1   **Physical properties and stability of starch-gelatin based films as**

2   **affected by the addition of esters of fatty acids.**

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26 **Abstract**

27 Cassava starch films containing 25 and 50 % (in the polymer blend) of bovine gelatin

28 were obtained using glycerol (30 %) as plasticizer. Film formulations were also

29 obtained with 15 % of lipid (blend of fatty acid-glycerol esters). Microstructural and

30 physical (optical, barrier and mechanical) properties were analysed in order to compare

31 blend films with neat starch or gelatin films. The incorporation of gelatin and lipids to

32 glycerol-plasticized cassava starch films notably affected their physical properties,

33 while they showed polymer-phase separation and a lipid-dispersed phase. Gelatine

34 incorporation gave rise to harder films with greater resistance to break and extensibility,

35 while the lipids decreased film hardness and resistance but enhanced the stretchability,

36 especially in films with 50 % gelatin content. Likewise, lipids improved water barrier

37 properties of the films, but they impaired the oxygen permeability at the same time as

38 they decreased the film gloss and transparency. After 5 weeks of storage, all the films

39 became harder and more resistant to break, especially those which did not contain

40 lipids, which enhanced the films' extensibility during storage. Cassava starch films with

41 50 % gelatine, both with and without lipids, exhibited very adequate properties for food

42 coating or packaging.

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45 **Keywords:** Starch, gelatin, physical properties, microstructure, storage.

46

1. Introduction.

In the last few years, the aim of many studies has been to obtain starch-based biodegradable materials for the purpose of substituting, at least partially, synthetic petroleum-based polymers (Ali, Rahman, Ibrahim & Kasmani, 2013; Dastidar & Netravali, 2013; López, Castillo, García, Villar & Barbosa, 2015; Pelissari, Grossman, Yamashita & Pineda, 2009). Starch is readily available from renewable sources and has a great ability to form transparent, tasteless, odorless films with very good oxygen barrier properties, which is very useful for food preservation. However, starch films exhibit some drawbacks derived from their great sensitivity to water and to retrogradation phenomena, which affect the film's mechanical properties (matrices become too brittle) and barrier capacity (Cano, Jiménez, Cháfer, González & Chiralt, 2014; Jiménez, Fabra, Talens & Chiralt, 2012a; Jiménez, Fabra, Talens & Chiralt, 2013a). Different strategies have been considered as a means of overcoming these problems. Among them: the plasma or chemical modification of starch (Bastos, Santos, da Silva & Simão, 2009; Zhou, Ren, Tong, Xie & Liu, 2009), the addition of hydrophobic compounds (Jiménez et al., 2012a; Jiménez et al., 2013a) as well as the formation of composites with other polymers or reinforcement materials (Nasri-Nasrabadi, Behzad & Bagheri, 2014; Ortega-Toro, Jiménez, Talens & Chiralt, 2014a). The chemical modification of starches includes not only the modification of starch chains via chemical reactions, but also crosslinking in combination with other polymers, such as PVA, or between amylose and amylopectin chains (Liu, Dong, Men, Jiang, Tong & Zhou, 2012; Reddy & Yang, 2010) by means of the formation of covalent bonds between polymer chains. Hydrophobic compounds may also be added to improve the characteristics of starch-based materials. These compounds include both molecular lipids and hydrophobic polymers, such as polycaprolactone or poly(lactic acid).

72   However, the lack of polymer compatibility (due to their different polarity) may give

73   rise to phase separation, as observed in PCL-starch blend films, which has a negative

74   impact on film properties (Kweon, Kawasaki, Nakayama, & Aiba, 2003). In some

75   cases, lipid materials have been observed to affect the crystallinity of the matrix through

76   the formation of V-type crystalline forms of the lipid-amylose complexes. Besides the

77   formation of these crystalline structures, lipids promoted the films brittleness,

78   negatively affecting the mechanical response of the material (Jiménez, Fabra, Talens &

79   Chiralt, 2013b; Ortega-Toro, Jiménez, Talens & Chiralt, 2014b). In starch composite

80   films with other polymers, the inhibition of starch crystallization and retrogradation was

81   deduced for sodium caseinate and HPMC (Jiménez, Fabra, Talens & Chiralt, 2012bc).

82   Starch-gelatin blend films have also been studied by other authors, who reported good

83   interactions of gelatin with polysaccharide matrices when the protein predominates in

84   the system (Al-Hassan & Norziah, 2012; Lee, Shim & Lee, 2004; Pérez-Gago &

85   Krochta, 2001; Pranoto, Lee & Park, 2007). These blends may offer advantages in terms

86   of oxygen and water vapor barrier properties, mechanical behavior and optical

87   parameters (Averous & Boquillon, 2004; Wang, Rakotonirainy & Padua, 2003). Even

88   though gelatin may improve the performance of starch-based materials, both polymers

89   are highly hydrophilic. In this sense, the addition of hydrophobic materials may be

90   considered a means of reducing the water sensitivity of the material. Ma, Tang, Yin,

91   Yang, Wang, Liu & Wei (2012) reported that olive oil was able to reduce the water

92   vapour permeability of fish gelatin films when added at 5-20 % (w/w). Meanwhile,

93   Pérez-Mateos, Montero & Gómez-Guillén (2009) and Andreuccetti, Carvalho, Galicia-

94   García, Martínez-Bustos & Grosso (2011) reported that sunflower oil and lecithin

95   (amphiphilic compound) significantly reduced the solubility in water of cod and pig

96   gelatin films, respectively. In addition, barrier and mechanical properties of gelatin

97   films have been improved by the addition of laponite ~~at different concentrations~~ and

98   transglutaminase (Li, Liu, Ye, Wang & Wang, 2015; Wang, Liu, Ye, Wang & Li,

99   2015).

100 Although different studies have recently been published on starch-gelatin blend films

101 (Fakhouri, Costa, Yamashita & Martelli 2013; Al-Hassan & Norziah, 2012), there are

102 no studies into the influence of hydrophobic substances, such as fatty acids or fatty

103 acids esters (obtained from the esterification of an alcohol with different fatty acids), on

104 these blend films. Previous studies in films with similar features, such as sodium

105 caseinate-starch films containing oleic acid and/or α-tocopherol, revealed an

106 improvement of the film stretchability by lipid addition (Jiménez et al., 2013a).

107 This work analyzes different microstructural and physical properties of films based on

108 different blends of cassava starch and bovine gelatin, with and without lipids (mono-

109 and di-glyceride of fatty acids: E471), after 1 and 5 weeks of storage at 25ºC and 53%

110 relative humidity.

111

112 **2. Materials and methods**

113 **2.1. Raw materials**

114 Cassava starch, used to obtain film-forming dispersions, was produced by Asia CO.,

115 LDT (Kalasin, Thailand) and purchased from Quimidroga S.A. (Barcelona, Spain).

116 Bovine skin type A gelatin (Bloom 220-240°), used in combination with starch, was

117 supplied by Sancho de Borja, S.L. (Zaragoza, Spain). Mono- and diglycerides of fatty

118 acids (E471) were obtained from Cargill (Barcelona, Spain), while glycerol, used as

119 plasticizer, was provided by Panreac Química S.A. (Barcelona, Spain).

120 The amylose-amylopectin ratio of cassava starch was determined in triplicate, by using

121 an amylose/amylopectin assay procedure enzymatic kit (Megazyme International

122   Ireland, Bray Business Park, Bray, Co. Wicklow, Ireland). Cassava starch contains an

123   amylose content of 9.28% (g/100g), with an amylose:amylopectin ratio of 1:9.78.

124

125   **2.2. Preparation of film-forming dispersions**

126   The film-forming dispersions were prepared from a 2% polymer suspension (w/w),

127   using different ratios of cassava starch (S) and gelatin (G). The proportions (%) of

128   starch:gelatin were 0:100, 50:50, 75:25 and 100:0, respectively. Every formulation

129   contained glycerol as plasticizer (a polymer:glycerol ratio of 1:0.25). Furthermore, four

130   more formulations were prepared by adding 15 % (w/w) of mono- and di-esters of

131   glycerol and fatty acids (E471). So, eight film formulations were considered, with and

132   without a lipid fraction. Sample codes were represented by the starch (S) gelatin (G)

133   ratio in the films. When they contained glycerol ester, "e" was added in the code.

134   Firstly, starch and gelatin dispersions were prepared separately. Starch aqueous

135   dispersions were maintained at 95∘C for 30 min to induce starch gelatinization.

136   Meanwhile, gelatin was dissolved in water at 40 ºC. Then, the glycerol was added to the

137   dispersions, which were mixed by magnetic stirring prior to homogenization. This

138   process was carried out by using a rotor-stator homogenizer (Ultraturrax D125, Janke

139   and Kunkel, Germany) at 13,500 rpm for 1 min and 20,500 rpm for 3 min at 95 ºC

140   under vacuum in agreement with previous studies (Jiménez et al., 2012abc; Cano et al.,

141   2014). For formulations containing lipids, these were added prior to the homogenization

142   step.

143   Controlled volumes of film-forming aqueous dispersions (equivalent to 1.5 g of solids)

144   were cast into leveled Teflon® casting plates (15 cm diameter) and dried at 25ºC and

145   45% RH for 48 h. Then, they were peeled intact from the plates and conditioned at 53%

146 RH and 25ºC in a chamber with magnesium nitrate-6-hydrate saturated solution

147 (Panreac Química, S.A., Barcelona, Spain). All the films were analyzed after one or five

148 storage weeks, according to previous studies (Jiménez et al., 2012abc).

149

150 **2.3. Characterization of the films**

151 2.3.1. Microstructure

152 The microstructural analysis of the films was carried out by using a scanning electron

153 microscope (SEM), JEOL model JSM-5410 (Japan). Prior to testing, films were

154 equilibrated in desiccators with P_2O_5 (theoretical relative humidity in the dessicator 0

155 %) in order to remove any water present in samples. SEM observations were carried out

156 by considering the surfaces and cross sections of film samples. Films were frozen in

157 liquid nitrogen, and then cryofractured to observe the cross section. Samples were fixed

158 on copper stubs, gold coated to make them conductive (for 1.5 minutes) and observed

159 directly with an accelerating voltage of 10 KV. Surface and cross section images were

160 captured from the different film samples after 1 and 5 weeks of storage.

161

162 2.3.2. Thickness

163 Film thickness, used in different analyses, was measured with a Palmer digital

164 micrometer to the nearest 0.0025 mm at 6 random positions.

165

166 2.3.3. Water vapor permeability

167 The water vapor permeability (WVP) of films was determined following the gravimetric

168 method ASTM E96-95 (1995) for a 53-100% relative humidity gradient at 25ºC by

169   using Payne permeabilty cups (Payne, Elcometer SPRL, Hermelle/s Argenteau,

170   Belgium) of 3.5 cm diameter. The RH of the cabinet was held at a constant 53-100%

171   using oversaturated solutions of $Mg(NO_3)_2$ and distilled water, respectively. The weight

172   of the cup was measured every 2 h using an analytical balance ($\pm$ 0.00001 g). The water

173   vapor transmission (WVTR) was determined from the slope obtained from the

174   regression analysis of weight loss data versus time, once the steady state had been

175   reached, divided by the film area. The reported results represent the mean values of at

176   least four samples.

177

178   2.3.4. Oxygen permeability

179   The oxygen permeability (OP) of the films was analyzed in film samples (50 cm^2) by

180   using an Oxtran system (Mocon, Minneapolis, USA), following the standard method

181   (ASTM D3985-05, 2002) at 53% RH and 25ºC. Films were exposed to pure nitrogen

182   flow on one side and pure oxygen flow on the other side. OP was calculated by dividing

183   the oxygen transmission rate by the difference in oxygen partial pressure between the

184   two sides of the film, and multiplying by the average film thickness. At least two

185   replicates per formulation were taken.

186

187   2.3.5. Mechanical behavior

188   The mechanical properties were measured using a universal test machine (TA.XT plus

189   model, Stable Micro Systems, Haslemere, England) and following the ASTM standard

190   method D882 (ASTM, 2001). Equilibrated samples (8 per formulation) were mounted

191   in the film-extension grips (model A/TG, Stable Micro System, Haslemere, England) of

192   the testing machine and stretched at a rate of 50 mm$\cdot$min^{-1} until breaking. The relative

193 humidity of the environment was held at nearly 53% during the tests, which were

194 performed at 25 ℃.

195 The force-distance data obtained in the test were transformed into stress-strain curves,

196 which allow the mechanical parameters to be calculated: elastic modulus (EM), tensile

197 strength at break (TS) and elongation at break (expressed as percentage: E).

198

199 2.3.6. Optical properties

200 The transparency of the films was determined by applying the Kubelka-Munk theory

201 (Hutchings, 1999) for multiple scattering to the reflection spectra. The surface

202 reflectance spectra of the films were determined from 400 to 700 nm with a

203 spectrocolorimeter CM-3600d (Minolta Co., Tokyo, Japan) on both white and black

204 backgrounds. As the light passes through the film, it is partially absorbed and scattered,

205 which is quantified by the absorption (K) and the scattering (S) coefficients. Internal

206 transmittance (Ti) of the films was quantified using eq 1. In this equation, R0 is the

207 reflectance of the film on an ideal black background. Parameters a and b were

208 calculated by eqs 2 and 3, where R is the reflectance of the sample layer backed by a

209 known reflectance Rg. Measurements were taken in triplicate for each sample on the

210 free film surface during its drying.

211
$$T_i = \sqrt{(a - R_0)^2 - b^2}$$
Equation 1

212
$$a = \frac{1}{2} \cdot \left(R + \frac{R_0 - R + R_g}{R_0 R_g} \right)$$
Equation 2

213
$$b = (a^2 - 1)^{1/2}$$
Equation 3

214 The gloss of the films was measured at a 60º incidence angle, using a flat surface gloss

215 meter (Multi.Gloss 268, Minolta, Germany), according to the ASTM standard D-523

216 (ASTM, 1999). Three films of each formulation were measured over a black matte

217 standard plate. Results were expressed as gloss units, relative to a highly polished

218 surface of standard black glass with a gloss value close to 100.

219

220 2.3.7. Statistical analysis

221 The analysis of data was performed through the analysis of variance (ANOVA) using

222 the Statgraphics Plus 5.1. software (ManugisticsCorp., Rockville, MD). Fisher′s least

223 significant difference (LSD) procedure was used at the 95% confidence level.

224

225 **3. Results**

226 **3.1. Microstructure**

227 The microstructure of films, which depends on the interactions between film

228 components and drying conditions, seriously affects the physical properties of the final

229 materials. Thus, film microstructure can be well correlated with their barrier,

230 mechanical and optical properties.

231 Figures 1 and 2 show the micrographs corresponding to the surfaces and cross-sections

232 of every studied film, respectively. The images corresponding to pure cassava starch

233 film were similar to those obtained by Chen, Kuo & Lai (2009), who observed

234 continuous, smooth surfaces. The presence of micro-cracks may be due to the electron

235 beam during observation, as explained by Jiménez et al. (2012c). The water present in

236 the films was eliminated prior to observation, thus giving rise to a brittle matrix, in

237 agreement with the observed structure. Net starch films were homogeneous, as can be

238 observed in both cross-section and surface images, which indicates that starch granules

239 were totally disrupted during heating.

240   The pure gelatin film was also quite homogeneous, with a relatively smooth surface, as

241   observed by Ahmad, Benjakul, Prodpran & Agustini (2012). Nevertheless, the presence

242   of round zones on the gelatin film surface and inside the gelatin matrix can be observed,

243   which can be attributed to the reorganization of protein chains during drying. These

244   chains tend to aggregate through helical forms, forming fibrous structures similar to

245   those of collagen. In this sense, Chambi & Grosso (2006) found that gelatin films

246   presented a fibrous structure, characteristic of collagen fibrils. This phenomenon occurs

247   because of the protein chain interweave recovering the native conformation of collagen

248   (triple helices). Composite films (75S-25G and 50S-50G) exhibited heterogeneous

249   structures in both surface and cross-section images. Round formations are also observed

250   on the surface of these films, while in film cross-sections, fibrous regions appear, both

251   of which are related with the gelatin chain conformation and association. These results

252   demonstrate that, although starch and gelatin seem to be macroscopically compatible

253   due to their similar hydrophilic character, phase separation occurs, leading to two

254   phases: a starch-rich one and a gelatin-rich one.

255   Lipid addition significantly affected the film microstructure leading to heterogeneous

256   matrices, thus indicating the lack of lipid phase integration in the matrix at the

257   concentration used. This effect was more marked in blend films, as can be observed in

258   the corresponding micrographs, where lipid droplets interrupt the polymer matrix. In

259   gelatin-containing films with lipid (75S-25Ge, 50S-50Ge and 0S-100Ge), the surfaces

260   showed both small lipid droplets and rounded gelatin formations, which indicates that

261   the helical association of gelatin also takes place for these blends. The lack of

262   miscibility between polymers and lipid components provoked creaming phenomena

263   during the film's drying step, giving rise to the presence of large hydrophobic particles

264   on the film's surface, as previously described for other films containing lipids

265 (Monedero, Fabra, Talens & Chiralt, 2010). Bertan, Tanada-Palmu, Siani & Grosso

266 (2005) studied the effect of triacetin incorporation in gelatin matrices and also observed

267 that the integration of lipids in the protein matrix was not homogeneous. They also

268 reported more than one glass transition by differential scanning calorimetry, thus

269 indicating that phase separation occurred. Wongsasulak, Toovidhya, Bhumiratana,

270 Hongsprabhas, McClements & Weiss (2006) also found a heterogeneous structure in

271 egg albumen-cassava starch films containing sunflower oil, where the polymers were

272 not integrated well-enough to form a continuous matrix and oil was in a separate phase.

273

274 **3.2. Optical properties**

275 The gloss and the transparency of films, which are directly related with their

276 microstructure, are the most relevant optical properties with which to describe the

277 impact of a film after this is applied on a coated product (Hutchings, 1999). Gloss is

278 directly related with the surface roughness, whereas transparency depends on the

279 internal microstructure of the matrix and the distribution of the components. Table 1

280 shows the values of gloss (60º) and internal transmittance (480 nm) of starch-gelatin

281 films containing, or not, fatty acid esters. At a wavelength of 480 nm, the greatest

282 differences among films were observed in the spectral distribution of internal

283 transmittance.

284 All the films may be considered transparent, as deduced from the Ti values which

285 ranged between 84 and 87 %. High Ti values were related with a marked structural

286 homogeneity and more transparent films (Villalobos, Chanona, Hernández, Gutiérrez &

287 Chiralt, 2005). Neat starch films were the most transparent, regardless of the storage

288 time, coinciding with the more homogeneous structure observed in Figures 1 and 2.

289   Lipid addition contributed to a decrease in the transparency of starch-gelatin films

290   regardless of the polymer ratio, which was coherent with the microstructural

291   observations. Lipid components, which were not compatible with hydrophilic polymers,

292   remain in a dispersed phase, promoting the light dispersion and leading to a lower

293   degree of transparency. Similar results were also observed by Jiménez et al. (2012a) for

294   corn starch films containing fatty acids.

295   The transparency of stored films significantly decreased in most of the cases, and was

296   more marked in the pure starch film. This effect has previously been reported in corn

297   starch films containing fatty acids (Jiménez et al., 2012a). The reduction in the

298   transparency was related with the increase in the starch crystallinity or retrogradation

299   phenomenon, both leading to the increase in the number of hydrogen bonds in the

300   polymer network (Gutiérrez, Tapia, Pérez & Famá, 2015). This leads to changes in the

301   refractive index in the matrix, thus promoting light dispersion.

302   Film gloss (Table 1) was greatly influenced by film composition. In this sense, at the

303   initial time, the films were glossier as the gelatin content increased, with the net gelatin

304   film being the glossiest. However, this effect was not found when the fatty acid esters

305   were added. This can be due to the presence of lipid particles on the film surface (Figure

306   1) which contributes to increase the surface roughness, thus decreasing the gloss and

307   masking the glossiness effect of gelatin. In fact, lipid incorporation significantly ($p <$

308   0.05) decreased the film gloss in every case. As previously commented on, lipid

309   coalescence and creaming during the film drying step lead to migration of droplets to

310   the film-air interface, thus increasing the surface heterogeneity. So, values of the optical

311   parameters are coherent with the previously described film microstructure (SEM

312   images).

313 The effect of storage time on gloss values depended on the film formulation. While in

314 pure starch films, gloss did not significantly change after ageing, in the other cases films

315 gloss decreased, mainly in films without lipids. These results could be attributed to the

316 progressive reorganization of gelatin chains, which would have a relevant effect at

317 surface level.

318

3.3. Barrier properties

320 3.3.1. Water vapour permeability

321 The values of the water vapour permeability (WVP) and moisture content (Xw) of the

322 studied films stored for 1 or 5 weeks under controlled conditions are shown in Table 2.

323 The moisture of films ranged from 7.7 to 11 %, with no notable changes taking place

324 during film storage in most cases. The moisture content increased when gelatin was

325 added and decreased when the films contained lipids, in agreement with the different

326 water affinity of both components and the higher water affinity of gelatin in comparison

327 with starch (Al-Hassan & Norziah, 2012).

328 At initial time, films without esters (including composites) did not show significant

329 differences in their WVP values. This result is coherent with that reported in previous

330 studies into starch blend films with sodium caseinate and

331 hydroxypropylmethylcellulose, where no significant modifications of the WVP of

332 starch were observed (Jiménez et al., 2012bc). On the contrary, Jongjareonrak,

333 Benjakul, Visessanguan & Tanaka (2006) reported that higher WVP was found in

334 gelatin films containing a greater protein content, which agrees with the greater

335 hydrophilic character of gelatin. Al-Hassan & Norziah (2012) did not find any

336 significant differences in the WVP for sago starch films with different ratios of

337 gelatin,either. So, the water vapour permeability is highly dependent not only on the

338 composition of the matrices but also on the interactions between components.

339 Lipid addition generally decreased the WVP of the studied films in agreement with its

340 more hydrophobic character, as has also been observed in previous studies (Bertan et

341 al., 2005; Jiménez et al., 2012a; Ma et al., 2012). The greatest reduction in the WVP

342 corresponded to the net starch film (100S-0Ge), probably due to the development of

343 interactions between starch and lipid hydrophobic chains. These interactions involve the

344 establishment of hydrogen bonds between the polymer chains (Gutiérrez, Tapia, Pérez

345 & Famá, 2015), and give rise to the formation of V-type crystalline structures in which

346 helical conformations of amylose and amylopectin entrap the lipid hydrophobic chains

347 (Jiménez et al., 2012a; 2013b). Previous studies pointed out that an improvement in the

348 water vapour barrier properties can take place as a result of interactions between

349 different components (Gontard, Duchez, Cuq & Guilbert, 1994; Yang & Paulson, 2000)

350 The effect of film ageing on the water vapour barrier capacity of films was different,

351 depending on the film formulation. In fact, while the WVP of pure starch films without

352 lipids increased with time, the WVP of lipid-containing films significantly decreased (p

353 < 0.05). This could be related with structural changes in the polymeric matrix, which led

354 to an increase in the tortuosity factor for water transfer in films with lipids.

355

356 3.3.2. Oxygen permeability

357 The oxygen permeability values of films are shown in Table 2. The OP of net gelatin

358 films (0S-100G) was significantly higher than that of starch films (100S-0G). Indeed,

359 the OP increased when the gelatin content rose, as previously observed for other

360 polymers in combination with starch (Jiménez et al., 2012bc). As expected, lipid

361 addition increased the OP values due to the higher degree of oxygen solubility in the

362 hydrophobic regions of the matrices (lipid droplets).

363 Film storage contributed to a decrease in the oxygen permeability of films, probably due

364 to the progress of the chain aggregations and the increase in the matrix compactness.

365 For films containing starch, the promotion of the oxygen barrier was related to the

366 increase in crystallinity, since crystallites increase the ability of the matrix against

367 oxygen transfer due to the fact that they may be considered impermeable to oxygen

368 transfer (Shen, Wu, Chen & Zhao, 2010).

369

370 **3.4. Tensile properties**

371 Figure 3 shows the typical stress-Hencky strain curves for the films containing, or not,

372 lipids and stored for 1 and 5 weeks under controlled conditions. The curves at initial

373 time showed that gelatin (with and without lipid) gave rise to more resistant and flexible

374 films in comparison with net starch films, as deduced from the curves. The values of

375 elastic modulus (EM), tensile strength (TS) and elongation at break (E) of films are

376 shown in Table 3. These parameters are related with the arrangement of different film

377 components in the matrix, observed in SEM micrographs, and cohesion forces in the

378 matrix, and are normally used to describe the mechanical performance of the films

379 (McHugh & Krochta, 1994). The values obtained for net starch (100S-0G) and net

380 gelatin films (0S-100G) were similar to those obtained by Al-Hassan & Norziah (2012)

381 and Chambi & Groso (2006), respectively.

382 In contrast with that reported by Al-Hassan & Norziah (2012), in lipid-free starch-

383 gelatin films, the values of all mechanical parameters increased as the gelatin content

384 rose, due to the high mechanical resistance of this material. In fact, the conformation

385 and arrangement of protein chains in the matrix led to strong interchain forces close to

386 the collagen structure (Chambi & Grosso, 2006), which enhances its mechanical

387 performance. Pranoto et al. (2007) also reported that polysaccharides, such as starch,

388 may interweave with gelatin, thus increasing the mechanical resistance of the material.

389 The values of EM and TS of lipid containing films were lower than those of lipid-free

390 films. This can be explained by the microstructural features shown in Figure 2, where

391 the interruption of the polymer matrix continuity by lipid droplets can be observed.

392 These discontinuities, as well as the possible weakening of the interchain forces in the

393 presence of lipid compounds, decrease the cohesion forces of the matrix and so its

394 mechanical resistance, as previously reported by other authors (Jiménez et al., 2012a;

395 Schmidt, Porto, Laurindo & Menegalli, 2013). Likewise, lipid addition increased the

396 values of elongation at break of the films, this being significant when the gelatin ratio

397 increased (up to 50%). The increase in the film extensibility for protein-starch matrices

398 by lipid addition has been previously observed and it was attributed to the lipid

399 plasticizing effect (Jiménez et al. 2013a). So, lipid had a plasticizing effect in gelatin-

400 starch films, probably due to the orientation of the functional groups in the matrix,

401 favoring lipid-polymer interactions and weakening the polymer chain attraction forces.

402 This effect implies a softening of the polymer network and facilitates the slippage of the

403 chains during the film stretching, increasing the extensibility. This effect was more

404 remarkable at the initial control time.

405 Except for the net starch film containing lipid, all films became stiffer and less

406 stretchable as they aged, thus indicating that chain rearrangement continued during

407 storage. Protein chains tend to aggregate through helical structures, while amylopectin

408 and amylose chains progressively aggregate though the formation of hydrogen bonds

409 (Cano et al. 2014). In films containing lipids, similar but less marked tendency was

410 observed. The mechanical behaviour of pure starch film with lipid (100S-0Ge) after 5

411 weeks storage is remarkable. Contrary to that previously observed by Jiménez et al.

412 (2012a) when analysing corn starch containing fatty acids, this film exhibited a greater

413 extensibility ($p < 0.05$) in comparison with the initial value. This unexpected value

414 could be due to the low amylose content of cassava starch, which has the ability to form

415 helical complexes with lipids. Free (non complexed) lipid molecules could

416 progressively be released to lubricate the starch matrix, thus allowing the polymer

417 chains to slip more easily during the tensile test. The way cassava starch was observed

418 to behave could indicate that the low amylose content, as well as the interactions

419 between the starch chains and the lipid molecules, plays a relevant role in the

420 mechanical performance of the films.

421

422 **4. Conclusion**

423 The incorporation of bovine gelatin and lipids (blend of mono- and di-glycerides of

424 fatty acids) to glycerol plasticized cassava starch notably affected the physical

425 properties of starch films, while they exhibited polymer phase separation and a lipid

426 dispersed phase. The mechanical properties of polymer blend films were significantly

427 better than those of pure starch films. Gelatin incorporation gave rise to harder films

428 with a higher resistance to break and greater extensibility, while lipids decreased the

429 film hardness and resistance but enhanced the stretchability, especially in films with 50

430 % gelatin. Likewise, lipids improved the water barrier properties of the films, but they

431 impaired oxygen permeability at the same time as they decreased the film gloss and

432 transparency. After 5 weeks of storage, all the films became harder and more resistant to

433 break, especially those that did not contain lipids. Lipids enhanced the film extensibility

434 during storage. Cassava starch films with 50 % gelatin, both with and without lipids,

435 exhibited very adequate properties for food coating or packaging although more studies

436 with real food systems are needed in order to design ready-to-market materials.

437

443

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Figure captions

Figure 1. SEM images of the surfaces of starch-gelatin films, containing or not lipids (mono and di-glycerides of fatty acids).

Figure 2. SEM micrographs of the cross-sections of the studied starch-gelatin films, containing or not lipids.

Figure 3. Stress-Hencky strain curves for the starch-gelatin films equilibrated for 1 or 5 weeks at 25 ºC and 53 % RH. A and B correspond to 1 week of storage and C and D correspond to 5 weeks of storage.

Figure 1.

100S-0G

100S-0Ge

75S-25G

75S-25Ge

50S-50G

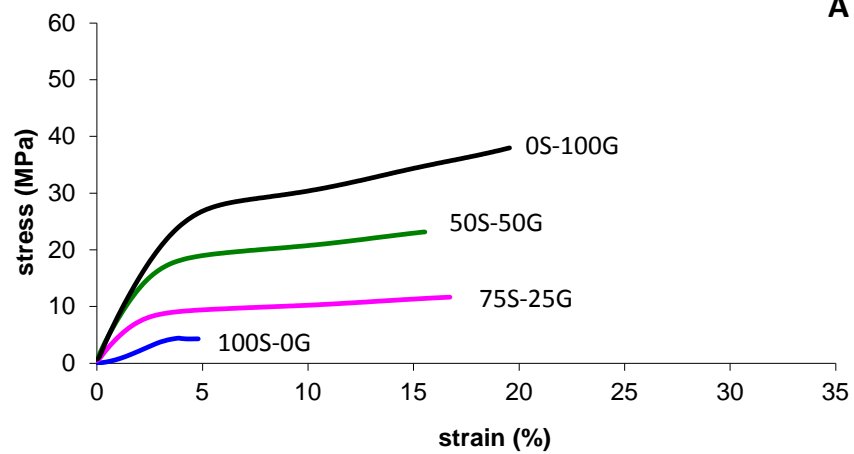50S-50Ge

0S-100G

0S-100Ge
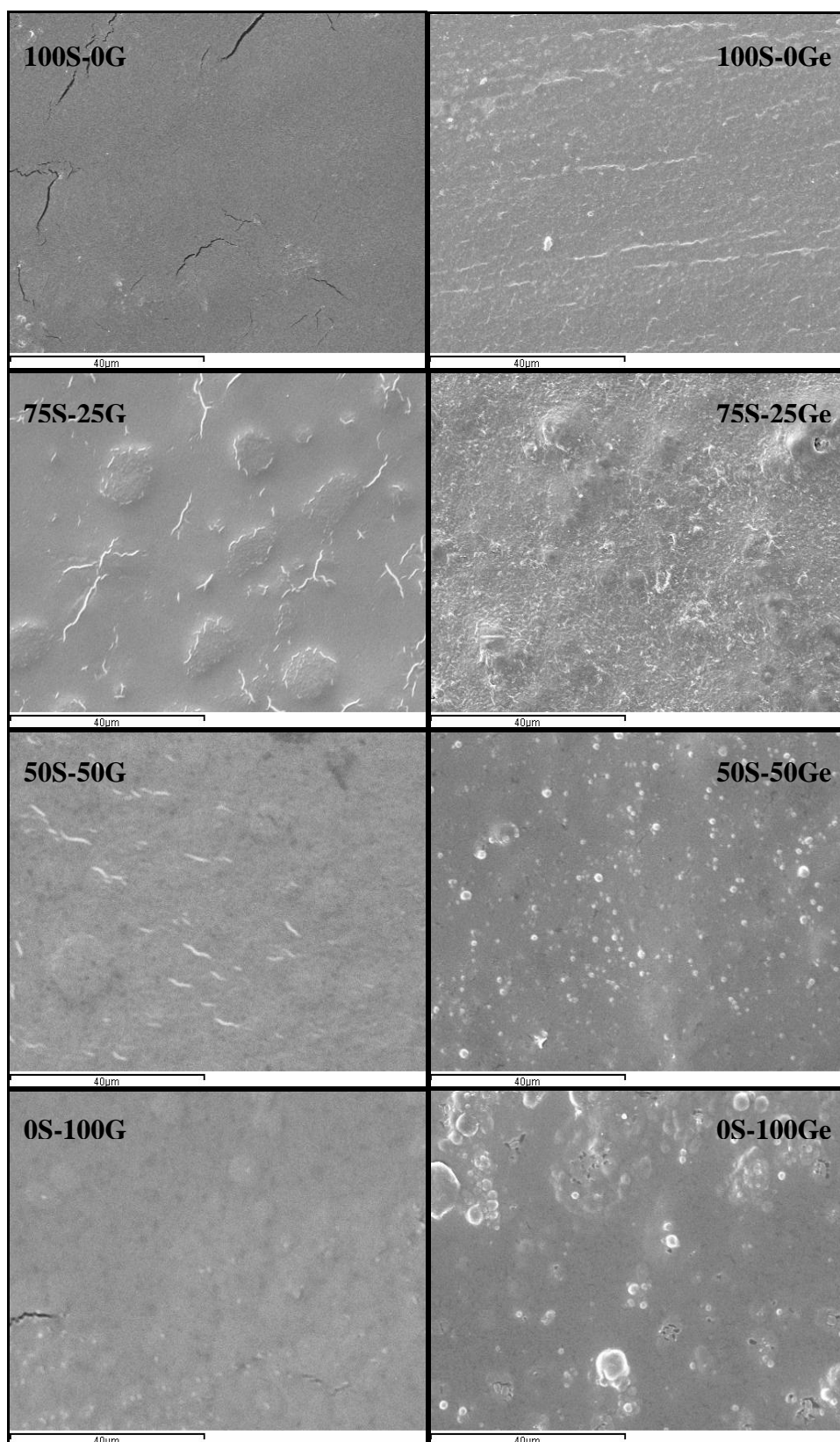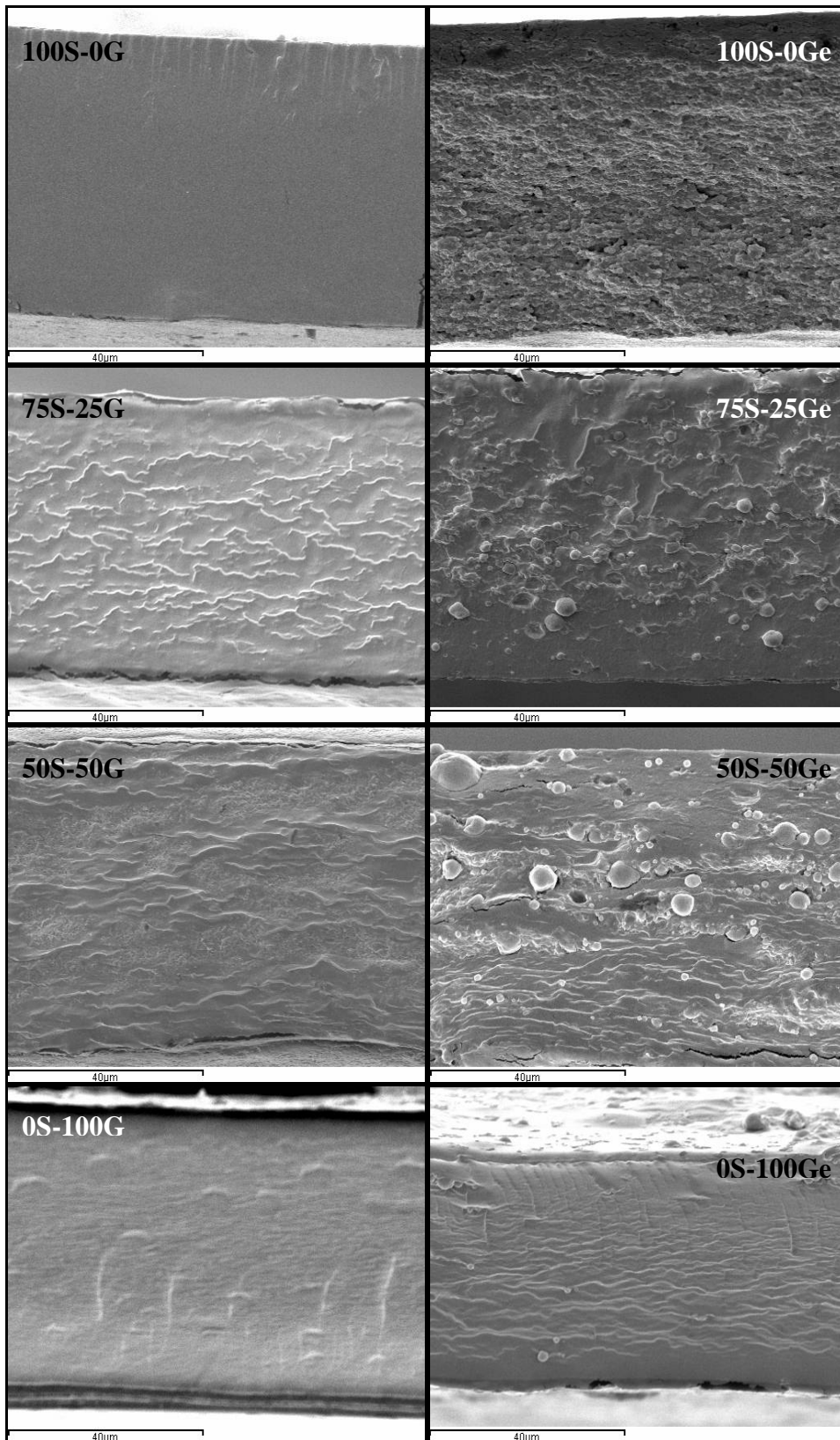
Figure 2.

Figure 3.
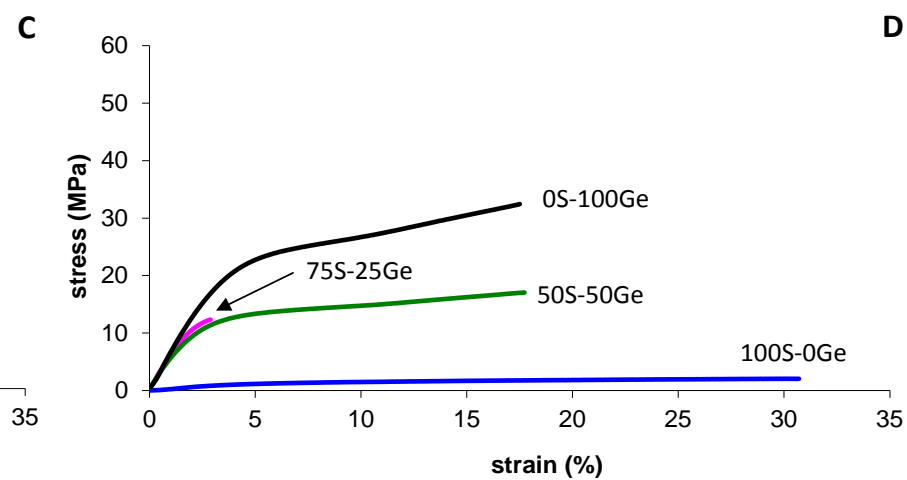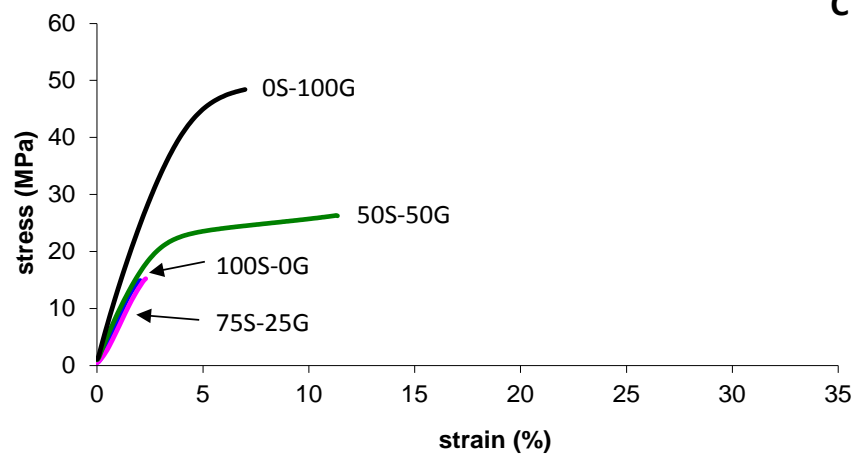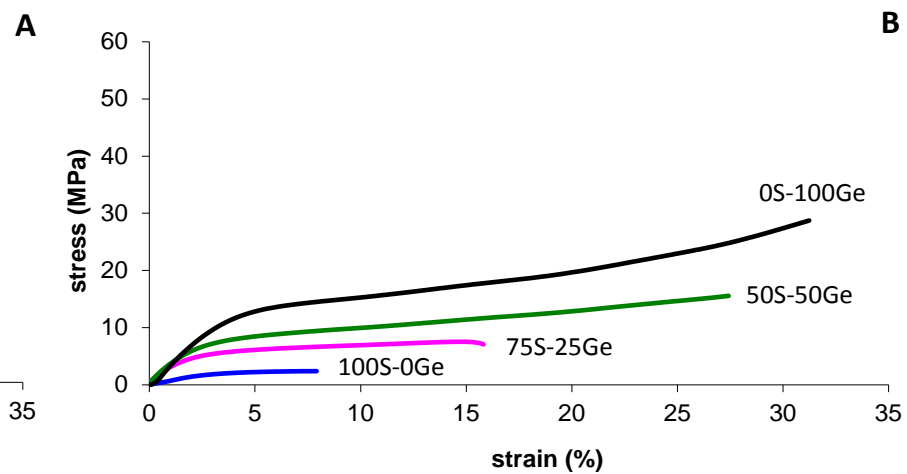
Table 1. Optical properties of starch-gelatin based films stored for both 1 week (initial time) and 5 weeks (final time). S (starch). G (gelatin). e (E471). Mean values and standard deviation.

Sample	Ti$_{480nm}$ (%)		Gloss 60°	
	1 week	5 weeks	1 week	5 weeks
100S-0G	$87.1 \pm 0.3^{(a)(x)(1)}$	$85.80 \pm 0.08^{(a)(x)(2)}$	$18 \pm 5^{(cd)(x)(1)}$	$16 \pm 4^{(a)(x)(1)}$
100S-0Ge	$84.86 \pm 0.09^{(c)(y)(1)}$	$84.66 \pm 0.12^{(b)(y)(1)}$	$11 \pm 2^{(e)(y)(1)}$	$16 \pm 2^{(a)(y)(2)}$
75S-25G	$85.8 \pm 0.3^{(b)(x)(1)}$	$85.8 \pm 0.2^{(a)(x)(1)}$	$24.6 \pm 1.8^{(c)(x)(1)}$	$9.6 \pm 0.8^{(bc)(x)(2)}$
75S-25Ge	$84.243 \pm 0.002^{(d)(y)(1)}$	$83.98 \pm 0.07^{(cd)(y)(1)}$	$11.3 \pm 0.9^{(e)(y)(1)}$	$7.4 \pm 0.8^{(c)(y)(2)}$
50S-50G	$85.80 \pm 0.04^{(b)(x)(1)}$	$84.54 \pm 0.15^{(bc)(x)(2)}$	$37 \pm 4^{(b)(x)(1)}$	$15 \pm 3^{(a)(x)(2)}$
50S-50Ge	$84.6 \pm 0.2^{(cd)(y)(1)}$	$83.7 \pm 0.2^{(d)(y)(2)}$	$14 \pm 2^{(de)(y)(1)}$	$10.7 \pm 0.3^{(b)(y)(2)}$
0S-100G	$85.5 \pm 0.2^{(b)(x)(1)}$	$84.7 \pm 0.2^{(b)(x)(1)}$	$75 \pm 15^{(a)(x)(1)}$	$32 \pm 3^{(a)(x)(2)}$
0S-100Ge	$83.6 \pm 0.1^{(e)(y)(1)}$	$82.79 \pm 0.09^{(e)(y)(1)}$	$10.0 \pm 0.2^{(e)(y)(1)}$	$8.7 \pm 0.3^{(bc)(y)(2)}$

[a–e] Different superscripts within the same column indicate significant differences among formulations ($p < 0.05$).

[x-y] Different superscripts for a same starch-gelatin ratio indicate significant differences among formulations ($p < 0.05$) due to lipid addition (E471).

[1–2] Different superscripts within the same line indicate significant differences among formulations stored for different times ($p < 0.05$).

Table 2. Barrier properties (WVP: water vapour permeability and OP: oxygen permeability), humidity and thickness of the films after storage for 1 or 5 weeks. Mean values and standard deviation.

	WVP (g·mm·KPa^{-1}·h^{-1}·m^{-2})		OP ·10^{-13} (cm^3·m^{-1}·seg^{-1}·Pa^{-1})		Xw (% w.b.)		Thickness (µm)	
Sample	**1 week**	**5 weeks**	**1 week**	**5 weeks**	**1 week**	**5 weeks**	**1 week**	**5 weeks**
100S-0G	5.4 ± 0.4[(bc)(x)(1)]	6.8 ± 0.5[(g)(x)(2)]	0.395 ± 0.002[(a)(x)(1)]	0.253 ± 0.0013[(a)(x)(2)]	8.8 ± 0.7[(ab)(x)(1)]	7.6 ± 0.2[(a)(x)(2)]	75 ± 4[(a)(x)(1)]	65 ± 4[(d)(x)(2)]
100S-0Ge	2.7 ± 0.3[(a)(y)(1)]	1.9 ± 0.4[(a)(y)(2)]	2.51 ± 0.01[(e)(y)(1)]	2.0 ± 0.3[(e)(y)(1)]	7.7 ± 0.8[(a)(x)(1)]	8.6 ± 0.8[(abc)(x)(1)]	76 ± 4[(a)(x)(1)]	74 ± 4[(a)(y)(1)]
75S-25G	6.2 ± 0.7[(cd)(x)(1)]	6.4 ± 0.5[(fg)(x)(1)]	0.49 ± 0.09[(a)(x)(1)]	0.38 ± 0.02[(abf)(x)(1)]	8.9 ± 1.1[(ab)(x)(1)]	8.8 ± 0.3[(bc)(x)(1)]	67 ± 5[(c)(x)(1)]	71 ± 6[(b)(x)(2)]
75S-25Ge	6.4 ± 0.8[(d)(x)(1)]	5.2 ± 0.2[(cd)(y)(2)]	1.330 ± 0.007[(c)(y)(1)]	0.600 ± 0.002[(bcd)(y)(2)]	8.5 ± 0.5[(ab)(x)(1)]	8.0 ± 0.6[(ab)(y)(1)]	70 ± 6[(b)(y)(1)]	74 ± 4[(a)(y)(2)]
50S-50G	5.9 ± 0.4[(bcd)(x)(1)]	6.0 ± 0.6[(ef)(x)(1)]	0.52 ± 0.09[(a)(x)(1)]	0.270 ± 0.001[(ab)(x)(2)]	10 ± 2[(bc)(x)(1)]	10.8 ± 1.3[(d)(x)(1)]	74 ± 3[(a)(x)(1)]	68 ± 3[(bc)(x)(2)]
50S-50Ge	5.2 ± 0.2[(b)(y)(1)]	3.76 ± 0.15[(b)(y)(2)]	1.520 ± 0.005[(c)(y)(1)]	0.860 ± 0.003[(cd)(y)(2)]	9.9 ± 0.4[(ab)(x)(1)]	9.3 ± 0.6[(c)(x)(1)]	71 ± 2[(b)(y)(1)]	60 ± 2[(e)(y)(2)]
0S-100G	6.3 ± 0.5[(d)(x)(1)]	5.5 ± 0.4[(de)(x)(2)]	0.75 ± 0.06[(b)(x)(1)]	0.487 ± 0.001[(abc)(x)(2)]	10.9 ± 1.9[(c)(x)(1)]	11 ± 0.5[(d)(x)(1)]	66 ± 3[(c)(x)(1)]	68 ± 4[(bc)(x)(1)]
0S-100Ge	5.3 ± 0.2[(b)(y)(1)]	4.8 ± 0.5[(c)(x)(1)]	1.88 ± 0.07[(d)(y)(1)]	0.975 ± 0.001[(d)(y)(2)]	9.9 ± 0.7[(bc)(x)(1)]	10.6 ± 0.6[(d)(x)(1)]	75 ± 3[(a)(y)(1)]	72 ± 4[(ab)(y)(2)]

[a–g] Different superscripts within the same column indicate significant differences among formulations (p < 0.05).
[x-y] Different superscripts for a same starch-gelatin ratio indicate significant differences among formulations (p < 0.05) due to lipid addition (E471).
[1–2] Different superscripts within the same line indicate significant differences among formulations stored for different times (p < 0.05).

Table 3. Mechanical properties of starch-gelatin based films, containing or not mono- and di-glycerides of fatty acids, both stored for 1 or 5 weeks. Mean values and standard deviation.

	EM (Mpa)		TS (MPa)		E (%)	
Sample	1 week	5 weeks	1 week	5 weeks	1 week	5 weeks
100S-0G	$131 \pm 31^{(ef)(x)(1)}$	$770 \pm 171^{(b)(x)(2)}$	$4.1 \pm 0.7^{(fg)(x)(1)}$	$13 \pm 2^{(c)(x)(2)}$	$4.5 \pm 0.9^{(e)(x)(1)}$	$1.9 \pm 0.6^{(e)(x)(2)}$
100S-0Ge	$73 \pm 17^{(f)(x)(1)}$	$26 \pm 7^{(e)(y)(2)}$	$2.2 \pm 0.5^{(g)(y)(1)}$	$1.9 \pm 0.3^{(e)(y)(1)}$	$6 \pm 2^{(de)(x)(1)}$	$28 \pm 5^{(a)(y)(2)}$
75S-25G	$354 \pm 51^{(c)(x)(1)}$	$724 \pm 57^{(b)(x)(2)}$	$11.2 \pm 1.4^{(e)(x)(1)}$	$16 \pm 3^{(c)(x)(2)}$	$15 \pm 1^{(bc)(x)(1)}$	$2.4 \pm 0.5^{(e)(x)(2)}$
75S-25Ge	$191 \pm 22^{(e)(y)(1)}$	$544 \pm 68^{(c)(y)(2)}$	$7 \pm 1^{(f)(y)(1)}$	$10 \pm 3^{(d)(y)(2)}$	$17 \pm 3^{(b)(x)(1)}$	$2.2 \pm 0.8^{(e)(x)(2)}$
50S-50G	$627 \pm 54^{(b)(x)(1)}$	$812 \pm 119^{(b)(x)(2)}$	$24 \pm 2^{(c)(x)(1)}$	$30 \pm 3^{(b)(x)(2)}$	$14 \pm 4^{(cd)(x)(1)}$	$12 \pm 3^{(c)(x)(2)}$
50S-50Ge	$271 \pm 46^{(d)(y)(1)}$	$392 \pm 58^{(d)(y)(2)}$	$16 \pm 2^{(d)(y)(1)}$	$16 \pm 2^{(c)(y)(2)}$	$30 \pm 4^{(a)(y)(1)}$	$18 \pm 7^{(b)(x)(2)}$
0S-100G	$703 \pm 23^{(a)(x)(1)}$	$1119 \pm 87^{(a)(x)(2)}$	$37 \pm 2^{(a)(x)(1)}$	$46 \pm 5^{(a)(x)(2)}$	$19 \pm 2^{(b)(x)(1)}$	$8 \pm 2^{(d)(x)(2)}$
0S-100Ge	$336 \pm 28^{(cd)(y)(1)}$	$599 \pm 35^{(c)(y)(2)}$	$28 \pm 5^{(b)(y)(1)}$	$31 \pm 3^{(b)(y)(1)}$	$31 \pm 5^{(a)(y)(1)}$	$17 \pm 4^{(b)(y)(2)}$