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**Effect of solid content and composition of hydroxypropyl methylcellulose-lipid edible coatings on physicochemical, sensory and nutritional quality of ‘Valencia’ oranges**

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Running title: Postharvest quality of HPMC-coated oranges

22 **Abstract**

23

24

25 ‘Valencia’ oranges were coated with edible coatings based on hydroxypropyl  
26 methylcellulose (HPMC), beeswax (BW) and shellac. Coatings were prepared at two  
27 BW:shellac ratios (1:3 and 3:1) and two solid content (SC) (4 and 8%). A commercial  
28 wax at a 10% SC, as a control of coated fruit, and an uncoated control were also tested.  
29 Oranges were stored up to 16 weeks at 5 °C, followed by 1 week at 20 °C. Although  
30 sensory quality was not negatively affected by coating application, care should be taken  
31 to the SC and shellac content of the formulations, since an increase of these parameters  
32 translated in a significant increase in the level of ethanol. Results indicate that HPMC-  
33 BW-Shellac coating with 4% SC and a BW:Shellac ratio 1:3 would provide the best  
34 compromise to extend shelf life of ‘Valencia’ oranges by reducing weight loss,  
35 providing gloss and maintaining the nutritional quality of the fruit.

36

37 **Keywords:** edible coating, hydroxypropyl methylcellulose, beeswax, shellac,  
38 postharvest quality, nutritional quality

39

40 **1. Introduction**

41 In the citrus industry, fruit coating is a normal practice to replace the natural  
42 waxes that are generally removed during washing with the purpose to reduce fruit  
43 weight loss, shrinkage and improve appearance. Coating application has also been  
44 proven to reduce the incidence of chilling injury and other rind disorders in citrus  
45 (Bajwa and Anjum, 2007). However, it has also been reported that coating of citrus can  
46 adversely affect fruit flavour (Hagenmaier, 2002), due to the overproduction of volatiles  
47 associated with anaerobic conditions.

48 Consumer interest in health, nutrition, and food safety combined with  
49 environmental concerns has renewed efforts in the development of new coating  
50 formulations to avoid the use of synthetic components used in many commercial  
51 coatings, such as polyethylene wax, and the use of ammonia or morpholine in the  
52 formulations. Major components of edible coatings include proteins, polysaccharides,  
53 and lipids. Additionally, some authors include shellac, which is a natural resin, as  
54 ingredient of natural coatings for fruits that are not consumed with peel like citrus fruit,  
55 even though it is not included in the GRAS (generally recognized as safe) ingredient list  
56 (Rhim and Shellhammer, 2005). These groups present advantages and disadvantages  
57 when used as coating ingredients. Generally, lipids and resins offer a good moisture  
58 barrier due to their hydrophobic nature, reducing water loss, shriveling and shrinkage of  
59 coated fruit. However, their non-polymeric nature limits their ability to form cohesive  
60 films. Proteins and polysaccharides are good film-formers and present an intermediate  
61 oxygen barrier between lipid and resin coatings at medium-high relative humidity,  
62 which helps controlling the gas exchange between the fruit and the environment  
63 reducing the appearance of off-flavour compared to commercial waxes (Baldwin and

64 Baker, 2002). However, their hydrophilic nature makes them poor moisture barriers. For  
65 this reason, most natural coatings for fruit contain a combination of ingredients forming  
66 what is called “edible composite coatings”. Several other compounds such as  
67 plasticizers and emulsifiers may be added to the formulations to improve coating  
68 integrity and form stable emulsions when lipids and hydrocolloids are combined.

69 Nowadays, nutritional and functional fruit quality has gained great interest. Citrus  
70 fruits are an important source of vitamin C, as well as other bioactive compounds such  
71 as polyphenolic compounds, mainly flavonoids, with high antioxidant properties  
72 (Sánchez-Moreno et al., 2003). Therefore, recent works have been focussed on the study  
73 of citrus postharvest treatments, such as cold and curing conditions, irradiation, cold  
74 quarantine treatments, and minimally processing, on their bioactive compounds (Del  
75 Caro et al., 2004; Patil et al., 2004; Perez et al., 2005; Biolatto et al., 2005; Vanamala et  
76 al., 2007; Girenavar et al., 2008; Rapisarda et al., 2008, Contreras-Oliva et al., 2011a).

77 In the literature, many works report the effect of edible composite coatings on the  
78 postharvest quality of citrus fruit (Hagenmaier et al., 2002; Pérez-Gago et al., 2002;  
79 Navarro-Tarazaga and Pérez-Gago, 2006; Navarro-Tarazaga et al., 2008a). Most of  
80 these studies provide information about the effect of coating composition, formulation  
81 solid content (SC), storage conditions and fruit cultivars on the physicochemical and  
82 sensory quality, however little information can be found on their effect on the  
83 nutritional quality of citrus fruit. Togrul and Arslan (2004) reported that ascorbic acid  
84 loss after storage was delayed when mandarins citrus were coated with carboxymethyl  
85 cellulose. However, application of a commercial chitosan to ‘Oronules’ mandarins did  
86 not affect either the internal quality or the bioactive compounds of the fruit (Contreras-  
87 Olivas et al., 2011b). Therefore, the objective of this work was to study the effect of

88 coating composition and formulation SC of hydroxypropyl methylcellulose (HPMC)-  
89 lipid edible coatings on the physicochemical, sensory and nutritional quality of  
90 'Valencia' oranges.

## 91 **2. Material and methods**

### 92 **2.1 Materials**

93 HPMC (Methocel E15) was purchased from Dow Chemical Co. (Midland, MI,  
94 USA). Shellac and beeswax (BW) (grade 1) were supplied by Fomesa Fruitech, S.L.  
95 (Beniparrell, Valencia, Spain). Oleic acid and glycerol were from Panreac Química,  
96 S.A. (Barcelona, Spain). Ammonia (25%) was from Scharlau (Sentmenat, Barcelona,  
97 Spain).

98 Reagents 2,2-diphenyl-1-picrylhydrazyl (DPPH<sup>•</sup>), potassium dihydrogen  
99 phosphate (KH<sub>2</sub>PO<sub>4</sub>), *meta*-phosphoric acid (MPA), phosphoric acid (H<sub>3</sub>PO<sub>4</sub>), folin-  
100 ciocalteu's phenolreagent, sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), gallic acid and standard L-  
101 ascorbic acid (AA) were purchased from Sigma (Sigma-Aldrich Chemie, Steinheim,  
102 Germany). Acetic acid glacial and dimethyl sulfoxide (DMSO) were from Scharlau  
103 (Sentmenat, Spain). Methanol was from BDH Prolabo (Poole, UK), 1,4-dithio-DL-  
104 threitol (DTT) and hesperidin (hesperitin-7-O-rutinoside, HES) were obtained from  
105 Fluka (Sigma Co., Barcelona, Spain). Narirutin (naringenin-7-rutinoside, NAT) and  
106 didymin (isosakuranetin-7-rutinoside, DID) were purchased from Extrasynthese  
107 (Genay, France). All solvents used were of HPLC-grade and ultrapure water (Milli-Q)  
108 was used for the analysis.

### 109 **2.2 Coating formulation**

110 Emulsion coatings consisted of HPMC and different ratios of BW and shellac  
111 suspended in water. Oleic acid and glycerol were added as emulsifier and plasticizer,

112 respectively. Ratios of HPMC-glycerol (2:1) (dry basis, db) and lipid components  
113 (BW/shellac)-oleic acid (5:1) (db) were kept constant throughout the study. BW and  
114 shellac content was 60% (db). Ammonia (15%, w/w, shellac/ammonia) was added to  
115 dissolve shellac. Formulations were prepared at two different BW:shellac ratios (1:3  
116 and 3:1) and two SC (4% and 8%). Table 1 shows the composition of the HPMC-based  
117 coatings (T3 to T6).

118       Emulsions were made in a 2-L stirred pressure cell (Parr Instrument Co., Moline,  
119 IL), in which glycerol, oleic acid, BW, shellac, NH<sub>3</sub>, and one-third of the water were  
120 added. The mixture was initially stirred at 100 rpm until the temperature reached 60 °C.  
121 Next, stirring was increased to 400 rpm until temperature reached 110 °C and remained  
122 at these conditions for 30 min. Afterwards, the remaining water, previously heated to 90  
123 °C, was pumped into the vessel maintaining the stirring conditions at 400 rpm for about  
124 10-15 min after the water was incorporated. The emulsion was then removed from the  
125 pressure vessel and mixed with a 5% HPMC solution previously prepared by dispersing  
126 the HPMC in hot water at 90 °C and later hydration at 20 °C for 45 min. Finally, the  
127 emulsions were cooled under agitation to a temperature lower than 20 °C by placing  
128 them in an ice water bath. Water was added to a final SC of 4% or 8% depending on the  
129 treatment.

### 130 **2.3 Fruit preparation–coating application**

131       ‘Valencia’ oranges (*Citrus sinensis*) were hand-harvested with an average  
132 maturity index of 8.7 from a local grove in Valencia (Spain) and transferred to the IVIA  
133 postharvest facilities where they were selected, randomised, washed with tap water, and  
134 dipped in a solution of imazalil (1,000 ppm) for 1 min.

135 The oranges were randomly divided into 6 groups: 4 experimental coating  
136 treatments, 1 uncoated (control), and 1 commercial wax (CW) (polyethylene-shellac)  
137 applied at 10% SC as a control of coated fruit (Table 1). The fruit was dip-coated by  
138 immersion in the coating solutions for 20 sec, drained of excess coating and dried in a  
139 drying tunnel at 50 °C for 2 min (Pérez-Gago et al., 2002). After coating, fruit were  
140 stored for 6, 8 and 16 weeks at 5 °C and 90-95% RH, followed by 1 additional week at  
141 20 °C to simulate retail storage conditions.

#### 142 **2.4. Physicochemical quality**

143 **Weight loss.** Lots of 30 fruit per treatment were used to measure weight loss. The  
144 same fruit were weighed at the beginning of the experiment and at the end of each  
145 storage period. The results were expressed as the percentage loss of initial weight.

146 **Internal gas concentration.** Ten fruit per treatment were used to calculate internal  
147 gas concentrations. Internal CO<sub>2</sub> and O<sub>2</sub> concentrations of each sample were obtained  
148 by withdrawing 1 mL internal gas sample from the orange central cavity with a syringe  
149 while the fruit was immersed under water. The gas sample was then injected into a gas  
150 chromatograph (Thermo Fisher Scientific, Inc., Waltham, MA) fitted with a Porapak QS  
151 80/100 (1.2 m x 0.32 cm) column, followed by a molecular sieve 5A 45/60 (1.2 m x  
152 0.32 cm) column. Temperatures were 35, 125 and 180 °C, respectively, for the oven,  
153 injector and thermal conductivity detector. Helium was used as carrier gas at 22 mL/min  
154 flow rate. Peak areas obtained from standard gas mixtures were determined before and  
155 after analysis of samples and results were expressed as kPa.

156 **Ethanol content.** Ethanol content (EC) in juice were determined by head-space  
157 gas chromatography according to the method described by Ke and Kader (1990). Ten  
158 fruit each in 3 replicates per treatment were analysed. Five mL orange juice were

159 transferred to 10 mL vials with crimp-top caps and TFE/silicone septum seals and  
160 frozen until analysis. EC was analysed using a gas chromatograph (Thermo Fisher  
161 Scientific, Inc., Waltham, MA, USA) equipped with an autosampler, a flame ionization  
162 detector and fitted with a Poropak QS 80/100 column (1.2 m x 0.32 cm). Temperatures  
163 of the oven, injector, and detector were 150, 175, and 200 °C, respectively. Helium was  
164 used as the carrier gas at a flow rate of 28 mL/min. A 1 mL sample of the head-space  
165 was withdrawn from each vial previously equilibrated in the autosampler incubation  
166 chamber for 10 min at 40 °C. EC concentration was calculated using peak areas of the  
167 samples relative to the peak area of standard solution. Results were expressed as mg/L  
168 juice.

## 169 **2.5 Sensory quality**

170 Sensory evaluation was conducted by 10 trained judges (5 females and 5 males),  
171 25 to 50 years old, at the end of each storage period. Judges evaluated overall flavour  
172 and off-flavour of mandarins. Overall flavour was rated on a 9-point scale, where 1 to 3  
173 represented a range of non-acceptable quality with the presence of off-flavour, 4 to 6  
174 represented a range of acceptable quality, and 7 to 9 represented a range of excellent  
175 quality. Off-flavour presence was evaluated using a 6-point intensity scale where 0=  
176 absence of off-flavour and 5= high presence of off-flavour. Six fruit per treatment were  
177 peeled and separated into individual segments. Two segments from two different fruit  
178 were presented to judges in trays labelled with 3-digit random codes and served at room  
179 temperature (25±1 °C). The judges had to taste several segments of each treatment in  
180 order to compensate, as far as possible, for biological variation of material. Mineral  
181 spring water was provided for rinsing between samples. External aspect of treated fruit  
182 (coating cracks, spots, etc.) was also evaluated by the panellist. A 3-point scale was



183 used, in which the aspect was classified as 1= bad, 2= acceptable, and 3= good.  
184 Panellists were also asked to rank visually the treatments from highest to lowest gloss.  
185 Sum of rankings were calculated (AENOR, 1997). The lowest sum of ranking indicates  
186 the highest gloss treatment. For visual aspect (external aspect and gloss ranking), four  
187 intact fruit per treatment were placed in trays labelled with 3-digit random codes and  
188 presented to the judges under the same conditions (light intensity and temperature) to  
189 minimize variations in human perception.

## 190 **2.6 Nutritional quality**

191 **Total antioxidant capacity ( $EC_{50}$ ).** The total antioxidant capacity was evaluated  
192 by the DPPH<sup>•</sup> assay. 0.4 ml of orange juice diluted with 0.8 mL of methanol was  
193 centrifuged at 12,000 rpm and 4 °C for 20 min. Six methanolic dilutions from the  
194 supernatant (0.075 mL) were mixed with 0.2925 mL of DPPH<sup>•</sup> (24 mg/L) and kept in  
195 darkness for 40 min. Afterwards, the change in absorbance at 515 nm was measured in a  
196 Multiskan spectrum microplate reader (Thermo Labsystem, USA). For each dilution,  
197 the percentage of remaining DPPH<sup>•</sup> was determined on the basis of the DPPH<sup>•</sup> standard  
198 curve. The amount of juice in each dilution was plotted against the amount of DPPH<sup>•</sup>  
199 radical remaining and  $EC_{50}$  value was calculated. This result expressed the amount of  
200 orange juice (L) needed to reduce 1 kg of DPPH<sup>•</sup> by 50%; thus, lower values mean  
201 higher antioxidant activity.

202 **Total ascorbic acid (TAA).** TAA was determined by the sum of AA plus L-  
203 dehydroascorbic acid (DHA), by using the reducing agent DTT (Sánchez-Mata et al.,  
204 2000). One mL of orange juice was diluted to 10 mL with 2.5% (w/v) MPA. Two mL of  
205 this solution were mixed with 0.4 mL of DTT (20 mg mL<sup>-1</sup>) for 2 h in darkness.

206 Afterwards, the extracts were filtered through a 0.45 µm Millipore filter before being  
207 HPLC analysed.

208 The HPLC analyses were performed on a Lachrom Elite HPLC (Merck Hitachi,  
209 Germany) equipped with a autosampler (Model L-2200), quaternary pump (Model L-  
210 2130), column oven (Model L-2300 ), and diode array detector (Model L-2450). A  
211 reversed-phase C18 LiChrospher®100 column (250 x 4 mm, 5 µm-particle, Merck,  
212 Darmstadt, Germany) preceded by a precolumn (4 x 4 mm) was used. System  
213 conditions were: injection volume 20 µL, oven temperature 25 °C, detector wavelength  
214 243 nm and flow rate 1 mL min<sup>-1</sup>. The mobile phase was 2% KH<sub>2</sub>PO<sub>4</sub> adjusted to pH  
215 2.3 with H<sub>3</sub>PO<sub>4</sub>. The flow rate was fixed at 1 mL min<sup>-1</sup> and the wavelength of  
216 measurement was 243 nm. AA was identified and quantified by comparison of peak  
217 areas with external standard and results were expressed as mg/L juice.

218 ***Flavanone glycosides (FGs)***. The main FGs identified in citrus fruit, HES, NAT  
219 and DID were determined by the method described by Cano et al. (2008) slightly  
220 modified. Two mL of orange juice were homogenized with 2 mL of DMSO:methanol  
221 (1:1 v/v) and centrifuged for 30 min at 12,000 rpm and 4 °C. The supernatant was  
222 filtered through one 0.45 µm nylon filter and analysed by HPLC-DAD using the HPLC  
223 equipment described above. System conditions were: injection volume 10 µL, oven  
224 temperature 25 °C, detector wavelength 280 nm and flow rate 1 mL min<sup>-1</sup>. The column  
225 Lichospher 100 RP-18 of 25x0.4 cm was preceded by a precolumn (4x4 mm) 5 µm  
226 particle size (Merck, Darmstadt, Germany). The mobile phase was acetonitrile (A):0.6%  
227 acetic acid (B) with initial condition of 10% A for 2 min, reaching 75% A in the  
228 following 28 min, then back to the initial condition in 1 min and held for 5 min prior to  
229 the next sample injection. The main FGs were identified by matching their respective

230 spectra and retention times with those of commercially obtained standards. NAT, HES  
231 and DID contents were calculated by comparing the integrated peak areas of each  
232 individual compounds to that of its pure standards. Results were expressed as mg/L.

233 **Total phenolic content (TPC).** The TPC of the orange juice was analysed by the  
234 Folin-Ciocalteu colorimetric method. 0.3 mL of orange juice was diluted with 1.7 mL of  
235 80% aqueous methanol. Appropriately diluted extract (0.4 mL) was mixed with 2 mL of  
236 folin ciocalteu commercial reagent (previously diluted with water 1:10, v/v) and  
237 incubated for 1 min before 1.6 mL sodium carbonate (7.5% w/v) was added. The  
238 mixture was incubated for 1 h at room temperature. The absorbance of the resulting blue  
239 solution was measured spectrophotometrically at 765 nm (Thermo UV1, Thermo  
240 Electron Corporation, UK) and the TPC was expressed as gallic acid equivalents per L  
241 (mg GAE/L).

242 Total antioxidant capacity, TAA, FGs and TPC were determined in juice from  
243 three replicates of 10 fruit each.

## 244 **2.8 Statistical Analysis.**

245 Two-way analysis of variance (ANOVA) was performed to determine the effect  
246 of each treatment and storage time on the quality attributes. Because of significant  
247 interactions, individual one-way ANOVA was also performed for each level of each  
248 factor. Significant differences between means were determined by least significant  
249 difference (LSD) at  $p \leq 0.05$ . Data were analysed using STATGRAPHICS Plus 4.1  
250 (Manugistics, Inc., Rockville, Maryland, USA).

251 For sensory gloss, specific differences were determined by Friedman test, which  
252 is recommended for ranking by the UNE 87 023 (AENOR, 1997). Significance  
253 differences were defined at  $p \leq 0.05$ .

254 **3. Results and discussion**

255 **3.1 Physicochemical quality**

256 *Weight loss.* Table 2 shows the weight loss of coated and uncoated oranges stored  
257 for 4, 8, and 16 weeks at 5 °C, followed by 1 week at 20 °C. Weight loss increased with  
258 storage time, increasing to nearly 12% after 16 weeks of storage at 5 °C plus 1 week at  
259 20 °C in control samples. After 8 weeks of storage, the CW (T2) and the HPMC-based  
260 coatings containing a BW:shellac ratio 1:3 (T3 and T4) were the most effective  
261 treatments controlling weight loss. However, after 16 weeks of storage at 5°C, the CW  
262 did not control fruit weight loss, being T3 the most effective coating controlling weight  
263 loss of ‘Valencia’ oranges.

264 Application of HPMC-based edible coatings has been reported both with and  
265 without significant effects on weight loss of some fruit. For example, Pérez-Gago et al.  
266 (2002) reported that HPMC–lipid composite coatings containing different types of  
267 lipids reduced weight loss of coated ‘Fortune’ mandarins. However, HPMC-lipid  
268 coatings containing food preservatives did not control weight loss of ‘Valencia’ oranges  
269 after 60 d at 5 °C followed by 7 d of shelf-life at 20 °C (Valencia-Chamorro et al.,  
270 2009). In ‘Angeleno’ plums, HPMC–BW coatings containing different types of  
271 plasticizers did not reduce weight loss of the fruit as compared with uncoated samples  
272 (Navarro-Tarazaga et al., 2008b). Similarly, HPMC coatings containing soybean oil or  
273 carnauba wax had minimal effect on water loss of coated cherries or cucumbers  
274 (Baldwin et al., 1997).

275 *Internal gas concentration.* All coatings increased the internal CO<sub>2</sub> and decreased  
276 the O<sub>2</sub> concentrations of the oranges compared to the control, which indicates that the  
277 coatings exerted a barrier to gas exchange (Table 2). In general, the HPMC-based

278 coatings exerted a higher gas barrier than the CW, although the effect depended on  
279 composition of the HPMC-based coatings. Up to 8 weeks of storage at 5 °C plus 1 week  
280 at 20 °C, an increase in SC of the HPMC-based coating increased the internal CO<sub>2</sub> level  
281 and decreased the O<sub>2</sub> level of the oranges. Many works have described a direct relation  
282 between the internal gas modification of coated fruit and coating thickness, which  
283 depends on SC, viscosity, and density of the coating formulation (Cisneros-Zevallos and  
284 Krochta, 2003; Navarro-Tarazaga and Pérez-Gago, 2006).

285 For similar SC, coatings containing more shellac (BW:shellac ratio 1:3) induced a  
286 higher modification of the orange internal atmosphere, which can be explained by the  
287 higher gas barrier than shellac provides compared to waxes such as BW (Hagenmaier,  
288 2000). In general, when comparing all the HPMC-based coatings, T4 was the treatment  
289 that induced the highest CO<sub>2</sub> and the lower O<sub>2</sub> accumulation in the fruit, since this  
290 coating had the highest SC and shellac content (8% SC and BW:shellac ratio 1:3),  
291 whereas, oranges coated with T5 (4% SC and BW:shellac ratio 3:1) did not show  
292 differences in internal atmosphere with those coated with the CW.

293 Among the different ingredients incorporated to coating formulations, shellac has  
294 been known to reduce gas exchange in a greater extend than waxes, creating in many  
295 cases an anaerobic/fermentative environment in the fruit (Hagenmaier, 2000). Although  
296 the HPMC-based coatings and the CW contained shellac in their formulations, the  
297 concentration of internal CO<sub>2</sub> and O<sub>2</sub> on coated oranges at the end of the storage  
298 reached values around 7-11 and 5-11 kPa, respectively. In general, these levels of  
299 internal O<sub>2</sub> could be considered not low enough to create anaerobic conditions inside the  
300 fruit (Baldwin et al., 1997).

301            *Ethanol content.* Coatings induce an increase in the amount of some internal  
302 volatiles associated with anaerobic conditions. Ethanol has been found to be the volatile  
303 component undergoing the greatest change occurring in citrus during storage (Baldwin  
304 et al., 1995). Table 2 shows the ethanol levels in juice for coated and uncoated oranges  
305 during storage. The results confirm the creation of a modified atmosphere, as can be  
306 seen by the lower ethanol accumulation during storage in uncoated fruit than in coated  
307 fruit.

308            As observed in the fruit internal atmosphere, the CW showed a moderate increase  
309 in EC compared to some HPMC-based coatings. Comparing HPMC-based coatings, an  
310 increase in SC significantly increased the ethanol level in the fruit, which correlated  
311 with the higher gas barrier that these coatings offered to the fruit. Citrus fruit coated  
312 with shellac-based coatings generally have been reported as having higher EC than  
313 those treated with wax-based coatings (Baldwin et al., 1995; Hagenmaier 2000). In our  
314 experiment, we found that in the HPMC-based coatings with 4% SC, an increase in  
315 shellac content did not affect the EC of oranges; whereas, at 8% SC an increase in  
316 shellac content significantly increase the EC. At the end of storage, 4% SC-coated  
317 mandarins (T3 and T5) showed EC close to the CW; meanwhile, mandarins treated with  
318 the highest SC and shellac content coating (T4) reached EC values above 5,000 mg/L.  
319 Different works have reported higher EC on coated fruit after prolonged cold storage of  
320 citrus fruit. For instance, ‘Fortune’ mandarins coated with HPMC:lipid (20% lipid  
321 content, db) reached ethanol values between 3,000 and 4,000 mg/L after 30 days at 9 °C  
322 plus 7 days at 20 °C (Pérez-Gago et al., 2002). In another study with ‘Ortanique’  
323 mandarins coated with HPMC:BW, the EC was higher than 4,000 mg L<sup>-1</sup> after 45 days  
324 at 5 °C plus 7 days at 20 °C (Navarro-Tarazaga et al., 2008a).

325 **3.2 Sensory quality**

326 Sensory quality of 'Valencia' oranges was evaluated within the range of  
327 acceptability after 16 weeks of storage at 5 °C plus 1 week at 20 °C, with values around  
328 4 and no differences were found among treatments (Table 2). Under this storage  
329 conditions, coated and uncoated oranges were evaluated as having very slight or slight  
330 off-flavour. Several works showed that the contribution to off-flavour of volatile  
331 content depends on citrus cultivar. Ke and Kader (1990) established the minimum EC  
332 associated with off-flavour in 'Valencia' oranges to be 2,000 mg/L; whereas, Pérez-  
333 Gago et al. (2002) found flavour degradation in 'Fortune' mandarin at an EC above  
334 3,000 mg/L and Navarro-Tarazaga and Pérez-Gago (2006) found that EC of 1,000 mg/L  
335 reduced flavour quality of 'Clemenules' mandarins. In this work, the ethanol level  
336 found in oranges coated with T4 (high shellac content and high SC) at the end of the  
337 storage period (5,465 mg/L) was well above the limit shown by other authors associated  
338 with off-flavour development. Although, the judges evaluated this treatment as having  
339 slight off-flavour and without significant differences with the rest of the treatments, care  
340 should be taken after prolonged cold storage of citrus fruit for the potential risk of off-  
341 flavour development.

342 The appearance of the oranges was evaluated as acceptable throughout all the  
343 storage period, without differences among treatments (data not shown). One of the aims  
344 of coating applications, together with the control of weight loss, is the enhancement of  
345 external citrus appearance by conferring gloss. Panellists were asked to rank the five  
346 treatments on the basis of perceived gloss (1= the most glossy and 6= the least glossy)  
347 and the sum of the rank values was calculated (Table 2). Therefore, treatments with low  
348 scores represent more shine. Among all the coatings, treatment T5 was not effective

349 providing gloss during storage. The experimental coatings that provided the highest  
350 gloss were T3 and T4 (BW:shellac ratio 1:3), being similar to that of the CW during  
351 storage, which makes these treatments a potential replacement of commercial waxes  
352 based on petroleum derivatives such as polyethylene. This could be related to its higher  
353 shellac content. It has been reported that shellac and other resins provide higher gloss to  
354 fruit than waxes, this being the main reason for their incorporation into many coating  
355 formulations (Baldwin et al., 1997).

### 356 **3.3 Nutritional quality**

357 Table 3 shows the EC<sub>50</sub> values of coated and uncoated 'Valencia' oranges stored  
358 at 5 °C for 6, 8 and 16 weeks plus 1 week at 20 °C. As mentioned earlier, the DPPH•  
359 radical decreases by reacting with antioxidants present in the sample; therefore, the  
360 highest the EC<sub>50</sub> value the lowest the total antioxidant capacity of the sample. In this  
361 work, no effect was observed by coating application in the total antioxidant capacity of  
362 'Valencia' oranges.

363 The TAA of 'Valencia' oranges was not affected by coating application or the  
364 storage length (Table 3). Togrul and Arslan (2004), however, reported that AA loss after  
365 storage was delayed when mandarins were coated with carboxymethyl cellulose. This  
366 result was explained by the gas barrier of the coatings which decreased the potential  
367 autoxidation of AA in the presence of oxygen. In our work, although the HPMC  
368 coatings and the CW reduced the level of internal O<sub>2</sub> (Table 2), these levels could be not  
369 low enough to affect the TAA of the oranges.

370 In citrus the major FGs are NAT, HES and DID. FGs contents in 'Valencia'  
371 oranges were in the range of those reported for citrus fruit (Table 3), being HES the  
372 most abundant flavanoid followed by NAT and DID (Dhuique-Mayer et al., 2005). The



373 content of the different flavonoids, were not affected by storage length. Similarly, these  
374 FGs were not affected after 3 months of storage at 5 °C in ‘Fortune’ mandarin (Palma et  
375 al., 2005) or 24 days of storage at cold-quarantine temperature at 1 °C in ‘Valencia’  
376 oranges (Contreras-Oliva et al., 2010). In general, coating application had not an  
377 important effect on the level of the different flavonoids, although some significant  
378 differences were found among treatments for NAT after 16 weeks of storage at 5 °C  
379 plus 1 week at 20 °C.

380 In addition to flavanones, the citrus fruit also contains other phenolic compounds,  
381 such as flavones and hydroxycinnamic acids (represented by ferulic, caffeic, synapic,  
382 and *p*-coumaric acids) that, although present in a lower concentration, contribute to the  
383 total phenolic concentration (Gil-Izquierdo et al. 2002). TPC of ‘Valencia’ oranges  
384 ranged from 625 to 887 mg/L juice (GAE) (Table 3). TPC of ‘Valencia’ oranges was  
385 not affected by storage time at 5 °C. Other works have shown that cold storage at  
386 quarantine temperatures of 1 °C increased TPC of ‘Valencia’ oranges (Contreras-Oliva  
387 et al., 2010). However, Rapisarda et al. (2008) found a decrease in TPC of ‘Valencia’  
388 oranges after 40 days of storage at 6 °C attributed to senescence phenomena during  
389 storage. Other works have shown either an increase during storage, attributed to an  
390 increase in the PAL activity during low temperature storage of citrus fruit (Patil et al.,  
391 2004) or no effect, such as in ‘Fortune’ mandarins after 90 d of storage at 5 °C (Palma et  
392 al., 2005). Although some significant differences were found among treatments after 4  
393 and 8 weeks of storage at 5 °C, no tendency was found due to coating application, which  
394 makes difficult to withdraw any conclusion regarding the effect of coating composition.

#### 395 **4. Conclusion**

396 Coating application had little effect controlling weight loss of ‘Valencia’ oranges.  
397 However, after 16 weeks of storage at 5 °C plus 1 week at 20 °C, the T3 coating (4% SC  
398 and BW:shellac ratio 1:3) was the most effective coating controlling weight loss, even  
399 better than the CW. SC and the BW:shellac ratio affected the internal orange  
400 atmosphere and EC during storage. Although sensory quality was not negatively  
401 affected by coating application, care should be taken to the SC and shellac content of  
402 the formulations, since an increase of these parameters translates in a significant  
403 increase in the level of ethanol. In general, the nutritional quality was not negatively  
404 affect by the application of the different coatings. Results indicate that HPMC-BW-  
405 Shellac coating with 4% SC and a BW:Shellac ratio 1:3 would provide the best  
406 compromise to extend shelf life of ‘Valencia’ oranges by reducing weight loss,  
407 providing gloss and maintaining the nutritional quality of the fruit.

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413

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525 Table 1. Treatments and composition of the HPMC-based coatings (% dry basis)  
 526 applied to 'Valencia' oranges.

| Treatment                    | HPMC | BW   | Shellac | Glycerol | Oleic acid |
|------------------------------|------|------|---------|----------|------------|
| <b>T1:</b> Uncoated          | -    | -    | -       | -        | -          |
| <b>T2:</b> CW – 10% SC       | -    | -    | -       | -        | -          |
| <b>T3:</b> 1:3 BW:Sh - 4% SC | 0.75 | 0.60 | 1.80    | 0.37     | 0.48       |
| <b>T4:</b> 1:3 BW:Sh - 8% SC | 1.49 | 1.20 | 3.60    | 0.75     | 0.96       |
| <b>T5:</b> 3:1 BW:Sh - 4% SC | 0.75 | 1.80 | 0.60    | 0.37     | 0.48       |
| <b>T6:</b> 3:1 BW:Sh - 8% SC | 1.49 | 3.60 | 1.20    | 0.75     | 0.96       |

527 T3, T4, T5 and T6 correspond to the HPMC-based edible coatings.

528 BW= beeswax, CW= commercial wax (polyethylene-shellac), HPMC= hydroxypropyl  
 529 methylcellulose, Sh= shellac, SC= solid content.

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537 Table 2. Physico-chemical and sensory quality of coated and uncoated ‘Valencia’ oranges after storage at 5 °C followed by 1 week at 20  
538 °C.

| Storage period            | Treatment | Weight loss (%) | Internal CO <sub>2</sub> (KPa) | Internal O <sub>2</sub> (KPa) | Ethanol content (mg/L juice) | Off-Flavour | Flavour  | Gloss visual rank (sum of ranks) |
|---------------------------|-----------|-----------------|--------------------------------|-------------------------------|------------------------------|-------------|----------|----------------------------------|
| At harvest                |           | -               | 2.81                           | 18.93                         | 39.8                         | 0.08        | 7.08     |                                  |
| 4 wk 5 °C +<br>1 wk 20 °C | T1        | 2.99 cd         | 2.60 a                         | 18.46 d                       | 279.8 a                      | 0.65 ab     | 5.55 bc  | 101 d                            |
|                           | T2        | 2.70 b          | 5.04 bc                        | 14.50 c                       | 386.3 a                      | 0.45 a      | 6.35 c   | 76 bcd                           |
|                           | T3        | 2.21 a          | 5.93 cd                        | 11.39 b                       | 791.5 b                      | 0.65 ab     | 6.30 c   | 46 ab                            |
|                           | T4        | 2.79 bc         | 8.90 e                         | 7.64 a                        | 1280.5 c                     | 2.05 c      | 4.15 a   | 36 a                             |
|                           | T5        | 3.03 d          | 4.19 b                         | 16.29 cd                      | 727.1 b                      | 1.35 bc     | 5.25 b   | 80 cd                            |
|                           | T6        | 2.74 b          | 6.22 d                         | 7.91 a                        | 2172.2 d                     | 1.95 c      | 3.90 a   | 59 abc                           |
| 8 wk 5 °C +<br>1 wk 20 °C | T1        | 6.30 c          | 2.94 a                         | 18.42 e                       | 832.0 a                      | 0.48 a      | 5.19 c   | 115 c                            |
|                           | T2        | 5.29 ab         | 5.14 b                         | 15.10 d                       | 976.4 a                      | 1.19 ab     | 4.81 bc  | 52 a                             |
|                           | T3        | 5.01 a          | 8.06 c                         | 7.24 b                        | 1503.3 b                     | 1.90 bc     | 3.90 ab  | 76 ab                            |
|                           | T4        | 5.14 a          | 11.64 d                        | 3.67 a                        | 3318.4 d                     | 2.24 c      | 4.05 ab  | 43 a                             |
|                           | T5        | 5.63 b          | 6.82 bc                        | 10.10 c                       | 1379.1 b                     | 1.52 bc     | 4.43 abc | 97 bc                            |
|                           | T6        | 6.11 c          | 7.30 c                         | 5.77 b                        | 2104.4 c                     | 2.33 c      | 3.57 a   | 56 a                             |
| 16 wk 5 °C+<br>1 wk 20 °C | T1        | 11.33 c         | 5.10 a                         | 15.97 c                       | 1085.9 a                     | 1.41 a      | 4.41 a   | 81 b                             |
|                           | T2        | 11.50 c         | 7.31 b                         | 11.38 b                       | 1745.4 b                     | 1.18 a      | 4.71 a   | 36 a                             |
|                           | T3        | 9.29 a          | 10.56 cd                       | 7.11 a                        | 2195.6 b                     | 2.06 a      | 4.35 a   | 45 a                             |
|                           | T4        | 11.59 c         | 10.71 d                        | 4.59 a                        | 5465.1 d                     | 1.82 a      | 3.82 a   | 50 ab                            |
|                           | T5        | 10.36 b         | 8.08 bc                        | 10.98 b                       | 1779.3 b                     | 1.24 a      | 4.53 a   | 62 ab                            |
|                           | T6        | 9.84 ab         | 8.47 bcd                       | 10.93 b                       | 3271.1 c                     | 1.24 a      | 4.24 a   | 61 ab                            |

539 T1= uncoated, T2= CW, T3= 1:3 BW:Sh-4% SC, T4= 1:3 BW:Sh-8% SC, T5= 3:1 BW:Sh-4% SC, T6= 3:1 BW:Sh-8% SC.

540 CW= commercial wax, BW= beeswax, Sh= shellac, SC= solid content

541 Flavour was rated from 1-9 and off-flavour from 0-5.

542 Panellists ranked visually the treatments from highest (1) to lowest gloss (6) and the sum of the rank is presented.

543 Means within each storage period with the same letter are not different (p ≤ 0.05).

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545 Table 3. Antioxidant activity (EC<sub>50</sub>), total ascorbic acid (TAA), flavonoids and total phenolics contents of coated and uncoated ‘Valencia’  
 546 oranges after storage.

| Storage period             | Treatment | EC <sub>50</sub><br>(L juice/Kg DPPH) | TAA<br>(mg/L juice) | Narirutin<br>(mg/L juice) | Hesperidin<br>(mg/L juice) | Didymin<br>(mg/L juice) | Total phenolics<br>(mg GAE/L juice) |
|----------------------------|-----------|---------------------------------------|---------------------|---------------------------|----------------------------|-------------------------|-------------------------------------|
| Initial                    |           | 233 ± 14                              | 337 ± 17            | 28.3 ± 2.3                | 217 ± 11                   | 9.1 ± 0.0               | 743 ± 80                            |
| 4 wk 5 °C +<br>1 wk 20 °C  | T1        | 338±25 a                              | 453±28 a            | 38.8±0.1 a                | 268± 4 a                   | 11.6±0.2 a              | 625±21 a                            |
|                            | T2        | 345±22 a                              | 445±32 a            | 34.9±3.8 a                | 253± 4 a                   | 10.8±1.0 a              | 787±23 b                            |
|                            | T3        | 339±22 a                              | 463±21 a            | 35.9±2.2 a                | 270±17 a                   | 12.1±1.1 a              | 811±32 bc                           |
|                            | T4        | 360±23 a                              | 420±30 a            | 37.0±0.9 a                | 250±11 a                   | 11.6±0.1 a              | 784±33 b                            |
|                            | T5        | 354±23 a                              | 434±24 a            | 36.8±2.1 a                | 288±23 a                   | 11.9±0.4 a              | 783±22 b                            |
|                            | T6        | 350±18 a                              | 417±21 a            | 34.2±2.0 a                | 276±17 a                   | 11.5±0.2 a              | 835±22 c                            |
| 8 wk 5 °C +<br>1 wk 20 °C  | T1        | 368±15 a                              | 366±24 a            | 37.2±1.1 a                | 220±75 a                   | 10.4±0.1 a              | 866±14 d                            |
|                            | T2        | 363±25 a                              | 342±18 a            | 39.7±1.0 a                | 262±11 a                   | 10.7±0.2 a              | 794± 8 ab                           |
|                            | T3        | 342±17 a                              | 358±15 a            | 37.0±1.8 a                | 263± 6 a                   | 9.6±1.1 a               | 768±27 a                            |
|                            | T4        | 338± 2 a                              | 355± 7 a            | 37.9±4.0 a                | 274± 4 a                   | 10.4±0.2 a              | 838±29 cd                           |
|                            | T5        | 325±31 a                              | 379±13 a            | 38.4±1.0 a                | 270±14 a                   | 10.2±0.1 a              | 827±30 bcd                          |
|                            | T6        | 365±15 a                              | 355±19 a            | 39.2±2.1 a                | 271±12 a                   | 10.3±0.1 a              | 824±11 bc                           |
| 16 wk 5 °C +<br>1 wk 20 °C | T1        | 386±16 a                              | 342±27 a            | 46.2±2.9 b                | 303±16 a                   | 12.0±1.0 a              | 844± 4 a                            |
|                            | T2        | 369±26 a                              | 360±19 a            | 44.9±2.0 b                | 296±12 a                   | 11.4±0.8 a              | 837±38 a                            |
|                            | T3        | 383±11 a                              | 348±10 a            | 39.4±0.3 a                | 300± 9 a                   | 10.4±0.1 a              | 863±14 a                            |
|                            | T4        | 345±26 a                              | 381±33 a            | 38.4±2.1 a                | 293± 9 a                   | 10.3±0.1 a              | 887±13 a                            |
|                            | T5        | 379±21 a                              | 352±26 a            | 44.4±3.2 b                | 323± 7 a                   | 11.8±0.9 a              | 834±12 a                            |
|                            | T6        | 352± 6 a                              | 357±14 a            | 39.6±2.0 a                | 293±22 a                   | 10.4±0.2 a              | 851±21 a                            |

547 T1=uncoated, T2=CW, T3= 1:3 BW:Sh-4% SC, T4= 1:3 BW:Sh-8% SC, T5= 3:1 BW:Sh-4% SC, T6= 3:1 BW:Sh-8% SC.

548 CW= commercial wax, BW= beeswax, Sh= shellac, SC= solid content.

549 GAE= gallic acid equivalents

550 Values give means±SD (n=3). For each storage period, different treatments with the same lower case letter are not different at p ≤ 0.05. For each treatment and

551 different storage period, means with the same letter are not different at p ≤ 0.05.