: plasticizer proportion 60:40.

563 **Figure 3.** Typical stress-strain curves of the films after 1 week (left) and 5 weeks (right)

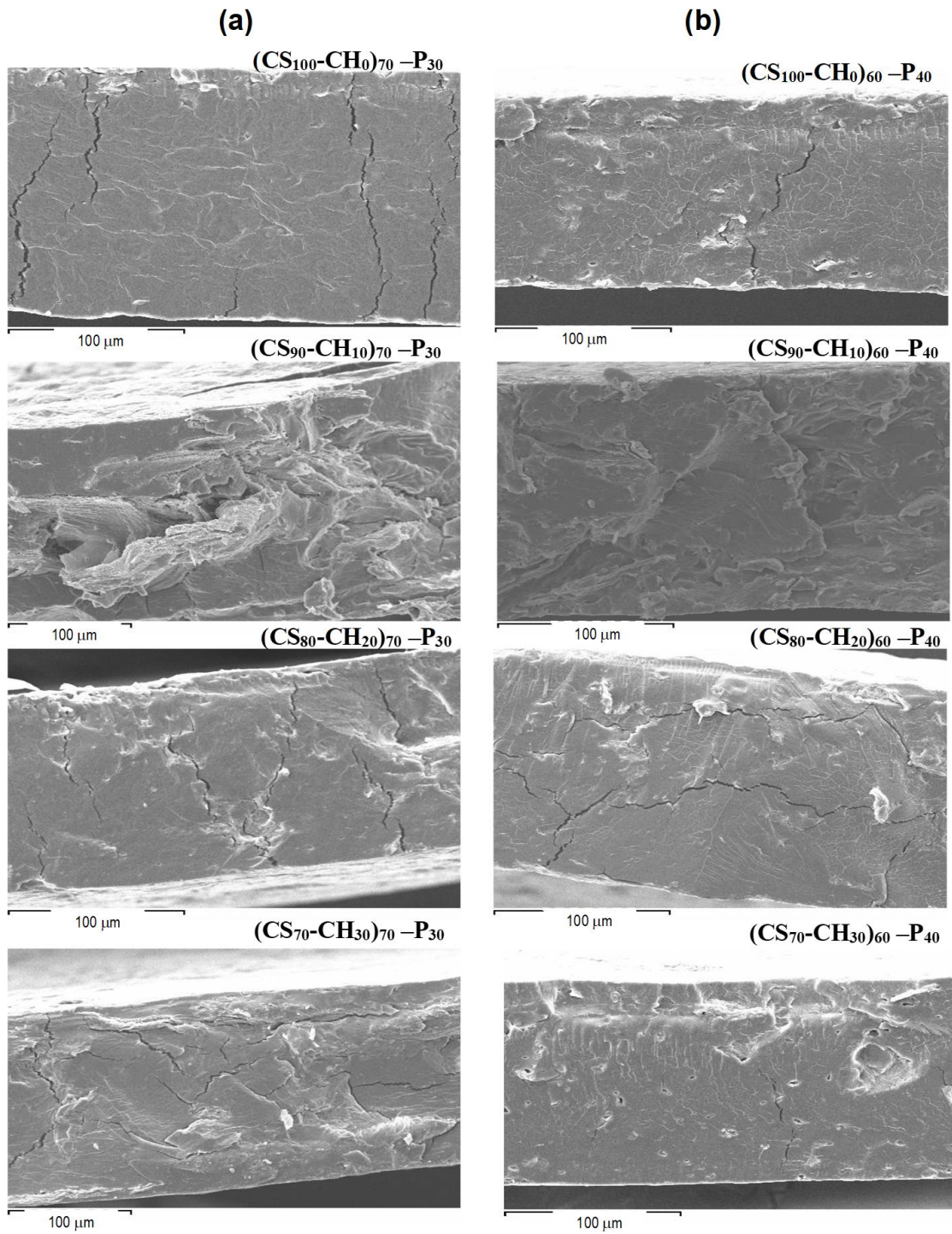
564 of storage at 53% RH.

565 **Figure 4.** Spectral distribution of the internal transmittance (T_i) of the films. **(a)** Ratio

566 polymer: plasticizer 70:30. **(b)** Ratio polymer: plasticizer 60:40. After 1 week and 5

567 weeks of storage.

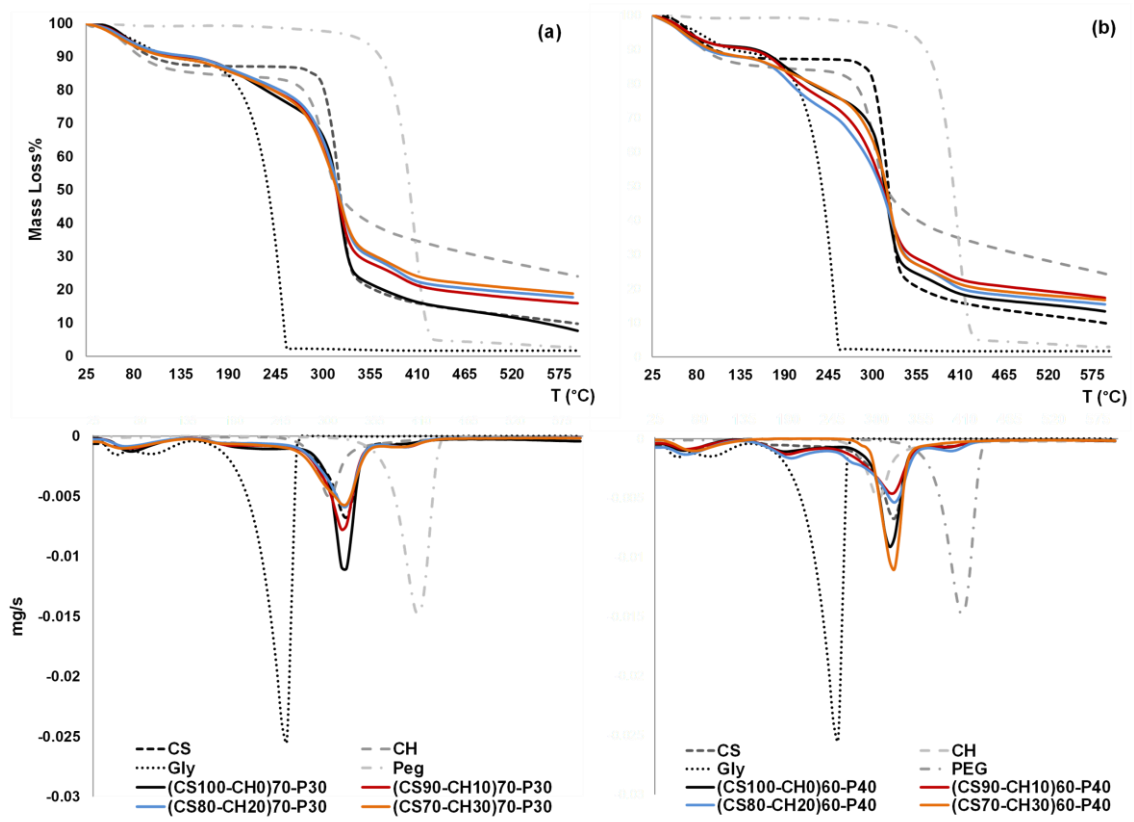
568



570

571

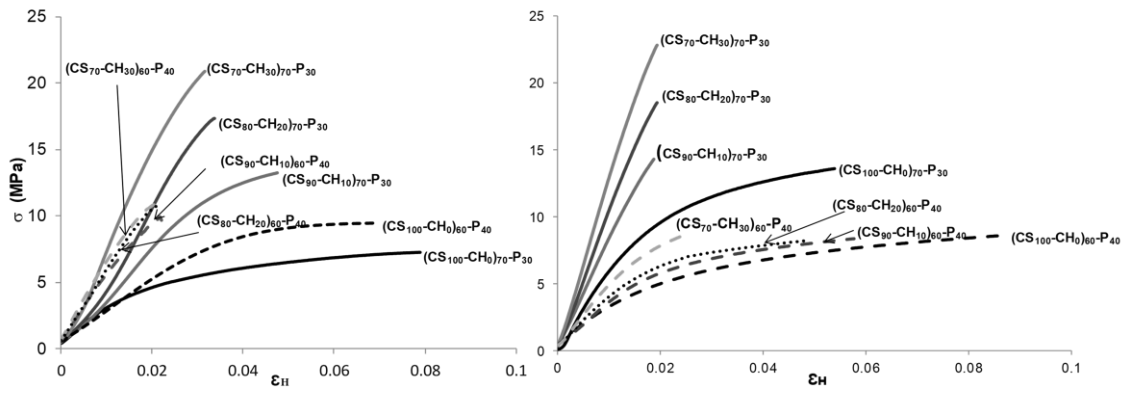
572 **Figure 2**



573

574

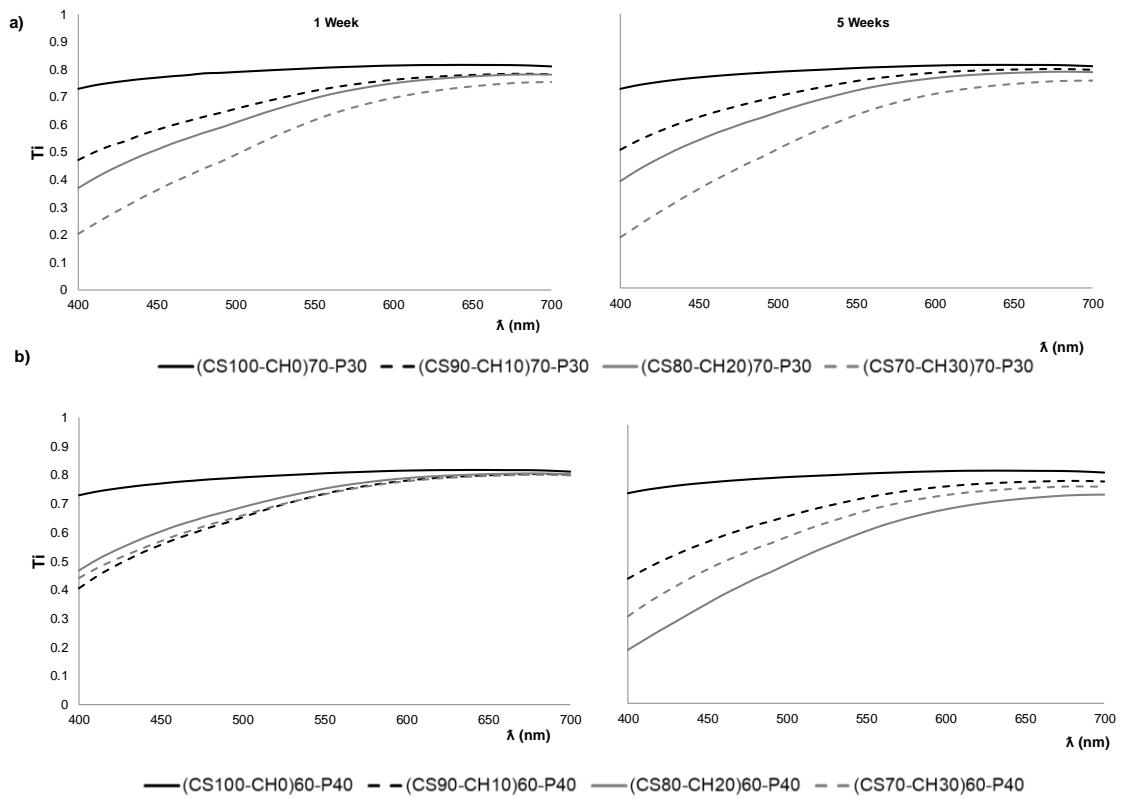
575 **Figure 3**



576

577

578 **Figure 4**



579