Int. J. Food Sci. Technol. 46: 2437-2445

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

ADRIANA CONTRERAS-OLIVA[1,2], CRISTINA ROJAS-ARGUDO[1], MARIA B. PÉREZ-GAGO[1,3*]

[1]Postharvest Department, Instituto Valenciano de Investigaciones Agrarias, 46113 Moncada, Spain, [2]Campus Córdoba, Colegio de Postgraduados, Carretera Federal Córdoba-Veracrúz Km 348, A.P. 94946, Amatlán de los reyes, Veracrúz, México and [3]Agroalimed, 46113 Moncada, Spain

[*] To whom correspondence should be addressed.
Telephone: (34) 96 342 4000
Fax number: (34) 96 342 4106
e-mail address: perez_mbe@gva.es

Running title: Postharvest quality of HPMC-coated oranges

Abstract

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

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

1. Introduction

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

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

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

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

In the literature, many works report the effect of edible composite coatings on the postharvest quality of citrus fruit (Hagenmaier et al., 2002; Pérez-Gago et al., 2002; Navarro-Tarazaga and Pérez-Gago, 2006; Navarro-Tarazaga et al., 2008a). Most of these studies provide information about the effect of coating composition, formulation solid content (SC), storage conditions and fruit cultivars on the physicochemical and sensory quality, however little information can be found on their effect on the nutritional quality of citrus fruit. Togrul and Arslan (2004) reported that ascorbic acid loss after storage was delayed when mandarins citrus were coated with carboxymethyl cellulose. However, application of a commercial chitosan to 'Oronules' mandarins did not affect either the internal quality or the bioactive compounds of the fruit (Contreras-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 ($DPPH^{\bullet}$), potassium dihydrogen
99 phosphate (KH_2PO_4), *meta*-phosphoric acid (MPA), phosphoric acid (H_3PO_4), folin-
100 ciocalteu's phenolreagent, sodium carbonate (Na_2CO_3), gallic acid and standard L-
101 ascorbic acid (AA) were purchased from Sigma (Sigma-Aldrich Chemie, Steinhein,
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-7-0-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., Molline,
119 IL), in which glycerol, oleic acid, BW, shellac, NH_3, and one-third of the water were
120 added. The mixture was initially stirred at 100 rpm until the temperature reached 60 ºC.
121 Next, stirring was increased to 400 rpm until temperature reached 110 ºC 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 ºC 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.

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

2.4. Physicochemical quality

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

Internal gas concentration. Ten fruit per treatment were used to calculate internal gas concentrations. Internal CO_2 and O_2 concentrations of each sample were obtained by withdrawing 1 mL internal gas sample from the orange central cavity with a syringe while the fruit was immersed under water. The gas sample was then injected into a gas chromatograph (Thermo Fisher Scientific, Inc., Waltham, MA) fitted with a Porapak QS 80/100 (1.2 m x 0.32 cm) column, followed by a molecular sieve 5A 45/60 (1.2 m x 0.32 cm) column. Temperatures were 35, 125 and 180 ºC, respectively, for the oven, injector and thermal conductivity detector. Helium was used as carrier gas at 22 mL/min flow rate. Peak areas obtained from standard gas mixtures were determined before and after analysis of samples and results were expressed as kPa.

Ethanol content. Ethanol content (EC) in juice were determined by head-space gas chromatography according to the method described by Ke and Kader (1990). Ten 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 ºC, 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 ºC. 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 ºC). 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 $DPPH^{\bullet}$ assay. 0.4 ml of orange juice diluted with 0.8 mL of methanol was
193 centrifuged at 12,000 rpm and 4 ºC for 20 min. Six methanolic dilutions from the
194 supernatant (0.075 mL) were mixed with 0.2925 mL of $DPPH^{\bullet}$ (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 $DPPH^{\bullet}$ was determined on the basis of the $DPPH^{\bullet}$ standard
198 curve. The amount of juice in each dilution was plotted against the amount of $DPPH^{\bullet}$
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 $DPPH^{\bullet}$ 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^{1}) 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 ºC, detector wavelength
214 243 nm and flow rate 1 mL min^{-1}. The mobile phase was 2% KH_2PO_4 adjusted to pH
215 2.3 with H_3PO_4. The flow rate was fixed at 1 mL min^{-1} 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 ºC. 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 ºC, detector wavelength 280 nm and flow rate 1 mL min^{-1}. 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

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

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

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

2.8 Statistical Analysis.

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

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

3. Results and discussion

3.1 Physicochemical quality

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

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

Internal gas concentration. All coatings increased the internal CO_2 and decreased the O_2 concentrations of the oranges compared to the control, which indicates that the 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 ºC plus 1 week
280 at 20 ºC, an increase in SC of the HPMC-based coating increased the internal CO_2 level
281 and decreased the O_2 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_2 and the lower O_2 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_2 and O_2 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_2 could be considered not low enough to create anaerobic conditions inside the
300 fruit (Baldwin et al., 1997).

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

As observed in the fruit internal atmosphere, the CW showed a moderate increase in EC compared to some HPMC-based coatings. Comparing HPMC-based coatings, an increase in SC significantly increased the ethanol level in the fruit, which correlated with the higher gas barrier that these coatings offered to the fruit. Citrus fruit coated with shellac-based coatings generally have been reported as having higher EC than those treated with wax-based coatings (Baldwin et al., 1995; Hagenmaier 2000). In our experiment, we found that in the HPMC-based coatings with 4% SC, an increase in shellac content did not affect the EC of oranges; whereas, at 8% SC an increase in shellac content significantly increase the EC. At the end of storage, 4% SC-coated mandarins (T3 and T5) showed EC close to the CW; meanwhile, mandarins treated with the highest SC and shellac content coating (T4) reached EC values above 5,000 mg/L. Different works have reported higher EC on coated fruit after prolonged cold storage of citrus fruit. For instance, 'Fortune' mandarins coated with HPMC:lipid (20% lipid content, db) reached ethanol values between 3,000 and 4,000 mg/L after 30 days at 9 ºC plus 7 days at 20 ºC (Pérez-Gago et al., 2002). In another study with 'Ortanique' mandarins coated with HPMC:BW, the EC was higher than 4,000 mg L^{-1} after 45 days at 5 ºC plus 7 days at 20 ºC (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 ºC plus 1 week at 20 ºC, 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

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

3.3 Nutritional quality

Table 3 shows the EC_{50} values of coated and uncoated 'Valencia' oranges stored at 5 ºC for 6, 8 and 16 weeks plus 1 week at 20 ºC. As mentioned earlier, the $DPPH^{\bullet}$ radical decreases by reacting with antioxidants present in the sample; therefore, the highest the EC_{50} value the lowest the total antioxidant capacity of the sample. In this work, no effect was observed by coating application in the total antioxidant capacity of 'Valencia' oranges.

The TAA of 'Valencia' oranges was not affected by coating application or the storage length (Table 3). Togrul and Arslan (2004), however, reported that AA loss after storage was delayed when mandarins were coated with carboxymethyl cellulose. This result was explained by the gas barrier of the coatings which decreased the potential autoxidation of AA in the presence of oxygen. In our work, although the HPMC coatings and the CW reduced the level of internal O_2 (Table 2), these levels could be not low enough to affect the TAA of the oranges.

In citrus the major FGs are NAT, HES and DID. FGs contents in 'Valencia' oranges were in the range of those reported for citrus fruit (Table 3), being HES the 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 ºC in 'Fortune' mandarin (Palma et
375 al., 2005) or 24 days of storage at cold-quarantine temperature at 1 ºC 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 ºC
379 plus 1 week at 20 ºC.

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 ºC. Other works have shown that cold storage at
386 quarantine temperatures of 1 ºC 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 ºC 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 ºC (Palma et
392 al., 2005). Although some significant differences were found among treatments after 4
393 and 8 weeks of storage at 5 ºC, 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**

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

Acknowledgements

This work was funded by the Consellería de Educación de la Generalitat Valenciana through the project GV/2007/187 and the European Social Fund. The authors thank Fontestad S.A. for supplying fruit. Adriana Contreras was also funded by a scholarship from the Consejo Nacional de Ciencias y Tecnología (CONACyT).

References

AENOR (Asociación Española de Normalización y Certificación) (1997). Ensayo de clasificación por ordenación. In: *Análisis sensorial. Tomo 1: Alimentación* (UNE 87 023) (edited by AENOR). Pp. 151-166. Madrid, Spain: AENOR.

418 Bajwa, B.E. & Anjum, F.M. (2007). Improving storage performance of *Citrus reticulata*
419 Blanco mandarins by controlling some physiological disorders. *International*
420 *Journal of Food Science and Technology*, **42**, 495-501.
421 Baldwin, E.A. & Baker, R.A. (2002). Use of protein in edible coating for whole and
422 minimally processed fruit and vegetables. In: *Protein-based films and coatings*
423 (edited by A. Gennadios). Pp. 501-515. Boca Raton, USA: CRC Press.
424 Baldwin, E.A., Nisperos-Carriedo, M.O., Shaw, P.E. & Burns, J. (1995). Effects of
425 coating and prolonged storage conditions on fresh orange flavor volatiles, degrees
426 brix, and ascorbic acid levels. *Journal of Agricultural and Food Chemistry*, **43**,
427 1321-1331.
428 Baldwin, E.A., Nisperos-Carriedo, M.O., Hagenmaier, R.D. & Baker, R.A. (1997). Use
429 of lipids in coatings for food products. *Food Technology*, **51**, 56-62.
430 Biolatto, A., Salitto, V., Cantet, R.J.C. & Pensel N.A. (2005). Influence of different
431 postharvest treatments on nutritional quality of grapefruits. *Lebensmittel*
432 *Wissenschaft und Technologie,* **38**, 131-134.
433 Cano, A., Medina, A. & Bermejo, A. (2008). Bioactive compounds in different citrus
434 varieties. Discrimination among cultivars. *Journal of Food Composition and*
435 *Analysis*, **21**, 377– 381.
436 Cisneros-Zevallos, L. & Krochta, J.M. (2003). Dependence of coating thickness on
437 viscosity of coating solution applied to fruits and vegetables by dipping method.
438 *Journal of Food Science*, **68**, 503-510.
439 Contreras-Oliva, A., Rojas-Argudo, C. & Pérez-Gago, M.B. (2010). Effect of
440 insecticidal atmospheres at high temperature combined with short cold-quarantine
441 treatment on quality of 'Valencia' oranges. *Hortscience*, **45**, 1496-1500.

442 Contreras-Oliva, A., Perez-Gago, M.B., Palou, L. & Rojas-Argudo C. (2011). Effect of
443 insecticidal atmosphere and low dose X-ray irradiation in combination with cold
444 quarantine storage on bioactive compounds of clementine mandarins cv.
445 'Clemenules'. *International Journal of Food Science and Technology*, 46, 612-
446 619.
447 Del Caro, A., Piga, A., Vacca, V. & Agabbio, M. (2004). Changes of flavonoids,
448 vitamin C and antioxidant capacity in minimally processed citrus segments and
449 juices during storage. *Food Chemistry*, 84, 99-105.
450 Dhuique-Mayer, C., Caris-Veyrat, C., Ollitrault, P., Curk, F. & Amiot, M.J. (2005).
451 Varietal and interspecific influence on micronutrient contents in citrus from the
452 Mediterranean area. *Journal of Agricultural and Food Chemistry*, **53**, 2140–2145.
453 Gil-Izquierdo, A., Gil, M.I. & Ferreres, F. (2002). Effect of processing techniques at
454 industrial scale on orange juice antioxidant and beneficial health compounds.
455 *Journal of Agricultural and Food Chemistry*, **50**, 5107-5114.
456 Girennavar, B., Jayaprakasha, G.K., Mclin, S.E., Maxim, J., Yoo, K.S., Patil, B.S.
457 (2008). Influence of electron-beam irradiation on bioactive compounds in
458 grapefruits (Citrus paradisi Macf.). *Journal of Agricultural and Food Chemistry*,
459 56, 10941-10946.
460 Hagenmaier, R.D. (2000). Evaluation of a polyethylene-candelilla coating for
461 'Valencia' oranges. *Postharvest Biology and Technology*, **19**, 147-154.
462 Hagenmaier, R.D. (2002). The flavor of mandarin hybrids with different coatings.
463 *Postharvest Biology and Technology*, **24**, 79-87.

464 Hagenmaier, R.D., Goodner, K., Rousseff, R. & Dou, H. (2002). Storage of 'Marsh'
465 grapefruit and 'Valencia' oranges with different coatings. *Proceedings of the*
466 *Florida State Horticultural Society*, **115**, 303-308.
467 Ke, D. & Kader, A.A. (1990). Tolerance of 'Valencia' oranges to controlled
468 atmospheres as determined by physiological responses and quality attributes.
469 *Journal of the American Society for Horticultural Science,* **115**, 779-783.
470 Navarro-Tarazaga, M.L. & Pérez-Gago, M.B. (2006). Effect of edible coatings on
471 quality of mandarins cv. Clemenules. *Proceedings of the Florida State*
472 *Horticultural Society*, **119**, 350-352.
473 Navarro-Tarazaga, M.L., del Río, M.A., Krochta, J.M. & Pérez-Gago, M.B. (2008a).
474 Fatty acid effect on hydroxypropyl methylcellolose-beeswax edible film propertis
475 and postharvest quality of coated 'Ortanique' mandarins. *Journal of Agricultural*
476 *and Food Chemistry*, **56**, 10689-10696.
477 Navarro-Tarazaga, M.L., Sothornvit, R. & Pérez-Gago, M.B. (2008b). Effect of
478 plasticizer type and amount on hydroxypropyl methylcellulose-beeswax edible
479 film properties and postharvest quality of coated plums (cv. Angeleno). *Journal of*
480 *Agricultural and Food Chemistry*, **56**, 9502-9509.
481 Palma, A., D´Aquino, S., Agabbio, M. & Schirra, S. (2005). Changes in flavonoids,
482 ascorbic acid, polyphenol content and antioxidant activity in cold-stored 'Fortune'
483 Mandarin. *Acta Horticulturae*, **682**, 617-622.
484 Patil, B.S., Vanamala, J. & Hallman, G. (2004). Irradiation and storage influence on
485 bioactive components and quality of early and late season 'Rio Red' grapefruit
486 (Citrus paradisi Macf.). *Postharvest Biololgy and Technology*, **34**, 53-64.

Perez, A.G., Luaces, P., Oliva, J., Rios, J.J. & Sanz C. (2005). Changes in vitamin C and flavour components of mandarin juice due to curing of fruits. *Food Chemistry,* **91**, 19-24.

Pérez-Gago, M.B., Rojas, C. & del Río, M.A. (2002). Effect of lipid type and amount of edible hydroxypropyl methylcellulose-lipid composite coatings used to protect postharvest quality of mandarins cv. Fortune. *Journal of Food Science*, **67**, 2903-2909.

Rapisarda, P., Lo Bianco, M., Pannuzzo, P. & Timpanaro, N. (2008). Effect of cold storage on vitamin C, phenolics and antioxidant activity of five orange genotypes [Citrus sinensis (L.) Osbeck]. *Postharvest Biology and Technology*, **49**, 348–354.

Rhim, J.W. & Shellhammer, T.H. (2005). Lipid-based edible films and coatings. In: *Innovations in food packaging* (edited by J. Han). Pp. 362-383. Amsterdam, The Netherlands: Elsevier Academic Press.

Sánchez-Mata, M.C., Camara-Hurtado, M., Diez-Marques, C. & Torija-Isasa, M.E. (2000). Comparison of high-performance liquid chromatography and spectrofluorimetry for vitamin C analysis of green beans (Phaseolus vulgaris L.). *European Food Research Technology*, **210**, 220-225.

Sánchez-Moreno, C., Plaza, L., de Ancos, B. & Cano, M.P. (2003) Quantitative bioactive compounds assessment and their relative contribution to the antioxidant capacity of commercial orange juice. *Journal of the Science of Food and Agriculture,* **83**,430-439.

Togrul, H. & Arslan, N. (2004). Carboxymethyl cellulose from sugar beet pulp cellulose as a hydrophilic polymer in coating of mandarin. *Journal of Food Engineering*, **62**, 271-279.

511 Valencia-Chamorro, S.A., Pérez-Gago, M.B., del Río, M.A. & Palou, L. (2009). Effect
512 of antifungal hydroxypropyl methylcellulose (HPMC)–lipid edible composite
513 coatings on postharvest decay development and quality attributes of cold-stored
514 'Valencia' oranges. *Postharvest Biology and Technology*, **54**, 72-79.
515 Vanamala, J., Cobb, G., Loaiza, J.,Yoo, K., Pike, L.M. & Patil, B.S. (2007). Ionizing
516 radiation and marketing simulation on bioactive compounds and quality of
517 grapefruit (*Citrus paradisi* cv. Rio Red). *Food Chemistry,* **105**, 1404-1411.
518
519

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

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

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

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

535

536

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

Storage period	Treatment	Weight loss (%)	Internal CO_2 (KPa)	Internal O_2 (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 ºC + 1 wk 20 ºC	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 ºC + 1 wk 20 ºC	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 ºC+ 1 wk 20 ºC	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 ($p \leq 0.05$).
544

Table 3. Antioxidant activity (EC_{50}), total ascorbic acid (TAA), flavonoids and total phenolics contents of coated and uncoated 'Valencia' oranges after storage.

Storage period	Treatment	EC_{50} (L juice/Kg DPPH)	TAA (mg/L juice)	Narirutin (mg/L juice)	Hesperidin (mg/L juice)	Didymin (mg/L juice)	Total phenolics (mg GAE/L juice)
Initial		233 ± 14	337 ± 17	28.3 ± 2.3	217 ± 11	9.1 ± 0.0	743 ± 80
4 wk 5 ºC + 1 wk 20 ºC	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 ºC + 1 wk 20 ºC	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 ºC + 1 wk 20 ºC	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

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.
CW= commercial wax, BW= beeswax, Sh= shellac, SC= solid content.
GAE= gallic acid equivalents
Values give means±SD (n=3). For each storage period, different treatments with the same lower case letter are not different at $p \leq 0.05$. For each treatment and different storage period, means with the same letter are not different at $p \leq 0.05$.