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Additional Information

1 **Effect of the incorporation of surfactants on the physical properties of corn starch**

2 **films**

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12 **Abstract**

13 The effect of surfactant addition on structural, mechanical, optical and barrier properties of

14 corn starch-glycerol based films was studied. Sorbitan monopalmitate, monostearate or

15 monooleate were incorporated into starch-glycerol (1:0.25) at a surfactant:starch ratio of

16 0.15:1. The film forming dispersions (FFD) were characterized as to rheology, ζ-potential,

17 particle size distribution and contact angle. Film characterization was carried out at 1 and 5

18 storage weeks (at 25ºC and 53 % relative humidity). Surfactants led to different particle

19 size distribution, zeta potential and viscosity in FFD, and film extensibility, depending on

20 their hydrophobicity and melting properties. Their incorporation to the corn starch-glycerol

21 films produced a coarser film microstructure due to the appearance of free surfactant

22 aggregates or V-amylose inclusion complexes which produce discontinuities in the

23 amorphous continuous matrix. The size of these crystalline complexes was smaller for the

24 surfactant with the lowest hydrophobicity with saturated fatty acid (span 40). This

25 contributed to decrease the WVP values with respect to surfactant-free film. Films

26 containing surfactants were less hard, resistant and extensible, and more permeable to

27 oxygen, than surfactant-free films, but they did not notably affect the film gloss and

28 transparency. Saturated fatty acid compounds with higher melting temperature are

29 recommended to ensure a finer microstructure in the final film which favours water barrier

30 efficiency.

31

32 Key words: starch, surfactants, physical properties, microstructure, ageing**.**

1. Introduction

The environmental conservation policies, the integral use of natural resources and the reduction of hydrocarbon reserves have generated particular interest in developing alternatives to petroleum synthetic polymers for different industrial uses, particularly for foodstuffs. Several studies focused on the development of biodegradable materials to replace, at least partially, conventional plastics. The materials obtained from natural biopolymers, such as polysaccharides, are an interesting alternative, of which, starch is one of the most promising materials for the manufacture of biodegradable plastics (Ma *et al*., 2009). This polymer is a renewable, low cost resource, readily available and has thermoplastic characteristics which permit it to be processed easily by using conventional synthetic polymer methods (Shah *et al*., 1995). However, starch presents worse physical characteristics than synthetic polymers. Its main weaknesses are its highly hydrophilic nature, which makes it a poor water vapour barrier, and the fact that it undergoes retrogradation processes, which implies that its mechanical properties vary over time. Nevertheless, their properties can be modified by adding small quantities of chemical compounds (García *et al*., 2000, Ma *et al*., 2009).

Plasticizers act by increasing the molecular mobility in the polymeric network, thus improving mechanical properties, but reducing the water vapour barrier properties (Rosen, 1993). Glycerol can be added as a plasticizer to improve the mechanical properties of the film, increasing the flexibility and tensile strength by lowering the glass transition temperature (Vieira *et al*., 2011).

Other components with potential capacity to improve some properties of the starch-based films are surfactants. Some studies have found that surfactants may enhance the wettability and stability of the dispersions (Ghebremeskel *et al*., 2007; Chen *et al*., 2009), reduce the

57 starch retrogradation (Jovanovich and Añón, 1999) and improve the water vapour barrier

58 properties (Villalobos *et al*., 2006). Vieira *et al*., 2011 have observed that if the surfactant

59 was added without glycerol, it had a significant effect on mechanical properties but did not

60 significantly modify the water vapour barrier properties. Nevertheless, if the surfactant was

61 added with glycerol, it provoked a reduction in the tensile strength and an increase in both

62 elongation and water vapour permeability. Surfactants have been added to different

63 polysaccharide matrices, such as tapioca starch/decolorized hsian-tsao leaf gum films

64 (Chen *et al*., 2009). When concentration and HLB of surfactant increased, there was an

65 observed improvement in the water vapour barrier properties, although films showed a loss

66 of mechanical resistance. Nevertheless, in matrices of corn starch reinforced with

67 microfibres of cellulose, the mechanical properties were improved with the addition of

68 glyceryl monostearate by the formation of complexes which increase the V-type

69 crystallinity (Mondragón *et al*., 2008).

70 Working on films of potato starch and glycerol, Rodríguez *et al*. (2006) observed that the

71 presence of surfactants increased the wettability of film forming dispersions, with a

72 synergistic effect between glycerol and surfactants. Similarly, in chitosan films, surfactants

73 showed a synergistic effect with the glycerol on the water vapour permeability (Ziani *et al*.,

74 2008). Zhong and Li (2011) also observed that the addition of surfactants and citric acid

75 diminished the surface tension in the film forming dispersions and the formation of

76 crystalline forms in kudzu starch films.

77 Despite several works have report the effect of different surfactants on biopolymer films,

78 no previous studies have been published in corn starch films containing this kind of

79 compounds, except that reported by Jimenez *et al.* 2012 where the effect of different fatty

80 acids, with very low (between 1-2) hydrophilic-lipophilic balance (HLB), was analysed. A

81   low HLB value indicates the predominant lipophilic balance in the molecular structure

82   which can contribute to limit the water vapour permeability of the films, although slightly

83   higher values could improve the better integration of these compounds in the hydrophilic

84   matrix. Likewise, the presence of saturated and unsaturated hydrocarbon chains could also

85   play a relevant role in the component interactions in the matrix. In this sense, the study of

86   the effect a family of surfactants with intermediate HLB values containing saturated or

87   unsaturated chains could give interesting information about the different effects on the

88   properties of the film forming dispersions and films, which are relevant for both coating

89   applications on a determined product and film formation for packaging ends. Surfactants

90   incorporated to film aqueous forming dispersions can act as carrier vehicles of non-polar

91   bioactive compounds (such as antimicrobials or antioxidants), making their dispersion easy

92   in the non-polar core of the formed micellar structures.

93   The aim of this work was to study the effect of addition of sorbitan esters of different fatty

94   acids (saturated and unsaturated), with low-intermediate HLB values (sorbitan

95   monopalmitate: 6.7, sorbitan monostearate: 4.7 and sorbitan monooleate: 4.3) on physical

96   properties of film forming dispersions (particle size distribution, ζ-potential, contact angle)

97   and films (mechanical, optical, structural and barrier properties) of corn starch-glycerol

98   based blends. The effect of storage time on film properties was also analyzed.

99

100   **2. Material and Methods**

101   **2.1 Materials**

102   Corn starch was obtained from Roquette (Roquette Laisa España, Benifaió, Spain). The

103   glycerol and surfactants (sorbitan monopalmitate: span 40, sorbitan monostearate: Span 60

104 and sorbitan monooleate: Span 80) were provided by Panreac Química, S.A. (Castellar del

105 Vallès, Barcelona, Spain).

106

107 **2.2 Preparation of film-forming dispersions (FFD)**

108 Four different dispersions based on corn starch, glycerol and surfactant were prepared by

109 using starch:glycerol:surfactant ratios of 1:0.25:0.15. The starch:surfactant ratio was chosen

110 on the basis of previous studies (Jimenez *et al*., 2012). Corn starch (1% w/w in distilled

111 water) was gelatinized in a thermostat-controlled bath at 95 °C for 30 min. Then, the

112 glycerol and the surfactants (previously melted) were added and the mixtures were

113 homogenized. The homogenization was carried out under vacuum, to avoid bubble

114 formation, by using a venturi vacuum pump connected to the homogenization chamber.

115 This step was performed at 95 ℃ using a rotor-stator homogenizer (Ultraturrax T25, Janke

116 and Kunkel, Germany) for 1 min at 13,500 rpm and for 3 min at 20,500 rpm. Melting

117 temperature of surfactants are 46-47, 54-57 and 0.98 ℃, respectively for Span 40, Span 60

118 and Span 80. Therefore, they remain in liquid state during the homogenization step, thus

119 making their dispersion easy. Prior to the characterization, FFD were cooled to 25 °C.

120 Sample control (FFD-C) was considered as a dispersion containing only starch and

121 glycerol. FFD with surfactants span 40, span 60 and span 80 were designated FFD-S40,

122 FFD -S60 and FFD-S80, respectively.

123

124 **2.3 Characterization of the film-forming dispersions**

125 **2.3.1 Rheological behaviour**

126 The rheological behaviour of FFD was analyzed in triplicate at 25 °C by means of a

127 rotational rheometer (Haake Rheostress1, Thermo Electric Corporation, Germany), by

128 using a coaxial cylinder sensor system (Z34DIN Ti). Rheological curves were obtained

129 after a sample resting time in the sensor of 5 min at 25 °C. The shear stress (σ) was

130 measured as a function of shear rate ($\dot{\gamma}$) from 0 to 512 s^{-1}, obtaining the up and down

131 curves taking 5 min for each one.

132

133 **2.3.2 Particle size, ζ-potential and surface tension measurements**

134 The droplet size distribution, volume-length mean diameter ($D_{4.3}$) and volume-surface

135 mean diameter ($D_{3.2}$) were determined in FFD, in triplicate, with a laser light scattering

136 instrument (Malvern Mastersizer, Malvern Instruments, Worcestershire, U.K.). To obtain ζ-

137 potential values, all FFD were diluted to a droplet concentration of 0.02 % using deionised

138 water. ζ-Potential was determined at 25 °C by mean of Zetasizer nano-Z (Malvern

139 Instruments, Worcestershire, UK). The Smoluchowsky mathematical model was used to

140 convert the electrophoretic mobility into ζ-potential values.

141 The surface tension was determined at 25 °C by means of the ring method using a ring

142 tensiometer (Mobil-K9 Tensiometer, Kruss GmbH, Hamburg, Germany) and a platinum-

143 iridium ring of 19.09 mm in diameter. Measurements were performed in triplicate in each

144 FFD.

145

146 **2.3.3 Contact angle**

147 Due to the potential application in multilayer packaging of these dispersions with synthetic

148 polymers, contact angle (θ) was measured on the surface of polyethylene terephthalate

149 (PET) (Cubil S. L. Barcelona, Spain) copolymer polypropylene (PP) (Cubil S. L.

150 Barcelona, Spain) and polystyrene (PS) (Cubil S. L. Barcelona, Spain). The shape of a

151   sessile drop (0.01 ml) was studied after 10 s by means of a Video-Based Contact Angle

152   Meter model OCA 20 (DataPhysics Instruments GmbH, Filderstadt, Germany). Image

153   analyses were carried out using SCA20 software. Ten replicates were made per

154   formulation.

155

156   2.4 Preparation and characterization of films

157   Films were obtained by casting method. The mass of the FFD containing 1 g of total solids

158   was spread evenly over a teflon casting plate of 15 cm diameter resting on a level surface.

159   Films were formed by drying for approximately 48h at 45% RH and 20 °C. Dry films could

160   be peeled intact from the casting surface and they were conditioned for 1 and 5 weeks at

161   53% RH and 25°C depending on the analysis. RH in the storage chamber was controlled by

162   using an oversaturated solution of Magnesium nitrate-6-hydrat (Panreac Quimica, S.A.).

163   Films without surfactant (control) are denominated F-C and films containing Span 40, Span

164   60 and Span 80, FS-40, FS-60 and F-S80, respectively. Film thickness was measured with a

165   Palmer digital micrometer to the nearest 0.0025 mm at 4-6 random position, depending on

166   the analysis.

167

168   2.4.1 Scanning electron microscopy (SEM)

169   Microstructural analysis of the films was carried out by SEM using a scanning electron

170   microscope JEOL JSM-5410 (Japan). Film samples were maintained in a desiccator with

171   P_2O_5 (Panreac Quimica, S.A.) for two weeks to ensure that water was not present in the

172   sample (theoretical relative humidity in desiccator 0). Afterwards, films were frozen in

173   liquid nitrogen and cryofractured to observe the cross-section and surface of the samples.

174 Films were fixed on copper stubs, gold coated, and observed using an accelerating voltage

175 of 10 kV.

176

177 **2.4.2 X-ray diffraction**

178 X-ray diffraction patterns were recorded using an X-ray diffractometer (XRD, Bruker

179 AXS/D8 Advance). All the aged samples (equilibrated for five weeks at 25 °C and 53%

180 RH) were analyzed between 2θ: 5º and 50º, using Kα Cu radiation (λ: 1.542 Ǻ), 40 kV and

181 40 mA with a step size of 0.05º. For this analysis, samples were cut into 4 cm squares, prior

182 to storage, in order to avoid breakage during handling. Span 40 and Span 60 reagents were

183 also analyzed in order to identify their characteristic peaks.

184

185 **2.4.3 Atomic force microscopy (AFM)**

186 The surface morphology of dried aged films (equilibrated in a desiccator with P_2O_5 for 1

187 week) was analyzed using an atomic force microscope (Multimode 8, Bruker AXS, Santa

188 Barbara, USA) with a NanoScope® V controller electronics. Measurements were taken

189 from several areas of the film surface (50 x 50 µm and 5 x 5 µm) using the Tapping mode.

190 According to method ASME B46.1 (ASME, 1995), the following statistical parameters

191 related with sample surface roughness were calculated: average roughness (Ra: average of

192 the absolute value of the height deviations from a mean surface), root-mean-square

193 roughness (Rq: root-mean-square average of height deviations taken from the mean data

194 plane), and factor of roughness (r: ratio between the three-dimensional surface and two-

195 dimensional area projected onto the threshold plane). Three replicates were considered to

196 obtain these parameters.

197 Phase Imaging mode derived from Tapping Mode, that goes beyond topographical data,

198 was also applied. Phase imaging is the mapping of the phase lag between the periodic

199 signal that drives the cantilever and the oscillations of the cantilever. Changes in the phase

200 lag indicate changes in the properties of the sample surface in composition, adhesion,

201 friction, viscoelasticity, and other properties, including electric and magnetic.

202

203 **2.4.4 Optical properties**

204 The film transparency was determined by applying the Kubelka-Munk theory for multiple

205 scattering to the reflection spectra (Hutchings, 1999). The surface reflectance spectra of the

206 films were determined from 400 to 700 nm with a spectrocolorimeter CM- 3600d (Minolta

207 Co., Tokyo, Japan) on both a white and a black background. As the light passes through the

208 film, it is partially absorbed and scattered, which is quantified by the absorption (K) and the

209 scattering (S) coefficients. Internal transmittance (Ti) of the films was quantified using eq.

210 (1). In this equation R_0 is the reflectance of the film on an ideal black background.

211 Parameters *a* and *b* were calculated by eqs. (2) and (3), where R is the reflectance of the

212 sample layer backed by a known reflectance R_g. Measurements were taken in triplicate for

213 each sample on the free film surface. Wavelength of 450 nm was considered for analysis.

214

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

216

$$a = \frac{1}{2}(R + \frac{R_0 - R + R_g}{R_0 R_g}) \tag{2}$$

218

219 $$b = \sqrt{a^2 - 1} \qquad\qquad (3)$$

220

221   The gloss was measured on the free film surface, at 60° incidence angle, according to the

222   ASTM standard D523 method (ASTM, 1999), by means of a flat surface gloss meter (Multi

223   Gloss 268, Minolta, Germany). Measurements were taken in triplicate for each sample and

224   three films of each formulation were considered. All results were expressed as gloss units,

225   relative to a highly polished surface of black glass standard with a value near to 100.

226   Transparency and gloss measurements were carried out with films conditioned for 1 week

227   (initial time) and 5 weeks (final time) in hermetic desiccators at 25 °C and 53% RH.

228

229   **2.4.5 Moisture content**

230   Films previously conditioned at 53% RH were dried for 24h at 60 °C in a natural

231   convection stove and subsequently placed in a desiccator with P_2O_5 at 25 °C for 2 weeks.

232   The reported results represent the mean values of at least three samples.

233

234   **2.4.6 Water vapour permeability (WVP)**

235   WVP measurements were carried out with films conditioned for 1 and 5 weeks in hermetic

236   desiccators at 25 °C and 53% RH. WVP of films was determined by using the ASTM E96-

237   95 (ASTM, 1995) gravimetric method, taking into account the modification proposed by

238   McHugh et al., 1993. Films were selected for WVP tests based on the lack of physical

239   defects. Distilled water was placed in Payne permeability cups (3.5 cm diameter, Elcometer

240   SPRL, Hermelle/s Argenteau, Belgium) to expose the film to 100% RH on one side. Once

241   the films were secured, each cup was placed in a relative humidity equilibrated cabinet at

242   25 °C, with a fan placed on the top of the cup in order to reduce resistance to water vapour

243   transport, thus avoiding the stagnant layer effect in this exposed side of the film. RH of the

244   cabinets (53%) was held constant using oversaturated solutions of magnesium nitrate-6-

245   hydrate. The free film surface during film formation was exposed to the lowest relative

246   humidity to simulate the actual application of the films in high water activity products when

247   stored at intermediate relative humidity. The cups were weighed periodically (0.0001 g)

248   and water vapour transmission (WVTR) was determined from the slope obtained from the

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

250   divided by the film area. From WVTR data, the vapour pressure on the film's inner surface

251   (p2) was obtained with eq. (4), proposed by McHugh et al. (1993), to correct the effect of

252   concentration gradients established in the stagnant air gap inside the cup.

253

$$WVTR = \frac{PDL_n\left[\dfrac{P-p_2}{P-p_1}\right]}{RT\Delta z} \tag{4}$$

254

255

256   where P, total pressure (atm); D, diffusivity of water through air at 25 °C (m2 /s); R, gas

257   law constant (82.057×10^{-3} m^3 atm $kmol^{-1}$ K^{-1}); T, absolute temperature (K); Δz, mean

258   stagnant air gap height (m), considering the initial and final z value; p_1, water vapour

259   pressure on the solution surface (atm); and p_2, corrected water vapour pressure on the film's

260   inner surface (atm). Water vapour permeance was calculated using eq. (5) as a function of

261   p_2 and p_3 (pressure on the film's outer surface in the cabinet).

262

263
$$permeance = \frac{WVTR}{p_2 - p_3} \qquad (5)$$

264

265 Permeability was obtained by multiplying the permeance by the average film thickness.

266

267 **2.4.7 Oxygen permeability (OP)**

268 The oxygen permeation rate of the corn starch films was determined at 53% RH and 25 °C

269 using an OX-TRAN Model 2/21 ML Mocon (Lippke, Neuwied, Germany). Film samples

270 were previously conditioned for 1 or 5 weeks at 25 ºC and 53% RH by using magnesium

271 nitrate-6-hydrate saturated solutions. Two samples were placed in the equipment for

272 analysis and they were conditioned in the cells for 6 h, then the transmission values were

273 determined every 20 min until the equilibrium was reached. The exposure area during the

274 tests was 50 cm^2 for each formulation. In order to obtain the oxygen permeability, film

275 thickness was considered in all cases. Measurements were carried out in triplicate.

276

277 **2.4.8 Tensile properties**

278 The mechanical behaviour of films was tested by using conditioned samples (1 or 5 weeks

279 at 25 ºC and 53 % RH). A universal test Machine (TA.XTplus model, Stable Micro

280 Systems, Haslemere, England) was used to determine the tensile strength (TS), elastic

281 modulus (EM), and elongation (E) of the films, according to ASTM standard method D882

282 (ASTM, 2001) EM, TS, and E were determined from the stress-strain curves, estimated

283 from force-distance data obtained for the different films (2.5 cm wide and 10 cm long).

284 Equilibrated samples were mounted in the film-extension grips of the testing machine and

285 stretched at 50 mm min^{-1} until breaking. The relative humidity of the environment was held

286   at nearly 53% during the tests, which were performed at 25 °C. At least seven replicates

287   were obtained from each sample.

288

289   **2.5 Statistical analysis**

290   Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, MD) was used to carry

291   out statistical analyses of data through analysis of variance (ANOVA). Fisher's least

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

293

294   **3. Results and discussion**

295   **3.1 Properties of film forming dispersions**

296   Figure 1 shows the particle size distribution for the film forming dispersions containing

297   corn starch and surfactants (Span 40, Span 60 and Span 80). The values of mean diameters

298    $D_{3,2}$ and $D_{4,3}$ are shown in Table 1. A similar distribution is observed in all cases. Most of

299   the volume fraction of the dispersed particles is under 1 µm in size, although there are large

300   particles (over 10 µm) in all cases that greatly contribute to the average size. $D_{4,3}$ values

301   indicate that the particle size of FFD-S80 was significantly smaller ($p < 0.05$) than those of

302   FFD-S40 and FFD-S60 whereas no significant differences ($p < 0.05$) were observed

303   between the particle sizes of these last FFD.

304   Surfactants, as polar lipids, form different kind of molecular association (association

305   colloids) in aqueous systems, where the hydrocarbon chains are oriented to minimize the

306   contact with water molecules. These aggregates (micellar structures) have different size and

307   shape depending on their relative water affinity (HLB) and the overall balance of molecular

308   interactions which minimize the total free energy of the system (Dickinson, 1992). Usually,

309   the lower the water affinity (HLB) the greater the aggregation number and so, the greater

310   the particle size. In this sense, it is remarkable that S80, with the lowest HLB, forms the

311   smallest particles which could be related with the double bond in the oleic acid chain,

312   which make the packing of molecules more difficult. This is also related with the liquid

313   state of this compound under room conditions, whereas S40 and S60 are solids under the

314   same conditions. There are a greater proportion of large particles in FFD-S60 and FFD-

315   S40, which may be associated with the formation of big aggregates of the surfactant

316   molecules (Villalobos et al., 2005) or even combined aggregates of surfactants and starch

317   polymer chains. Associations of helical amylose which give rise to gel zones in the FFD

318   could also be detected as particles in the measurements. Surfactant molecules can also

319   participate in these associations through the formation of complexes with helical

320   conformation of amylose (Wokadala et al., 2012).

321   Table 1 shows the rheological behaviour, ζ-potential, surface tension and contact angle with

322   different polymer films (PET: Polyethylene terephthalate, PP: polypropylene copolymer,

323   PS: polystyrene) of FFDs containing corn starch and surfactants. All film-forming

324   dispersions showed similar rheological behaviour. At low shear rates, Newtonian behaviour

325   was observed, but from a determined shear rate, FFDs exhibited shear thickening

326   behaviour. No differences were observed between up and down which means that no

327   tixotropic effects occur during the fluid flow.

328   Table 1 shows the Newtonian viscosity values in the first step and the shear rate values

329   where the change in behaviour occurs, which range between 130 and 173 s^{-1}. The shear

330   thickening behaviour from a determined shear rate can be explained by the induced

331   orthokinetic flocculation of particles. Particle aggregates retain a part of the continuous

332   phase and this, together with the greater flow resistance of the bigger aggregates, defines

333   the higher shear stress-shear rate relationship or flow resistance (Peker and Helvaci, 2007).

334 Non-Newtonian behaviour has previously been observed by other authors in starch

335 dispersions and pastes (Zhong *et al*, 2009) where the viscosity varied according to the shear

336 rate applied. Table 1 shows that the presence of surfactants caused a significant increase

337 ($p<0.05$) in the Newtonian viscosity in all cases; the FFD-S40 dispersion exhibiting the

338 highest values. No significant differences were observed between the FFD-S60 and FFD-

339 S80 formulations despite their different particle sizes. This viscosity increase provoked by

340 the addition of surfactants is explained by the greater concentration of dispersed/dissolved

341 species in the aqueous media.

342 All dispersions had negative ζ-potential values with significant differences ($p<0.05$) among

343 formulations (Table 1). All FFDs containing surfactants showed higher values of ζ-

344 potential than that containing only starch. This can be due to the promotion of the

345 adsorption of negative ions on the polar heads of the surfactant associations. Some authors

346 (Ho and Ahmad, 1999; Hsu and Nacu, 2003) have reported that at pH near 7, non-ionic

347 surfactants attract OH^- ions of the aqueous medium, thus increasing the surface charge of

348 the lipid associations while a pH decrease is promoted.

349 In Table 1, the lower pH of FFD-S60 and FFD-S80 can be observed, while these FFDs also

350 showed the highest surface charge. Variations in ζ-potential could also be due to the

351 different degree of hydrolysis in sorbitan esters, giving rise to free fatty acids with ionisable

352 groups. The ζ-potential is defined as the electrostatic potential in the layer of the particle

353 electrophoretic mobility and greatly affects the stability of the colloidal systems by

354 electrostatic forces (Ravina and Moramarco, 1993). Accordingly, the most stable FFD

355 would be FFD-S80 and FFD-S60 because the contribution of repulsive forces between

356 particles to the stability would be greater. However, it should be noted that colloidal

357 stabilization is the result of the interaction of numerous factors. In the case of FFD, the

358   particle charge may not be enough to ensure stability of the dispersion because it is a

359   complex system in which the hydrocolloid interactions (amylose and amylopectin) also

360   play an important role. In particular, the viscosity of the continuous phase, which depends

361   on the molecular interactions, greatly affects the system stability. So, as FFD-S80 and FFD-

362   S60 are less viscous, destabilization phenomena during film drying could take place more

363   easily, promoting coalescence and creaming, despite their greater surface charge. Other

364   factors, such as the physical state of the lipid, may affect the coalescence phenomena, this

365   being inhibited in solid lipids.

366   Surface tension values showed significant differences ($p<0.05$) among formulations. The

367   control dispersion (FFD-C) exhibited similar values to pure water (72 mN/m at 25 °C), but,

368   as expected, the presence of surfactants reduced the surface tension of the dispersions to

369   values below 47.6 mN/m at 25 °C. The surface tension reduction in the FFDs is interesting

370   from the point of view of their application on a determined surface as coatings, since this

371   favours their extensibility during the product coating (Fernandez et al., 2006) and the

372   possible formation of multi-layer materials with other polymers (Chen *et. al.,* 2010)

373   The contact angle values of FFD on the surface of different polymer films (PET:

374   Polyethylene terephthalate, PP: polypropylene copolymer, PS: polystyrene) are shown in

375   Table 1. Values lower than 90º indicate surface wettability and therefore greater

376   extensibility of the dispersion on the polymer. The polymer which was least wettable with

377   the studied dispersions was PP, since the greatest contact angles were obtained in this case

378   ($p<0.05$). PET had the lowest contact angles and therefore it offers the best possibilities to

379   be coated with the studied FFDs, for instance as vehicles of active compounds, thus

380   obtaining multi-layer films. Nevertheless, the obtained values were dependent on the

381 composition of the FFDs and it was remarkable that the formulations without surfactant

382 were those which showed the greatest extensibility despite their higher surface tension.

383

384 **3.2 Microstructure of the films**

385 Films obtained had 50 ± 5 μm in thickness, regardless of the FFD composition. The final

386 structure of the film depends on both the interactions of film components and the drying

387 conditions of the film-forming dispersion and has a great impact on the different film

388 properties (Villalobos et al., 2005). The microstructural analysis of the films gives relevant

389 information about the arrangement of the components, which, in turn, allows us to explain

390 the differences in the barrier, mechanical or optical properties of the films.

391 Figure 2 shows the SEM micrographs of the surface (left) and the cross-section (right) of

392 starch based films with and without surfactants, conditioned for one week. Control films

393 (F-C) showed an homogeneous structure, coherent with the formation of a compact

394 arrangement of polymer chains. The addition of surfactants resulted in less homogeneous

395 films whose differences can be observed in the cross-section images. Films with surfactants

396 showed a coarser cross section than the control films, which suggests that polymer chain

397 packaging was interrupted by other structural elements present in the matrix. These

398 elements may be free surfactant particles, resulting from the micellar structure in the

399 aqueous FFD and surfactat particles of V-amylose inclusion complexes which produce

400 discontinuities of different magnitude in the amorphous continuous matrix, thus

401 contributing to weaken the bond strength between the polymer chains in the amorphous

402 matrix. Interactions of polar lipids with V-amylose leads to complex formation, where the

403 helical conformation of amylose traps the hydrocarbon chains of lipids, as has been

404   previously described by other authors (Singh et al., 2002). V-amylose forms small particles

405   with size in the nano or micro scale, depending on medium conditions (Lesmes et al.,

406   2009).

407   In F-S80, micro-droplets of span 80 (liquid at room temperature) can be seen in the matrix,

408   whereas no droplets can be observed for F-S40 and F-S60, but finer no spherical

409   formations. Lipid droplets also appear on the F-S80 film surface, even larger than in the

410   cross-section due to the coalescence and creaming phenomena occurring in the drying step.

411   Similar phenomena were observed by other authors (Zhong and Li, 2011) in kudzu starch

412   films. The presence of droplets in F-S80 films is related with the liquid state of span 80 at

413   room temperature and reveals the no total integration of the lipid in the starch matrix, and

414   its separation in a dispersed liquid phase. Span 40 and span 60 seem also separated

415   (discontinuities in the matrix) but their solid state at room temperature did not allow

416   droplets formation, but crystalline or amorphous solid particles.

417   Figure 3 shows the phase imaging analysis obtained from the Tapping Mode AFM for films

418   conditioned for five weeks. The phase image is providing material property contrast on the

419   film surface since this technique is sensitive to variations in composition, adhesion, friction,

420   viscoelasticity, and other surface properties (Baker et al., 2001). In control film (F-C), the

421   image reveals the presence of lighter zones which can be attributed to crystalline aggregates

422   of helical amylose (Baker et al., 2001). In films containing span 40 the presence of lighter

423   spots reveals the presence of lipid particles on the film surface due to lipid coalescence and

424   creaming during film drying and subsequent solidification. The excess of lipid contributed

425   to its separation in the matrix and its migration to the film surface where solidifies,

426   according to its high melting temperature (46-47 ºC). In the case of span 60, no big lipid

427   formations were observed on the film surface probably due to its higher melting

428 temperature (54-57ºC), which contribute to inhibit coalescence phenomena during the film

429 drying step, which also limits creaming, due to the restrictions in the particle size growth.

430 For the film containing span 80 (liquid at room temperature, melting point: 0.98 ºC) a great

431 proportion of lipid formations can be observed on the film surface, coherent with a high

432 progress of the coalescence and creaming phenomena during the film drying. The irregular

433 shape of lipid droplets reveals that they are droplet aggregates, whose surface is covered by

434 a thin polymer layer, which entraps them.

435 The melting properties and molecular characteristics of the surfactant had an impact on the

436 film structure and surface morphology which will affect the film properties, mainly its

437 mechanical and optical parameters.

438 Figure 4a shows X-ray diffraction (XR) spectra of corn starch films with and without

439 surfactants, equilibrated at 25°C and 53% RH for 5 weeks. Generally, the native starch

440 crystallinity is types A and B, type C being an intermediate form (Jiménez et al., 2013);

441 crystalline V type could also be observed, corresponding to the amylose helical

442 associations, which are the result of the amylose complexation with other substances, such

443 as lipids (Jiménez et al., 2013). The addition of surfactants affected the crystallization

444 pattern of the films. Control film showed the typical peaks of amylose type B, with a peak

445 of maximum intensity at 2θ 17º and other peaks at 2θ about 15º, 20º and 22 º (Lesmes et al.,

446 2009). When surfactants were added, two main peaks are shown at 2θ about 13° and 20°, as

447 has been observed for V-type amylose hosting complexes with long chain fatty acids

448 (Lesmes et al., 2009). So, the XR spectra reveal that surfactants in the film induce the

449 formation of complexes with amylose, as observed for other lipids. Wokadala et al., (2012)

450 reported peaks at 2θ 7.5º, 13º and 20º, attributable to V-type crystallinity as a result of the

451 presence of amylose-lipid complexes in hydrolyzed maize starch in the presence of stearic

452 acid.

453 By considering the XR diffraction pattern of the pure surfactants (Figure 4b), where a very

454 sharp peak appears at 2θ 22º, in both cases, the non-crystalline forms of the non-complexed

455 (free) lipid in the films must be deduced, since at this angle no sharp peak was observed in

456 the film RX diffraction spectra.

457 These results indicate that in the structure of surfactant-starch films, V-type amylose

458 complexes are formed with surfactant molecules giving rise to crystalline aggregates,

459 whereas the excess of lipid is not crystallized. These crystalline aggregates interrupt the

460 starch matrix producing discontinuities in the same way as the free surfactant molecules. In

461 the case of span 80, free lipids are clearly observable in the SEM micrographs as liquid

462 droplets dispersed in the starch matrix, with a high degree of lipid creaming on the film

463 surface. Film F-S40 showed the lowest peak intensity in the XR spectra, which suggests

464 that V-type crystalline forms were the smallest in size, which could have a positive effect

465 on the film properties.

466

467 **3.3 Physical properties of films**

468 Table 2 shows the optical properties (gloss at incidence angle of 60° and internal

469 transmittance at 450 nm) of corn starch films with and without surfactants, conditioned for

470 one and five weeks. The gloss values obtained were less than 35 units and, therefore, films

471 can be considered of low gloss (Villalobos et al., 2005). The addition of surfactants did not

472 have a significant effect (p>0.05) on the gloss values as compared with the control

473 formulation. In the case of F-S80, the gloss significantly increased during the storage time,

474 which did not occur in the other cases. This increase can be attributed to a progressive

475 migration of the liquid droplets to the film surface, in line with the progressive chain

476 aggregations and the reduction of the matrix volume. The fluid lipid acted as a filler of the

477 micro-pores in the surface, thus reducing the apparent roughness and increasing the gloss.

478 Table 2 shows the values of roughness parameters (R_a, R_b and r) analyzed through AFM in

479 films stored for five weeks. No significant differences in the film roughness were detected,

480 except for F-S40 where higher values were obtained. Nevertheless, this difference was not

481 coherent with the gloss values, unlike that reported by other authors (Villalobos et al.,

482 2005). This can be due to the liquid state of span 80 which interferes with the cantilever

483 signal in terms of the height of peaks on the surface.

484 All films were highly transparent, as deduced by the values of the internal transmittance

485 (Ti) in Table 2. This is associated with a greater homogeneity in the film structure. The

486 addition of surfactants resulted in a slight decrease of the transparency with respect to the

487 control film, in line with a more anisotropic structure due to the formation of crystalline

488 forms and the presence of dispersed surfactants aggregates, with a different refractive

489 index, which enhance light scattering, as observed by Jimenez et al. (2012) in corn starch

490 films containing fatty acids. The F-S80 films showed a significantly ($p<0.05$) lower Ti,

491 which can be attributed to the liquid droplet dispersion observed in the film microstructure.

492 During film storage, no notable changes in Ti could be observed, although control films

493 showed a slight decrease which could be attributed to the greater progress of amylose

494 crystallization, thus inducing a greater degree of anisotropy (Jimenez et al., 2012).

495 Table 3 shows the moisture content, water vapour permeability (WVP), oxygen

496 permeability (OP) and mechanical properties of corn starch films, with and without

497 surfactants. The incorporation of surfactants greatly decreased the equilibrium water

498 content of the films, even if this is considered in the lipid free basis (4.3, 4.9 and 5.4,

499   respectively for the films with span 40, span 60 and span 80). This agrees with the changes

500   in the packing of a part of the polymer chains involved in the V-type crystalline forms

501   complexing the lipids, which reduces the water binding capacity of these chains.

502   The WVP was measured at a gradient of 53-100% RH. At one week of storage, the

503   addition of span 40 implied a significant ($p < 0.05$) reduction of WVP, although this was

504   not observed for films containing span 60 (without significant differences with respect to

505   the control) and span 80, where a significant increase was observed, more markedly at 5

506   weeks' storage. The improvement of the water vapour barrier properties due to the addition

507   of surfactants has been observed in different matrices, such as tapioca starch with sucrose

508   esters (Chen et al., 2009), hydroxypropyl methylcellulose with span 60 and sucrose esters

509   (Villalobos et al., 2006) and kudzu starch and ascorbic acid based films with Tween 20

510   (Zhong and Li, 2011). The scarce effect of surfactants on WVP observed in these films,

511   regardless of their hydrophobicity, could be explained by the formation of crystalline

512   structures in all cases (even in control films) which could be a strong limitation factor for

513   transfer of water molecules.

514   In terms of WVP, the different behaviour induced by each of the three studied surfactants

515   must be related with the structural differences reached in the matrix for each case. F-S40

516   showed the smallest V-type crystalline forms, as deduced from RX spectra, which could

517   imply a great increase in the tortuosity factor for mass transfer in the matrix, thus reducing

518   WVP. On the contrary, the increase in WVP provoked by the addition of span 80 could be

519   related with the greater size of crystalline complexes. Lesmes et al. (2009) demonstrated

520   that increased fatty acid unsaturation affects the microscopic and nanoscopic arrangement

521   of V-amylose, and fatty acid unsaturation leads to bigger and more disperse populations.

522   This can suppose a more open structure where water molecules could diffuse more easily.

523   No significant differences (p<0.05) between WVP of the films stored for one and five

524   weeks were observed, except for F-S80 where WVP increased over time. This could be

525   explained by the above mentioned migration of the free liquid droplets of span 80 to the

526   film surface, leaving fewer hydrophobic areas or void nanopores in the matrix, which

527   facilitate transfer of water molecules.

528   Oxygen permeability values (Table 3) significantly (p<0.05) increased when surfactants

529   were incorporated into the films, as usually happens when hydrophobic components are

530   added, since these substances are more permeable to gases due to their greater chemical

531   affinity and solubility (Miller and Krochta, 1997). The oxygen permeability did not vary

532   with the storage time, except in the control film, where it decreased significantly (p <0.05).

533   This can be explained by the progressive increase in the film crystallinity, as observed in

534   previous works (Donhowe and Fennema, 1993; Jimenez et al. 2012).

535   Table 3 shows the values of the parameters used to describe the mechanical properties of

536   starch based films at 25 °C and 53% RH: elastic modulus (EM), tensile strength (TS) and

537   elongation at break (E). In general, the addition of surfactants caused the decrease of EM,

538   TS and E, in agreement with the formation of a more anisotropic structure with reduced

539   cohesion forces. Similar results were observed by Jimenez et al. (2012) in corn starch films

540   when fatty acids were added. Rodriguez, et al. (2006) also observed that the addition of

541   surfactants in the presence of glycerol causes a significant reduction in tensile strength and

542   elongation at break with respect to the control formulation. TS and EM initial values for F-

543   S80 were significantly higher (p<0.05) than for the other films with surfactants, which

544   showed very similar values. This indicates that bonding forces in the matrix were more

545   intense in the F-S80 films. Nevertheless, all films were very short, especially F-S60 and F-

546   S80, which showed the lowest deformation at break, thus indicating the low capacity of the

547 structural elements to slippage during tensile test without structural failure. This can be

548 attributed, in part, to the formation of crystalline zones with low deformability.

549

550 **4. Conclusions**

551 Incorporation of span 40, 60 or 80 into the corn starch-glycerol film forming dispersions

552 led to different particle size distribution, zeta potential, viscosity and extensibility on other

553 films, depending on their hydrophobicity and melting properties (related with the

554 unsaturation in the fatty acid chain). These aspects affected the final film microstructure

555 and its surface morphology, since the growing of the surfactant molecule aggregates during

556 the film drying (loss of water availability) occurred to a different extent, depending on the

557 dispersion stability. When the surfactant melting temperature was the highest (span 60),

558 creaming of surfactants in the film surface was not observed and the size of the final lipid

559 aggregates in the film were lower. Likewise, the size of the type-V crystalline amylose

560 complexes was smaller for the surfactant with the highest HLB value with saturated fatty

561 acid (span 40). This contributed to decrease the WVP values with respect to surfactant-free

562 film. Nevertheless, films containing surfactants were less hard, resistant and extensible than

563 surfactant-free films, but they did not notably affect the film gloss and transparency. The

564 use of surfactants in film formulation can be useful to incorporate non-polar bioactive

565 compounds in the aqueous film forming dispersions. In this sense, the obtained results

566 allow us to recommend saturated fatty acid compounds with higher melting temperature to

567 ensure a finer microstructure in the final film which favour water barrier efficiency.

568

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