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Additional Information

1 **The role of some antioxidants in the HPMC film properties and lipid protection in coated**
2 **toasted almonds.**

3
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7
8 **Abstract**

9 Hydroxypropylmethylcellulose based edible films with and without antioxidant additives
10 were characterised as to their microstructure, water vapour and oxygen permeability,
11 mechanical behaviour, optical properties and protective ability against lipid oxidation. The
12 corresponding film forming dispersions were also used to coat toasted almonds in order to test
13 their effectiveness at protecting against rancidity development. The efficiency of three additives
14 (ascorbic acid, citric acid or ginger essential oil) was tested and compared with antioxidant-free
15 coatings. A cross-linking effect in the film matrices containing ascorbic or citric acid was
16 detected through the analysis of the film microstructure, mechanical behaviour and barrier
17 properties to oxygen and water vapour. These films were the most effective protectors against
18 oxidation of almonds, due to both their antioxidant effect and the tighter structure which leads to
19 lower oxygen permeability. In films with ginger oil, the hydrophobic effect markedly reduced
20 water vapour permeability at low temperatures, but protection against lipid oxidation was less
21 effective at long storage times.

22
23 **Keywords:** edible coatings, hydroxypropylmethylcellulose, ascorbic acid, citric acid, ginger oil,
24 almond

25
26 **1. Introduction**

27 Nuts have been claimed as a healthy food choice, mainly because of their proven
28 cardioprotective effects. Numerous clinical studies have revealed that low-density lipoprotein
29 cholesterol reductions of 10-15% have been observed where walnuts, almonds, macadamias,
30 hazelnuts, pecans or peanuts were incorporated into the diet (Kurlandsky & Stote, 2006).

31 According to Ahmad (2010), almonds are a highly nutritional source of vitamins (B complex) and
32 minerals (Mg, P, K). Despite containing high levels of fat (51% w/w.), they have a favourable
33 fatty acid profile (64-82% oleic acid, 8-28% linoleic acid, 6-8% palmitic acid). The oxidation
34 process of unsaturated fatty acids results in rancid off-flavours, thus shortening the shelf-life of
35 this product under ambient conditions.

36 The potential interest in the use of biodegradable films has been extensively reviewed
37 by numerous authors (Guilbert, Gontard & Gorris, 1996; Han & Gennadios, 2005; Nussinovitch,
38 2009). Edible coatings provide a semipermeable barrier against the transfer of moisture, gases
39 and other agents, thus extending the shelf-life and raising the quality of foods, by limiting the
40 deteriorative reactions. Films made from proteins and carbohydrates are excellent barriers to
41 oxygen, because of their tightly packed, ordered hydrogen-bonded network structure (Yang &
42 Paulson, 2000). On the other hand, these materials have poor water vapour resistance due to
43 their hydrophilic nature.

44 According to Mauer, Smith & Labuza (2000), the interest in biodegradable packaging
45 stems from the drive to find environmentally friendly packaging, to improve the quality of food
46 products and to find new markets for existing materials. Additionally, edible films may be
47 effective carriers of food additives (Lee, Park, Lee & Choi, 2003). Baldwin & Wood (2006)
48 demonstrated the efficacy of cellulose derivative films and several additives (α -tocopherol, citric
49 acid, BHA, BHT) in prolonging the shelf-life of pecan kernels. Ayranci & Tunc (2003) succeeded
50 in reducing the oxygen permeability of methyl cellulose films with the inclusion of ascorbic acid
51 or citric acid, thus slowing down the browning reactions when the coatings were applied.

52 The aim of this work was to study the impact of some additives with proven antioxidant
53 properties (ascorbic acid, citric acid and ginger oil) on the effectiveness of
54 hydroxypropylmethylcellulose (HPMC) films and coatings as protectors against lipid oxidation.
55 Additionally, the obtained films were characterised as to their relevant functional characteristics
56 like mechanical behaviour, water vapour permeability and optical properties, to know the effect
57 of the additives on the polymer matrix properties.

58

59 **2. Materials and methods**

60

61 2.1. Materials

62 The film-forming dispersions were prepared with hydroxypropylmethylcellulose (HPMC,
63 Sigma-Aldrich, Co. St. Louis, USA), ascorbic acid (Panreac Química, S.A. Barcelona, Spain),
64 citric acid (Fisher Scientific Afora, Valencia, Spain), ginger essential oil (Essential aròms, Torre-
65 Serona, Lleida, Spain) and Tween 80 (Panreac Química, S.A. Barcelona, Spain).

66

67 2.2. Preparation of the film-forming dispersions and rheological characterization

68 HPMC (5% w/w) (average molecular weight 748 g/mol) was dispersed in deionised
69 water at 80°C, and left stirring over night. Ascorbic acid (A), citric acid (C) or ginger oil (G) were
70 added under magnetic stirring at 0.5% w/w (ratio HPMC: additive 1:0.1). Tween 80 was
71 incorporated to reduce the surface tension (HPMC: Tween 80 1:0.05) and contact angle with
72 the almond surface. Homogenization was carried out by using a vacuum high-shear probe
73 mixer (Ultraturax T25, Janke & Kunkel, Germany) at 13,500 rpm for 3 min, and the dispersions
74 were degasified at room temperature. From here onwards, the four formulations will be referred
75 to as H (HPMC), HA (HPMC-ascorbic acid), HC (HPMC-citric acid) and HG (HPMC-ginger oil).
76 Their viscosity (η) was determined in triplicate at 25 °C, with a rotational rheometer (HAAKE
77 Rheostress 1, Thermo Electric Corporation, Karlsruhe, Germany), fitted with a type Z34DIN Ti
78 sensor system of coaxial cylinders. The shear stress (σ) was measured as a function of shear
79 rate ($\dot{\gamma}$) from 0 to 512 s⁻¹.

80

81 2.3. Film casting

82 The amount of emulsions that would provide 1g of total solids was spread evenly over
83 each casting plate (150mm diameter), thus resulting in a solids distribution of 57 g solids/m².
84 Films were formed by drying on a level surface for approximately 24 h at 45% RH and 20 °C,
85 and were peeled off from the casting surface. The films were stored in cabinets at 25°C at two
86 levels of relative humidity (RH), namely 33% and 53%, using oversaturated solutions of MgCl₂
87 and Mg(NO₃)₂, respectively (Panreac química, S.A., Castellar del Vallés, Barcelona, Spain).

88

89 2.4. Characterization of the films

90

91 2.4.1. *Thickness*

92 A Palmer digital micrometer (Comecta, Barcelona, Spain) was used to measure film
93 thickness to the nearest 0.001 mm. Six random positions were considered for WVP tests, and
94 four measurements were taken along the previously cut strips for the tensile tests.

95

96 2.4.2. *Microstructure*

97 The microstructure of the films was observed by Scanning Electron Microscopy (SEM)
98 using a JEOL JSM-5410 (Japan) electron microscope. Surface and cross images of films were
99 captured and analysed. To obtain the cross-section images, film samples were immersed in
100 liquid nitrogen and cryofractured. After coating with gold, the samples were observed using an
101 accelerating voltage of 10kV.

102

103 2.4.3. *Water vapour permeability*

104 Water vapour permeability (WVP) was determined at three different temperatures (5,
105 20, 35°C) and two RH gradients using a modification of the ASTM E96-95 gravimetric method
106 (1995) for flexible films (McHugh *et al.*, 1993). Payne permeability cups of 3.5 cm in diameter
107 (Elcometer SPRL, Hermelle /s Argenteau, Belgium) were filled with oversaturated solutions of
108 $Mg(NO_3)_2$ (RH: 51-57% in the tested temperature range) or NaCl (RH: 75-76% in the teste
109 temperature range). Films were selected due to the lack of defects, three round samples per
110 formulation were cut and thickness was measured. Once the films were secured, each cup was
111 placed in a pre-equilibrated cabinet fitted with a fan to provide a strong driving force across the
112 film for water vapour diffusion. The RH of the cabinet was held constant using over-saturated
113 solutions of $MgCl_2$ (RH: 32-33%) or $Mg(NO_3)_2$ (RH: 51-57%). The shiny side of the films was
114 exposed to the ambient at the highest RH, and the cabinets were stored at the aforementioned
115 temperatures. The cups were weighed periodically (± 0.00001 g) after steady state had been
116 reached. The slope of the weight loss vs. time plot was divided by the exposed film area to
117 calculate the water vapour transmission rate (WVTR). WVP was quantified using the equations
118 described by Fabra *et al.* (2009a).

119

120 2.4.4. *Mechanical behaviour*

121 Rectangular samples (25 x 100 mm) were cut and stored at either 33 or 53% RH and
122 25°C, in cabinets containing MgCl₂ or Mg(NO₃)₂ saturated solutions. Once equilibrated, film
123 specimens were mounted in the film-extension grips and stretched at 50 mm min⁻¹ using a
124 universal test machine (TA.XTplus model, Stable Micro Systems, Haslemere, England). The RH
125 and temperature (25°C) of the testing environment were registered with a thermohygrometer
126 (testo 60-H2, Testo GmbH & Co., Lenzkirch, Germany). Nine replicates of each formulation
127 were tested. The elastic modulus (EM), tensile strength at break (TS) and percentage
128 elongation (%E) were determined according to ASTM standard method D882 (ASTM 2001).

129

130 2.4.5. Optical properties

131 Optical properties were determined on the shiny side of films pre-equilibrated at 33%
132 RH. The reflectance spectra were obtained with a spectrophotometer MINOLTA, model CM-
133 3600d (Minolta CO. Tokyo, Japan). Both black and white backgrounds were used and the
134 diameter of the illuminated sample area was 8mm. The internal transmittance (T_i) and
135 reflectance for an infinite thickness (R_∞) was quantified as described by Fabra *et al.* (2009a).

136 Colour CIE-L* a* b* parameters (CIE, 1986) were obtained using illuminant D₆₅ and
137 observer 10° from R_∞ values. Colour coordinates chroma (C_{ab}*) and hue (h_{ab}*), as well as the
138 whiteness index (WI) of the samples (equation 1), were calculated.

$$139 \quad WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (1)$$

140

141 The gloss of the films was measured at 20, 60 and 85° angles from the normal to the
142 coating surface, according to the ASTM standard D-523 (ASTM, 1999) using a flat surface gloss
143 meter (Multi.Gloss 268, Minolta, Germany). Ten replicates were taken per formulation and
144 angle. Results were expressed as gloss units, relative to a highly polished surface of black
145 glass standard with a value near to 100.

146

147 2.4.6. Oxygen permeability

148

149 The oxygen barrier properties of the films were evaluated by measuring oxygen
150 permeability (OP) with an Ox-Tran 1/50 system (Mocon, Minneapolis, USA) at 20°C (ASTM

151 Standard Method D3985-95, 2002). Measurements were carried out at 56% RH, in order to
152 avoid cracking at low RH. Film specimens were placed in precut aluminium masks that allowed
153 a 5 cm² exposure area. Masked films were exposed to pure nitrogen flow on one side and pure
154 oxygen flow on the other side. OP was calculated by dividing the oxygen transmission rate by
155 the difference in oxygen partial pressure between the two sides of the film, and multiplying by
156 the average film thickness. Three replicates per formulation were made.

157

158 *2.4.7. Protective ability against lipid oxidation*

159 The protective capacity of the films against lipid oxidation was quantified with an
160 accelerated test of oxidative rancidity (Osés *et al.*, 2008). Specially designed methacrylate cells
161 were filled with 18 g of almond oil (Biocesta S.L., Valencia, Spain), covered with the films and
162 hermetically secured. The headspace in each cell was 18.65 cm³, and the oxygen in it was
163 displaced by bubbling nitrogen into the oil. Two films were tested per formulation. Two
164 additional cells were covered with aluminium foil (17 µm thickness) and two more were left
165 uncovered. Each cell was placed in a cabinet at 50% RH and stored in darkness for 85 days at
166 40°C. Samples of 1 ml almond oil were periodically taken using a syringe through a septum
167 placed in the cell wall. Simultaneously, 1 ml of nitrogen was introduced in the cell through a
168 second septum to maintain the internal pressure constant. The progression of oil oxidation was
169 monitored by analysing the peroxide value (PV) according to the method described in the norm
170 UNE 55-023. PV was expressed as mEq of oxygen per kg of sample.

171

172

173 2.5. Application of the coatings on roasted peeled almonds

174 *2.5.1. Coating procedure*

175 Raw peeled almonds were purchased at a local market, roasted at 160°C for 15
176 minutes and cooled down using forced air at -20°C. Roasted almonds were coated by dipping in
177 the film-forming formulations for 1 minute, drained for 1 minute and gently air-dried. Surface
178 solid density (SSD), calculated as the ratio between the mass of dry coating and that of the non-
179 coated almond, was determined in quintuplicate for each formulation. All the almonds selected

180 were similar in size and shape. After coating and drying, almonds were stored at 25°C and two
181 levels of RH, namely 33 and 53%, using MgCl₂ or Mg(NO)₃ oversaturated solutions.

182

183 *2.5.2. Lipid oxidation throughout storage*

184 The lipid fraction of the almonds was extracted from 5 g ground nut samples by Soxhlet
185 extraction equipment (SoxtecT 2055 FOSS Analytical AB, Höganäs, Sweden) using petroleum
186 ether as a solvent, for 1.5 h. Oil oxidation was monitored by analysing the peroxide value (norm
187 UNE 55-023), periodically up to 17 weeks storage time. Peroxide value (PV) was expressed as
188 mEq of oxygen per kg of extracted oil. All analysis was performed in triplicate.

189

190 2.6. Statistical analysis

191 The statistical analysis of the data was performed through analysis of variance
192 (ANOVA) using Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, Md.) Fisher's
193 least significant difference (LSD) procedure was used.

194

195 **3. Results and discussion**

196 3.1. Characterization of the films

197

198 *3.1.1. Microstructure*

199 Figures 1 and 2 show, respectively, the surface and cross-section micrographs of all the
200 obtained films. Small irregular particles on the surface of the films, specially for pure HPMC
201 films (H), can be observed, which can be attributed to some amount of non-dissolved polymer
202 which remains non integrated in the matrix (figure 1). With the addition of acids (HA and HC) the
203 films surface became smoothed, which indicates that a good integration of these compounds
204 occurred into the HPMC matrix, resulting in a very homogenous, tighter structure. Cross-section
205 micrographs (figure 2) are coherent with this; homogenous and smooth cross-section surface
206 are observed. Nevertheless, the structure of HG films show discontinuities in both surface and
207 cross-section images (figures 1 and 2, corresponding to the ginger oil droplets, whose size
208 reached about 20 µm. This reveals that films with ginger oil had a heterogeneous structure, with
209 oil droplets dispersed in the polymer matrix. Moreover, HG films appeared thicker than the other

210 film, as can be seen in figure 2, which can be attributed to the fact that the oil presence made
211 the polymer chain aggregation more difficult, given rise to a more open film structure. In fact the
212 measurement of the film thickness revealed that those containing ginger oil were significantly
213 ($p < 0.05$) thicker (HG $62 \pm 14 \mu\text{m}$) than pure H films ($55 \pm 9 \mu\text{m}$). The ginger oil droplets can also
214 be observed in the irregular surface of HG films (figure 1). The statistical analysis of the film
215 thickness values also indicated that HA and HC films were very similar (HA: $53 \pm 8 \mu\text{m}$; HC: 53
216 $\pm 9 \mu\text{m}$) and significantly ($p < 0.05$) thinner than pure H films. This could be related to some
217 cross-linking effect caused by these acids, which favours the polymer chain aggregation and
218 tightens the structure of the matrix.

219

220 3.1.2. Water vapour permeability

221 The effect of the RH on WVP was relevant, and the values at RH gradients of about 33-
222 53% were significantly ($p < 0.05$) lower than those obtained at about 53-75% RH gradients, as
223 shown in table 1. This is coherent with the results of Villalobos *et al.* (2006) who reported the
224 water sorption isotherm of HPMC films at 10°C , and observed that the equilibrium moisture
225 increased slowly below $0.62 a_w$, whereas a substantial water gain occurs from $0.62 a_w$. Hence,
226 the hydrophilic nature of the HPMC, whose structure became plasticized at the highest moisture
227 content, allows for more intense water transport at high RH. To assess the effect of temperature
228 and formulation on WVP, the results at each RH gradient were treated separately for the
229 statistical analysis. It was found that the effect of both temperature and formulation on WVP was
230 similar, regardless of RH.

231 Formulation had a significant impact on WVP ($p < 0.05$) under any RH-T conditions. In
232 the case of films without additives, the obtained results were consistent with previous studies
233 (Ayranci *et al.*, 1997; Sánchez-González *et al.*, 2009), showing poor water barrier properties,
234 coherent with the hydrophilic nature of carbohydrates. The incorporation of A or C resulted in an
235 improvement of the water barrier properties ($p < 0.05$) and films with both acids behaved similarly
236 under all conditions ($p > 0.05$). This could be due to the cross-linking between the polymer chains
237 and the carboxylic acids, a phenomenon previously described by Reddy & Yang (2010).
238 According to these authors, carboxylic acids cross-link the hydroxyl groups in cellulose. This

239 would result in the formation of a tighter structure (coherently to the microstructure observed by
240 SEM), thus restricting the movement of the molecules and leading to a WVP decrease.

241 HG films had a variable response. At low temperatures, G was the most effective WVP
242 depressor due to its hydrophobic contribution to the matrix, whereas at 35°C it had no significant
243 effect on the poor water barrier properties of the HPMC films, whose structure seemed to
244 undergo some loosening. This could be due to the structural characteristics of HG films which
245 show a heterogeneous structure with a liquid oil dispersed phase and interfaces (see figure 2).
246 Similar results were observed by Atarés *et al.* (2010) in sodium caseinate films. The liquid state
247 of ginger oil could favour molecular mobility at high temperature, thus promoting the transport of
248 water molecules through the emulsified films.

249

250 3.1.3. Mechanical behaviour

251 Table 2 shows the mechanical parameters of all the films tested. RH had a reduced
252 impact on the mechanical behaviour of the samples. As RH increased from 33% to 53%, the
253 average values of mechanical parameters was either not significantly affected ($p>0.05$) or
254 showed little variations. Chick & Hernández (2002) found that casein-wax films were 5-fold
255 weaker at 75% RH than at 50% RH. Fabra *et al.* (2009a) studied the mechanical behaviour of
256 sodium caseinate films incorporated with fatty acids or beeswax, and reported that both EM and
257 TS were significantly lower at 53%RH than those at 33%. However, such studies refer to protein
258 edible films and no studies have been found dealing with the effect of moisture content on the
259 mechanical properties of cellulose derivative films. A possible explanation for this mild effect of
260 moisture content on the mechanical properties of HPMC films could be the limited water content
261 of the films, even at 53% RH, which was 5.2 ± 1.6 (db) without any significant effect of
262 formulation ($p>0.05$). Villalobos *et al.* (2006) reported the water sorption isotherm of HPMC films
263 at 10°C, and observed that the equilibrium moisture increased slowly between 0 and 0.62 a_w ,
264 remaining below 0.05 (db) even at 0.53 a_w . The substantial water gain occurs from 0.62 a_w ,
265 according to the reported sorption isotherm. So, no important changes in the moisture content
266 of the films occurred between equilibrium at 33% and 53% to provoke a notable plasticization
267 effect that can substantially modify mechanical behaviour.

268 The addition of A or C resulted in a significant EM increase at low RH, probably due to
269 the afore-mentioned cross-linking with the polymer. This effect results in a more compact, rigid
270 and brittle structure (coherently with that revealed in the microstructural analysis), whose
271 fracture occurs at lower TS and E. On the other hand, films with G tended to be weaker and
272 showed the lowest TS, which must be related to the presence of discontinuities in the film
273 structure, as described in section 3.1.1. The highest elongation percentage was observed in
274 films without additives (H), and it was reduced when A or C were added. The addition of G
275 entailed greater extensibility, which may be due the weakening of the polymer chain
276 aggregation associated to the oil presence and to the liquid state of G at room temperature and
277 the elongation of oil droplets as the structure is stretched. Similar results were obtained with the
278 addition of oleic acid to soy protein films (Monedero *et al.*, 2009).

279

280 3.1.4. Optical properties

281 Optical properties are greatly relevant to the film functionality due to their great impact
282 on the appearance of the coated products (Villalobos *et al.*, 2005). The spectral distribution of
283 the internal transmittance (%) between 400 and 700 nm is shown in figure 3. The film
284 formulation had a significant effect ($p < 0.05$) on this parameter at 400, 500, 600 and 700 nm
285 (table 3). The shape of the T_i curves corresponding to H, HC and HG films was very similar and
286 mostly parallel. HA films behaved differently from the rest, especially at low wavelengths. This
287 relates to the increased absorption in the blue region, caused by ascorbic acid which imparts
288 yellowness to the films. Apparently, the cross-linking taking place in HA films affected the
289 chromophore groups of this acid. The addition of ginger oil reduced the internal transmittance of
290 the HPMC films more than any other additive, as can be observed in figure 3. In fact, HG films
291 were the most opaque of the films, which is related to the presence of lipid droplets in the
292 polymer matrix, which implies anisotropy in the material refractive index.

293 Table 3 reports the colour coordinates L^* , C_{ab}^* , h_{ab}^* and WI of the films. All of them
294 were significantly affected by the film formulation ($p < 0.05$). There were few differences in clarity,
295 whereas ginger oil provoked a slight film darkening, ascorbic and citric acids enhanced clarity,
296 probably due to the structural effects (more compact matrix) which promote the light surface
297 reflection. Films with ascorbic acid had the highest colour saturation ($p < 0.05$) and the lowest

298 hue ($p < 0.05$), coherent with the light absorption behaviour described above for T_i . HC films
299 showed the highest WI because of their low colour saturation (the lowest chrome values).

300 Norm ASTM D523 (1999) defines gloss as the ratio of the luminous flux reflected from a
301 specimen to the luminous flux reflected from a standard surface under the same geometric
302 conditions. As recommended by the aforementioned norm, the geometry 60° was selected from
303 the different incidence angles (table 3), given that most of the values were comprised between
304 10 and 70 units. H and HG films were the least glossy, whereas A and especially C significantly
305 improved ($p < 0.05$) the gloss of the films. As described in section 3.1.1., the presence of these
306 acids entailed structural changes linked with some smoothing effect on the surface topography
307 of the HPMC films. In the case of HG films, the presence of oil dispersed droplets causes
308 irregularities on the film surface that reduce the specular light reflection (Villalobos *et al.* 2005;
309 Fabra *et al.* 2009b). Figure 1 supports these results.

310

311 3.1.5. Oxygen permeability

312 The values of oxygen permeability (OP) are shown in table 3. OP of HPMC films was in
313 the same range as that reported by Miller & Krochta (1997). The addition of ascorbic or citric
314 acids had a relevant impact on OP, which was significantly reduced ($p < 0.05$). This is in
315 agreement with the results of Ayranci & Tunc (2003), who included varying amounts of these
316 acids in cellulosic films and observed the improvement of oxygen barrier properties as the
317 content of both acids increased. The cross-linking taking place between these acids and the
318 polymer chains affected the film structure (as already commented in section 3.1.1.), and led to
319 the reduction of OP.

320 An opposite effect was observed for films containing ginger oil which showed the
321 highest OP values ($p < 0.05$). The occurrence of ginger oil droplets interrupting the HPMC matrix
322 and the apparent weakening of the polymer chain aggregation in the matrix, give rise to an
323 important OP increase. The liquid state of ginger oil, as well as its more hydrophobic nature,
324 also facilitated the oxygen transport in the matrix and worsened the oxygen barrier properties of
325 the films.

326

327 3.1.6. Protective ability against lipid oxidation

328 Figure 4 shows the PV development as a function of the storage time in almond oil
329 protected with HPMC films (with and without additives), aluminium foil and without any film. The
330 protection provided by aluminium foil was so effective that the corresponding almond oil did not
331 undergo any PV increase throughout time, remaining as low as 4.3 meq O₂/kg oil, even after 85
332 days of storage. In the rest of the cases, PV rose as storage time progressed after an induction
333 period. After 42 days, all PV values were below 20 and the differences between films and
334 control were not significant ($p>0.05$). At the end of the storage period, unprotected oil had
335 undergone the most intense oxidation ($p<0.05$), thus reaching a mean value of 99 meq O₂/kg
336 oil. At the same time, the presence of additives in the films resulted in some PV reduction as
337 compared with HPMC films.

338 Such a trend towards a better protection of lipids in films with additives could be due to
339 different effects. The antioxidant properties of ascorbic and citric acids are widely known, but
340 their chemical nature may not be the only factor affecting the antioxidant effect of HA and HC
341 films. As commented on above, the cross-linking between acids and polymer chains reduced
342 the oxygen permeability in these films, which would play a key role in protecting the oil against
343 oxidation. In the case of ginger oil films, their oxygen barrier properties were not improved
344 (section 3.1.6.), so their antioxidant effect may be necessarily related to the specific action of
345 the oil antioxidant compounds. According to Golebiowski *et al.* (2008), the main components of
346 ginger oil are zingiberene, β -bisabolene, β -sesquiphellandrene and curcumene. Shan *et al.*
347 (2005) reported that phenolic volatile oils, such as gingerol and shogaol are present in ginger
348 essential oil, as well as caffeic acid and other phenolic acids. These authors obtained a highly
349 positive linear relationship ($R^2>0.96$) between the antioxidant capacity and the total phenolic
350 content of 26 spices.

351 Figure 4 shows how the oil protected with HG films remained very well preserved
352 against lipid oxidation until 64 storage days. At such time, only aluminium foil was more
353 effective than HG films as a protector against lipid oxidation. However, the protective ability of
354 films with ginger oil diminished markedly after this period, and lipid oxidation exhibited some
355 acceleration between 64 and 85 storage days. This is in accordance with the aforementioned
356 antioxidant mechanism in films with ginger oil, since its antioxidant compounds will become
357 increasingly oxidized as time progresses, thus losing their protective ability.

358

359 3.2. Application of the coatings on roasted peeled almonds

360 *3.2.1. Coating efficiency*

361 HPMC proved to be a suitable material for the coating of roasted peeled almonds. The
362 coatings were continuous and did not develop visible cracks under any storage conditions,
363 which permitted their capability for protecting the nut against lipid oxidation to be tested.

364 The application of all the formulations markedly improved the appearance of the
365 almonds by increasing their gloss, at the same time as all the formulations effectively coated the
366 totality of the almond surface, which was easily appreciated with the naked eye. Nevertheless,
367 the surface solid density (SSD), ranging between 6 and 12 mg dry coating / g uncoated almond,
368 was significantly affected by the formulation ($p < 0.05$), as shown in table 4. Several factors affect
369 the final SSD, such as the size and shape of the almonds, the extensibility of the film forming
370 dispersion on the almond surface and their viscosity. The latter influences the gravitational
371 losses of the applied dispersion during the drainage and drying period after dipping. In all cases,
372 dispersions showed a Newtonian behaviour ($R^2 > 0.9996$), the η values ranging between 0.121
373 and 0.143 Pa s. These results are similar to those obtained by Jiménez *et al.* (2010) and
374 Villalobos-Carvajal *et al.* (2009), who also observed a Newtonian behaviour of HPMC
375 dispersions up to 4.5% (w/w). HA dispersion, being the least viscous, gave rise to the lowest
376 SSD and so, thinner coatings. On the other hand, the highest viscosity value was obtained for
377 HG emulsions ($p < 0.05$), which relates to the presence of oil droplets, and led to a high SSD
378 value (on average, twice that of HA). Differences in the coating thickness may also affect the
379 film protection capacity.

380

381 *3.2.2. Lipid oxidation throughout storage*

382 Table 5 shows the development of PV throughout storage time, as a measurement of
383 the lipid oxidation in almonds stored at 33 and 53% RH. Given that nuts are low-moisture
384 products, and as a simulation of the actual storage conditions, the lowest RH (33%) was tested
385 for longer. A shorter storage period at 53% RH (2 weeks) was tested in order to evaluate the
386 role of RH in the protective ability of the films and the oxidation kinetics, aiming to simulate the
387 contact of the product with normal ambient conditions.

388 RH had a relevant effect on the protective ability of the coatings without additives (H),
389 even after only 14 days of storage. At intermediate RH (53%), the oxidation was promoted as
390 compared to low RH (33%). Such a trend may relate to the hydrophilic nature of HPMC and the
391 increase of the oxygen permeability of this kind of films when their moisture content increases
392 (Hong & Krochta, 2006). In the absence of antioxidant additives, the mechanism of protection of
393 H coatings relies on their barrier properties against oxygen transport through the film, which are
394 extremely dependent on water availability. Hence, the oxidation is more delayed at low RH,
395 because of the reduction of oxygen permeability and the oxygen availability within the nut. Maté
396 *et al.* (1996) applied WPI/glycerol coatings to dry roasted peanuts, and demonstrated that they
397 were more effective at lower RH. In addition, the kinetics of lipid oxidation was dependent on
398 the water availability; intermediate RH promotes this process to a greater extent than low RH
399 (Labuza, 1980).

400 Almonds coated with formulations including additives (HA, HC and HG) did not show
401 the same trend. The presence of these agents seems to compensate for the negative effect of
402 water availability on the protection ability of the HPMC films. At intermediate RH, almonds
403 coated with HA, HC or HG remained better preserved ($p < 0.05$) than those without additives.
404 This indicates that, under these conditions and in the proportions tested, these agents act
405 against lipid oxidation, thus preserving the quality of coated almonds and extending their shelf-
406 life. As already pointed out, this could be related with two effects: the structural changes of the
407 matrix due to acid addition which limits the oxygen permeability (as commented in sections
408 3.1.1. and 3.1.6.) and the specific action of antioxidant components, which appears crucial in
409 the case of HG films.

410 At low RH, even after 118 days of storage, PV of all coated almonds remained, on
411 average, lower than 10, and rancid smell was not detectable. At this time, differences between
412 the treatments were statistically non-significant ($p > 0.05$). However, the pattern of PV as storage
413 time progressed differed between treatments. In almonds coated with HA or HC, the PV values
414 at all tested times (0, 14, 56 and 118 days) were statistically similar ($p > 0.05$), whereas the PV of
415 almonds coated with H or HG showed a progressive increase ($p < 0.05$). At the end of the
416 storage, the almonds coated with HA or HC showed a tendency to remain better preserved than
417 those coated with H or HG (table 5).

418 The differences between treatments may be related to the different protection
419 mechanisms taking place in the coatings against lipid oxidation. As commented on above,
420 ascorbic and citric acids may interact with the polymer matrix and cause cross-linking, which
421 results in a reduced permeability and a lower transport of oxygen across the coating (table 3).
422 Additionally, these compounds are known as effective antioxidant agents, and their presence in
423 the coating may reduce the rate of lipid oxidation in the almonds. This would not be the case in
424 almonds coated with HPMC without additives, which are preserved by the mere presence of the
425 polymer network acting as a barrier to oxygen at low moisture content. The presence of ginger
426 oil in HPMC coatings did not improve the film protection capacity against lipid oxidation at low
427 RH conditions. This could be due to two opposing phenomena: at short times, the presence of
428 antioxidant compounds in the oil composition contributes to slow down kinetics of lipid oxidation;
429 in a second period when antioxidants are oxidized, the heterogeneous structure of the HG
430 coating does not imply additional protection against the oxygen transport. So, the antioxidant
431 effect of the ginger oil becomes less and less effective as the antioxidant compounds become
432 increasingly oxidized.

433

434 **4. Conclusions**

435 HPMC coating of almonds improved their appearance and the addition of antioxidant
436 agents was effective against lipid oxidation. The presence of the antioxidant additives was able
437 to compensate for the negative effect of intermediate relative humidity on lipid oxidation. The
438 protective effect of films and coatings is linked to both the presence of active antioxidant
439 compounds and the reduction of the oxygen permeability, closely related to the structure of the
440 film or coating. Coatings with ascorbic or citric acids showed the best barrier efficiency against
441 oxygen transport, whereas the antioxidant activity of coatings containing ginger oil was
442 exclusively related to the chemical action of the antioxidant compounds. The structural effect
443 allows for a maintained protective effect against the action of oxygen, whereas the action of the
444 mere presence of antioxidant agents is limited in time. More work is necessary in order to
445 improve the efficiency of the coatings to delay rancidity development.

446

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453

454 6. References

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