J. Sci. Food & Agric. 92: 794-802

Effect of solid content and composition of hydroxypropyl methylcellulose-lipid edible coatings on physicochemical and nutritional quality of 'Oronules' mandarins

Running title: HPMC-lipid edible coatings applied on 'Oronules' mandarins

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Abstract

BACKGROUND: Citrus fruit represent an important source of vitamin C, as well as other bioactive compounds. Edible coatings have the potential to extend shelf life of citrus by providing a semi-permeable barrier to water and gases, that depends on coating composition, solid content (SC), and cultivar. However, little is known about the effect of coatings on citrus nutritional quality. This work studies the effect of coating composition and SC of hydroxypropyl methylcellulose (HPMC)-beeswax (BW)-shellac coatings on the physicochemical, sensory and nutritional quality of 'Oronules' mandarins. Coatings prepared at the same lipid content, differed in the BW:shellac ratio (1:3 and 3:1) and SC of the formulations (4 and 8 g Kg^{-1}).

RESULTS: The coating with 1:3 BW:shellac ratio and 8 g Kg^{-1} SC was the most effective controlling weight loss, although it was less effective than the commercial wax tested. Increasing SC had a greater effect than the BW:shellac ratio in fruit internal atmosphere and sensory quality, with the presence of off-flavor when coatings were applied at 8 g Kg^{-1} SC. Nutritional quality was not affected by the application of the different treatments.

CONCLUSION: HPMC-lipid coatings have the potential to extend shelf life of 'Oronules' mandarins. However, care should be taken controlling formulation SC to avoid off-flavor build-up.

Keywords: edible coating, nutritional quality, postharvest quality, HPMC, shellac, beeswax.

INTRODUCTION

Consumers demand higher quality and longer shelf-life in foods, while reducing disposable packaging materials and increasing recyclability. Such concerns have caused an increased interest in the development of new edible films and coatings. Coatings are used in fresh fruits to retard moisture loss, improve appearance, act as carriers for natural antimicrobials, and create a barrier for gas exchange between the commodity and the external atmosphere.[1] However, if the coating offers a high gas barrier, anaerobic conditions can be induced with the build-up of volatile compounds and the development of off-flavor.[2]

Edible fruit coatings are made with food-grade ingredients, generally recognized as safe (GRAS) for human consumption. Major components include polysaccharides, proteins, and lipids.[3] They present advantages and disadvantages when used as coating ingredients. Generally, lipids 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 O_2 barrier at medium-high relative humidity. However, their hydrophilic nature makes them poor moisture barriers. For this reason, most natural coatings for fruits contain a combination of ingredients forming what is called “edible composite coatings”.

There are many studies reporting the effect of edible composite coatings on the postharvest quality of citrus fruits. The combination of hydroxypropyl methylcellulose (HPMC) and lipids has been shown to reduce weight loss and retain firmness of different citrus fruit cultivars.[4-7] In these studies, coating performance depended on composition, storage conditions and fruit cultivar. Lipid type and content, and solid

content (SC) seemed to be the main factors affecting the final quality of coated citrus fruits. In general, HPMC-beeswax (BW) coatings provided a good weight control for 'Fortune' mandarins [4], 'Clemenules' mandarins[5] and 'Ortanique' mandarins[7]. However, these coatings did not improve fruit appearance. Shellac, which is a natural resin, is usually used as ingredient of natural coatings in fruits that are not consumed with peel like citrus fruits in order to provide gloss.[8] However, the higher gas barrier of resins compared to waxes may induce anaerobic conditions and increase the level of volatile components modifying fresh citrus flavor.[2]

Nowadays, nutritional and functional fruit quality has gained great interest, being a component of the overall quality that is very much valued by consumers. 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.[9] Therefore, post-harvest technologies should maintain both functional and nutritional citrus fruit quality until they reach the consumer. Most of the works found in the literature provide information about the effect of edible coatings on the physicochemical and sensory quality, but few studies can be found on their effect on the nutritional quality of coated citrus fruits. Therefore, the objective of this work was to study the effect of SC and BW:shellac ratio of HPMC-lipid edible coatings on the physicochemical, sensory and nutritional quality of 'Oronules' mandarin.

MATERIAL AND METHODS

Materials

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

Reagents 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), potassium dihydrogen phosphate (KH_2PO_4), *meta*-phosphoric acid (MPA), phosphoric acid (H_3PO_4), folin-ciocalteu's phenolreagents, sodium carbonate (Na_2CO_3), gallic acid and standard L-ascorbic acid (AA) were purchased from Sigma (Sigma-Aldrich Chemie, Steinhein, Germany). Acetic acid glacial and dimethyl sulfoxide (DMSO) were from Scharlau (Sentmenat, Barcelona, Spain). Methanol was from BDH prolabo (Poole, UK), 1,4-dithio-DL-threitol (DTT) and hesperidin (hesperitin-7-0-rutinoside, HES) were obtained from Fluka (Sigma Co., Barcelona, Spain). Narirutin (naringenin-7-rutinoside, NAT) and didymin (isosakuranetin-7-rutinoside, DID) were purchased from Extrasynthese (Genay, France). All solvents used were of HPLC-grade and ultrapure water (Milli-Q) was used for the analysis.

Coating Formulation

Emulsion coatings consisted of HPMC and different ratios of BW and shellac suspended in water. Oleic acid and glycerol were added as emulsifier and plasticizer, respectively. Coating formulations were prepared with the same lipid content (60 g Kg^{-1} of BW and shellac, dry basis) and the same HPMC content (18,7 g Kg^{-1} of HPMC, dry basis). Ratios of HPMC-glycerol (2:1) (dry basis, db) and lipid components (BW/shellac)-oleic acid (5:1) (db) were kept constant throughout the study. NH_3 (15 g

116 Kg^{-1} w/w, shellac/NH_3) was added to dissolve shellac. Formulations were prepared at
117 two different BW:shellac ratio (1:3 and 3:1) and two SC (4 and 8 g Kg^{-1}). Table 1
118 shows the treatments applied to 'Oronules' mandarins and the composition of the
119 HPMC-based coatings in wet basis.

120 Emulsions were made in a 2-L stirred pressure cell (Parr Instrument Co., Molline,
121 IL). Glycerol, oleic acid, BW, shellac, NH3, and one-third of the water were added to
122 the pressure cell. The mixture was initially stirred at 100 rpm until the temperature
123 reached 60 ºC. Next, stirring was increased to 400 rpm until temperature reached 110 ºC
124 and remained at these conditions for 30 min. Afterwards, the remaining water,
125 previously heated to 90 °C, was pumped into the vessel maintaining the stirring
126 conditions at 400 rpm for about 10-15 min after the water was incorporated. The
127 emulsion was then removed from the pressure vessel and mixed with a 5 g Kg^{-1} HPMC
128 solution previously prepared by dispersing the HPMC in hot water at 90 ºC and later
129 hydration at 20 °C for 45 min. Finally, the emulsions were cooled under agitation to a
130 temperature lower than 20 °C by placing them in an ice water bath. Water was added to
131 a final SC of 4 or 8 g Kg^{-1} depending on the treatment.

132 **Emulsion viscosity**

133 Emulsion viscosity was measured with a viscometer Synchro-Lectric viscometer Model
134 LVF (Brookfield Engineering Laboratories, Inc., Mass, USA). Three measurements
135 were made per emulsion and results were expressed as centipoises (cp). Sample
136 viscosity was measured at 20 ºC.

137 **Fruit preparation–coating application**

138 'Oronules' mandarins were hand-harvested with an average maturity index (ratio
139 between total soluble solids content and titratable acidity) of 11.8 from a local grove in

Valencia (Spain) and transferred to the IVIA postharvest facilities where they were selected, randomized, washed with tap water, and dipped in a solution of imazalil (1,000 mg/L) for 1 min.

The mandarins were randomly divided into 6 groups: 4 experimental coating treatments, 1 uncoated (control), and 1 commercial wax (CW) (polyethylene-shellac) applied at 10 g Kg^{-1} SC as a control of coated fruit (Table 1). The fruits were 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.[4] After coating, fruit were stored for 0, 1, 2, 3 and 4 weeks at 5 ºC and 90-95% RH, followed by 1 additional week at 20 ºC to simulate retail storage conditions.

Physicochemical quality

Weight loss

Lots of 30 fruits 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.

Fruit firmness

Firmness of 20 mandarins per treatment was determined at the end of each storage time using an Instron Universal Testing Machine (Model 3343, Instron Corp., Canton, MA, USA). The instrument gave the deformation (length) after application of a compression load of 10 N to the equatorial region of the fruit at a rate of 5 mm/min. Results were expressed as percentage deformation related to initial diameter.

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 mandarin 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 Poropak 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 and acetaldehyde content

Ethanol and acetaldehyde content in juice were determined by headspace gas chromatography according to the method described by Ke and Kader.[10] Ten fruits each in 3 replicates per treatment were analyzed. Five mL mandarin juice were transferred to 10 mL vials with crimp-top caps and TFE/silicone septum seals and frozen until analysis. Ethanol and acetaldehyde content were analyzed using a gas chromatograph (Thermo Fisher Scientific, Inc., Waltham, MA, USA) equipped with an autosampler, a flame ionization detector and fitted with a Poropak QS 80/100 column (1.2 m x 0.32 cm). Temperatures of the oven, injector, and detector were 150, 175, and 200 ºC, respectively. Helium was used as the carrier gas at a flow rate of 28 mL/min. A 1 mL sample of the headspace was withdrawn from each vial previously equilibrated in the autosampler incubation chamber for 10 min at 40 ºC. Ethanol and acetaldehyde concentrations were calculated using peak areas of the samples relative to the peak areas of standard solutions. Results were expressed as mg/L juice.

Sensory quality

Sensory evaluation was conducted by 10 trained panelists (5 females and 5 males), 25 to 50 years old, at the end of each storage period. Panelists evaluated overall flavor and off-flavor of mandarins. Overall flavor was rated on a 9-point scale, where 1 to 3 represented a range of non-acceptable quality with the presence of off-flavor, 4 to 6 represented a range of acceptable quality, and 7 to 9 represented a range of excellent quality. Off-flavor presence was evaluated using a 6-point intensity scale where 0= absence of off-flavor and 5= high presence of off-flavor. Six fruit per treatment were peeled and separated into individual segments. Two segments from two different fruit were presented to panelists in trays labeled with 3-digit random codes and served at room temperature (25±1 ºC). The panelists had to taste several segments of each treatment in order to compensate, as far as possible, for biological variation of material. Mineral spring water was provided for rinsing between samples. External aspect of treated fruit (coating cracks, spots, etc.) was also evaluated by the panelists. A 3-point scale was used, in which the aspect was classified as 1= bad, 2= acceptable, and 3= good. Panelists were also asked to rank visually the treatments from highest to lowest gloss. Sum of rankings were calculated.[11] The lowest sum of ranking indicates the highest gloss treatment. For visual aspect (external aspect and gloss ranking), four intact fruit per treatment were placed in trays labeled with 3-digit random codes and presented to the panelists under the same conditions (light intensity and temperature) to minimize variations in human perception.

Bioactive compounds

Total antioxidant capacity (EC_{50})

209 The total antioxidant capacity (EC_{50}) was evaluated by the DPPH$^{\bullet}$ assay.[9] 0.4 ml of
210 mandarin juice diluted with 0.8 mL of methanol was centrifuged at 12,000 rpm and 4 ºC
211 for 20 min. Six methanolic dilutions from the supernatant (0.075 mL) were mixed with
212 0.2925 mL of DPPH$^{\bullet}$ (24 mg/L) and kept in darkness for 40 min. Afterwards, the
213 change in absorbance at 515 nm was measured in a Multiskan spectrum microplate
214 reader (Thermo Labsystem, USA). For each dilution, the percentage of remaining
215 DPPH$^{\bullet}$ was determined on the basis of the DPPH$^{\bullet}$ standard curve. The amount of juice
216 in each dilution was plotted against the amount of DPPH$^{\bullet}$ radical remaining. Using the
217 curve obtained, the EC_{50} value was calculated. This result expressed the amount of
218 mandarin juice (L) needed to reduce 1 kg of DPPH$^{\bullet}$ by 50%; thus, lower values mean
219 higher antioxidant activity.

220 Total ascorbic acid (TAA)

221 TAA was determined as the sum of AA plus L-dehydroascorbic acid (DHA), by using
222 the reducing agent DTT.[12] One mL of mandarin juice was diluted to 10 mL with 2.5 g
223 L^{-1} (*w/v*) MPA. Two mL of this solution were mixed with 0.4 mL of DTT (20 g L^{-1}) for
224 2 h in darkness. Afterwards, the extracts were filtered through a 0.45 µm Millipore filter
225 before being HPLC analyzed.

226 The HPLC analyses were performed on a Lachrom Elite HPLC (Merck Hitachi,
227 Germany) equipped with autosampler (Model L-2200), quaternary pump (Model L-
228 2130), column oven (Model L-2300), and diode array detector (Model L-2450). A
229 reversed-phase C18 LiChrospher®100 column (250 x 4 mm, 5 µm-particle, Merck,
230 Darmstadt, Germany) preceded by a precolumn (4 x 4 mm) was used. System
231 conditions were: injection volume 20 µL, oven temperature 25 ºC, detector wavelength
232 243 nm, and flow rate 1 mL min^{-1}. The mobile phase was 2 g Kg^{-1} KH_2PO_4 adjusted to

pH 2.3 with H_3PO_4. AA was identified and quantified by comparison of peak areas with external standard and results were expressed as milligrams of AA per L of juice.

Flavanone glycosides (FGs)

The main FGs identified in citrus fruit (HES, NAT, and DID) were determined by the method described by Cano et al.[13] slightly modified. Two mL of mandarin juice were homogenized with 2 mL of DMSO:methanol (1:1 *v/v*) and centrifuged for 30 min at 12,000 rpm and 4 ºC. The supernatant was filtered through one 0.45 µm nylon filter and analyzed by HPLC-DAD using the HPLC equipment described above. System conditions were: injection volume 10 µL, oven temperature 25 ºC, detector wavelength 280 nm, and flow rate 1 mL min^{-1}. The column Lichospher 100 RP-18 of 25x0.4 cm was preceded by a precolumn (4x4 mm) with 5 µm particle size (Merck, Darmstadt, Germany). The mobile phase was acetonitrile (A):0.6% acetic acid (B) with initial condition of 10% A for 2 min, reaching 75% A in the following 28 min, then back to the initial condition in 1 min and held for 5 min prior to 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 mandarin juices were analyzed for TPC by the Folin-Ciocalteu colorimetric method. 0.3 mL of mandarin juice was diluted with 1.7 mL of 80 ml L^{-1} 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 g L^{-1} *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.

Statistical analysis.

A complete randomized design was used to perform the analysis of the samples. 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 analyzed 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 AENOR.[11] Significance differences were defined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Physicochemical quality

Weight loss

Figure 1 shows the weight loss of coated and uncoated mandarins stored for 0, 1, 2, 3, and 4 weeks at 5 ºC, followed by 1 week at 20 ºC. Weight loss increased with storage time, increasing to nearly 25% after 4 weeks at 5 ºC plus 1 week at 20 ºC on uncoated samples. The CW was the most effective coating controlling weight loss of 'Oronules'

281 mandarins during storage, probably due to its higher hydrophobic character. The
282 HPMC-based coatings had no effect controlling weight loss of 'Oronules' mandarins
283 stored 1 week at 5 ºC plus 1 week at 20 ºC. After 2 weeks at 5 ºC plus 1 week at 20 ºC,
284 these coatings reduced fruit weight loss by 30% compared to the control with no
285 differences among the edible coatings. However, for longer storage periods, the HPMC-
286 based coatings lost effectiveness, being T4 (BW:shellac ratio 1:3 and 8 g Kg^{-1} SC) the
287 most effective HPMC-based coating controlling weight loss of the fruit. All the HPMC-
288 based coatings had the same content of hydrophobic components (BW-Shellac), but
289 differed in the BW:Shellac ratio and SC. The small differences found among the
290 HPMC-based coatings could be due to the similar content of hydrophobic components
291 (BW-Shellac), indicating that changes in BW:Shellac ratio had little effect on weight
292 loss control of the mandarins.

293 Application of HPMC-based edible coatings has been reported both with and
294 without significant effects on weight loss of some fruit. For example, Pérez-Gago et al.[4]
295 reported that HPMC-lipid composite coatings containing different lipids reduced weight
296 loss of coated 'Fortune' mandarins. Other works also reported that HPMC-lipid edible
297 coatings were effective reducing weight loss of 'Ortanique' mandarins[14], whereas
298 similar coatings did not reduce weight loss of 'Valencia' oranges.[15] In 'Angeleno'
299 plums, HPMC-BW coatings containing different types of plasticizers did not reduce
300 weight loss of the fruit as compared with uncoated samples.[16] Similarly, HPMC
301 coatings containing soybean oil or carnauba wax had minimal effect on water loss of
302 coated cherries or cucumbers.[17]

303 Fruit firmness

In general, the firmness of 'Oronules' mandarins was slightly improved by coating application compared to uncoated mandarins (Figure 2). Even though some significant differences in firmness were found among treatments, no tendency was observed between BW:shellac ratio or SC of coating formulations and firmness. The lack of tendency between coating type and fruit texture has also been reported by Rojas et al.[18] in 'Fortune' mandarins.

Despite the good weight loss control offered by the CW, this coating did not show any effect controlling firmness loss of 'Oronules' mandarins during storage. Some authors have observed a correlation between citrus fruit weight loss and firmness[7,19], whereas others have found no correlation.[4,20] Differences in the results might indicate that in order to see an effect on fruit texture due to coating application, the coatings should provide sufficient weight loss control. Moreover, fruit cultivar and storage conditions could be contributing factors for the observed differences.

Internal gas concentration

Figure 3 shows the internal gas concentration of coated and uncoated 'Oronules' mandarins. The concentration of internal CO_2 and O_2 on coated mandarins reached values around 6-11 and 4-12 kPa, respectively, at the end of the storage.

In general, the CW increased the internal CO_2 and decreased the O_2 level of coated mandarins compared to uncoated samples stored up to 2 weeks at 5 ºC plus 1 week at 20 ºC, whereas no differences were found for longer storage periods. For short storage periods (up to 2 weeks at 5 ºC plus 1 week at 20 ºC), slight differences were found between the HPMC-based coatings and the CW. However, an important increase in CO_2 and a decrease in O_2 were observed as SC of the HPMC-based coatings increased in coated mandarins stored 4 weeks at 5 ºC plus 1 week at 20 ºC. Many works

have described a direct relation between the internal gas modification of coated fruit and coating thickness, which depends on SC, viscosity, and density of the coating formulation.[5,21,22]

For similar SC, the BW:shellac ratio seemed to have little or no effect on the mandarin internal atmosphere. This contrasts with the higher gas barrier that resins, such as shellac, provide compared to waxes such as BW.[23] Therefore, when comparing all the HPMC-based coatings, T4 and T6 were the coatings that induced the highest CO_2 and the lower O_2 accumulation in the fruit, indicating that SC of the HPMC-based coatings had a greater effect on internal atmosphere that the ratio of the hydrophobic ingredients. Mandarins coated with T3 and T5 coatings did not show differences in internal atmosphere with those coated with the CW and the control.

Ethanol and acetaldehyde contents

Figure 4 shows the ethanol levels in coated and uncoated mandarins with storage time. The HPMC-based and CW coatings increased both ethanol and acetaldehyde levels in coated mandarins compared to uncoated ones, which confirms the creation of a modified atmosphere into the fruit. As observed in the fruit internal atmosphere, the CW showed a moderate increase in ethanol level compared to some HPMC-based coatings. Comparing the 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 provided to the fruit.

Citrus fruit coated with shellac-based coatings generally have been reported as having higher ethanol content than those treated with wax-based coatings.[20,23,24] In our experiment, in mandarins stored up to 2 weeks at 5 ºC plus 1 week at 20 ºC and coated with 4 g Kg^{-1} SC coatings, an increase in shellac content did not affect the ethanol level

of 'Oronules' mandarins; whereas, at 8 g Kg^{-1} SC an increase in shellac content significantly increased the ethanol level. In general, mandarins coated with T4 (BW:shellac ratio 1:3 with 8 g Kg^{-1} SC) had the highest levels of ethanol and mandarins coated with T5 (BW:shellac ratio 3:1 with 4 g Kg^{-1} SC) had the lowest levels of ethanol. The same behavior was observed in acetaldehyde levels (data not shown).

At the end of the storage, the levels of ethanol in coated samples reached values between 1650-2460 mg/L juice. Different works have reported higher levels of ethanol on coated citrus fruit after prolonged cold storage. For instance, 'Fortune' mandarins coated with HPMC:lipid (20 g Kg^{-1} lipid content, db) reached ethanol values between 3000 and 4000 mg/L juice after 30 days at 9 ºC plus 7 days at 20 ºC.[4] In another study with 'Ortanique' mandarins coated with HPMC:BW, the ethanol content was higher than 4000 mg/L after 45 days at 5 ºC plus 7 days at 20 ºC.[7] In this work, however, ethanol concentration in coated mandarins did not exceed 3000 mg/L.

Sensory quality

Sensory quality of 'Oronules' mandarins was affected by coating and storage period (Table 2). Flavor evaluation of uncoated mandarins decreased with storage time from 7 at harvest time to 4 at the end of the storage. Several works showed that the contribution of fermentative volatiles to off-flavor depends on citrus cultivar. Ke and Kader[10] established the minimum ethanol content associated with off-flavor in 'Valencia' oranges to be 2000 mg/L; whereas, Pérez-Gago et al.[4] found flavor degradation in 'Fortune' mandarin at an ethanol content above 3000 mg/L and Navarro-Tarazaga and Pérez-Gago[5] found that ethanol content of 1000 mg/L reduced flavor quality of 'Clemenules' mandarins. In our experiment, mandarins coated with the HPMC-based coatings at 8 g Kg^{-1} SC showed an important decrease in flavor and an increase in off-

flavor compared to those coated at 4 g Kg^{-1} SC at the end of the storage period. These coatings induced the highest ethanol production (Figure 4), exceeding slightly the limit observed by some authors to induce off-flavor. Therefore, the lower ethanol content for mandarins coated with the HPMC-based coatings at 4 g Kg^{-1} SC, made them more appropriate to coat 'Oronules' mandarins under these storage conditions.

The appearance of the mandarins was evaluated as acceptable throughout all the storage period, without differences among treatments (data not shown). One of the aims of coating applications, together with the control of weight loss, is the enhancement of external appearance by conferring gloss. Panelists were asked to rank the five treatments on the basis of perceived gloss (1=the most glossy and 6=the least glossy). Therefore treatments with low scores represent shinier mandarins. The CW was the coating that provided more gloss to 'Oronules' mandarins, while the HPMC-based coatings did not significantly improved fruit gloss compared to uncoated samples (Figure 5). Among the HPMC-based coatings, T3 (BW:shellac ratio 1:3 with 4 g Kg^{-1} SC) was the most effective coating increasing mandarin gloss, approaching to the gloss provided by the CW coating. 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.[17,23]

Many reports show a lower effectiveness of edible composite coatings providing gloss than commercial waxes. These differences could be related to differences in the lipid particle size. It has been observed that in order to obtain high gloss, wax coatings need to be prepared as microemulsions, so that when water evaporates the emulsion will have a smooth surface.[25] The small lipid particle size of microemulsions makes the emulsion transparent to translucent[26] and as lipid particle size increases, emulsions lose

transparency.[27] In our experiment, all coating formulations were characterized by being translucent, and, therefore, it would be expected to have reduced gloss compared to commercial wax microemulsions. Although an increase in shellac content showed a slight increase in fruit gloss, the higher lipid particle size did not translate in a high gloss similar to the CW. In addition, the increase in the SC of the coating would have translated in an increase in coating thickness reducing transparency and gloss.

Bioactive compounds

Antioxidant capacity was expressed as EC_{50} or juice quantity necessary to reduce by 50% the $DPPH^{\bullet}$, thus the lower the value the higher the antioxidant capacity of the citrus fruit. The results showed that the EC_{50} of 'Oronules' mandarins was not affected by coating application or storage length.

The TAA of 'Oronules' mandarins increased as storage time increased (Table 3). Although significant differences were found among treatments during storage, no tendency can be observed, which makes difficult to draw any conclusion regarding the effect of coating composition. This variability in the results during storage can be due to biological variation of the fruit. After 3 and 4 weeks of cold storage plus 1 week at 20 ºC, mandarins coated with T3 (BW:shellac ratio (1:3) and 4 g Kg^{-1} SC) presented the highest TAA content. Togrul and Arslan[28] 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 ascorbic acid in the presence of oxygen.

The results showed that HES was the most abundant FGs in 'Oronules' mandarins followed by NAT and DID (Table 3). The contents of the different FGs were not affected or slightly affected by storage length. Similarly, these FGs were not affected

after 3 months of storage at 5 ºC in 'Fortune' mandarin[29] or 24 days of storage at cold-quarantine temperature (1 ºC) in 'Valencia' oranges.[30] In general, coating application had not an important effect on the level of the different FGs, although some significant differences were found among treatments for HES after 3 and 4 weeks at 5 ºC plus 1 week at 20 ºC.

In addition to flavanones, citrus fruit also contains other phenolic compounds, such as flavones and hydroxycinnamic acids (represented by ferulic, caffeic, synapic, and p-coumaric acids) that, although present in a lower concentration, contribute to the TPC.[31] Although some significant differences were found among treatments after 3 and 4 weeks of storage at 5 ºC, no tendency was found on TPC due to coating application, which makes difficult to draw any conclusion regarding the effect of coating composition on this parameter. However, some differences were observed with storage time. After 1 week of storage at 20 ºC and 1 week at 5 ºC plus 1 week at 20 ºC, the TPC of 'Oronules' mandarins showed an increase over the initial value. However, during the next storage periods the TPC decreased to values close to the initial value. Some works have shown that cold storage either did not influence or decreased the citrus TPC. For example, Palma et al.[29] did not find differences in the TPC of 'Fortune' mandarins after 90 d of storage at 5 ºC; whereas, Rapisarda et al.[32] found a decrease of total phenolics in 'Valencia' oranges after 40 d of storage at 6 ºC, which was attributed to senescence during storage.

CONCLUSION:

The coating with 1:3 BW:shellac ratio and 8 g Kg^{-1} SC was the most effective controlling weight loss, although it was less effective than the commercial wax tested.

448 Increasing SC had a greater effect than the BW:shellac ratio in fruit internal atmosphere
449 and sensory quality, with the presence of off-flavor when coatings were applied at 8 g
450 Kg^{-1} SC. Nutritional quality was not affected by the application of the different
451 treatments. HPMC-lipid coatings have the potential to extend shelf life of 'Oronules'
452 mandarins. However, care should be taken controlling formulation SC to avoid off-
453 flavor build-up.
454

455 **Acknowledgements**

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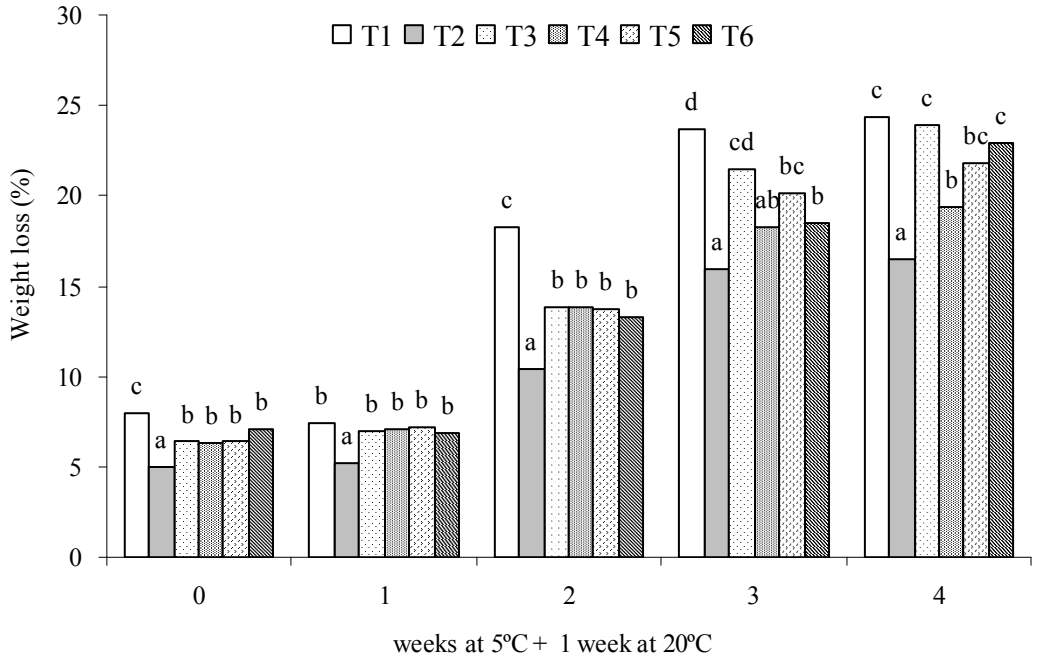

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560 Figure 1. Weight loss of coated and uncoated 'Oronules' mandarins during storage.

561 T1=uncoated, T2=CW, T3= 1:3 BW:Sh-4 g Kg^{-1} SC, T4=1:3 BW:Sh-8 g Kg^{-1} SC,

562 T5=3:1 BW:Sh-4 g Kg^{-1} SC, T6=3:1 BW:Sh-8 g Kg^{-1} SC.

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

564 Means within each storage with the same letter are not different by the least significant

565 difference (LSD) test ($p \leq 0.05$).

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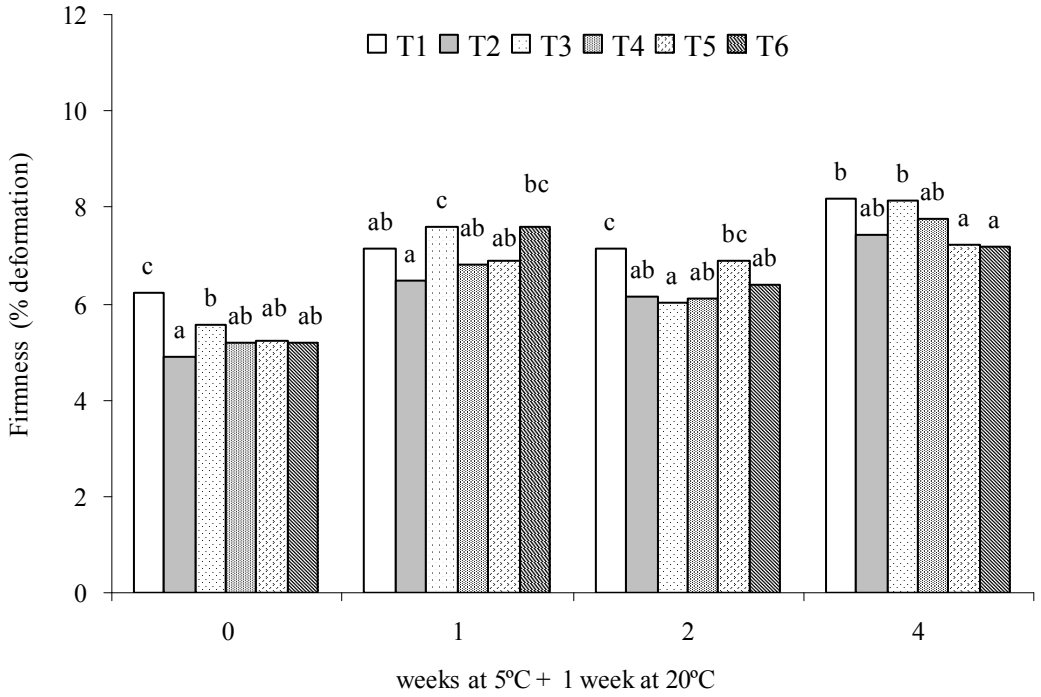

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575 Figure 2. Firmness of coated and uncoated 'Oronules' mandarins during storage.

576 T1=uncoated, T2=CW, T3= 1:3 BW:Sh-4 g Kg^{-1} SC, T4=1:3 BW:Sh-8 g Kg^{-1} SC,

577 T5=3:1 BW:Sh-4 g Kg^{-1} SC, T6=3:1 BW:Sh-8 g Kg^{-1} SC.

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

579 Firmness at harvest was 6.3% deformation.

580 Means within each storage with the same letter are not different by the least significant

581 difference (LSD) test ($p \leq 0.05$).

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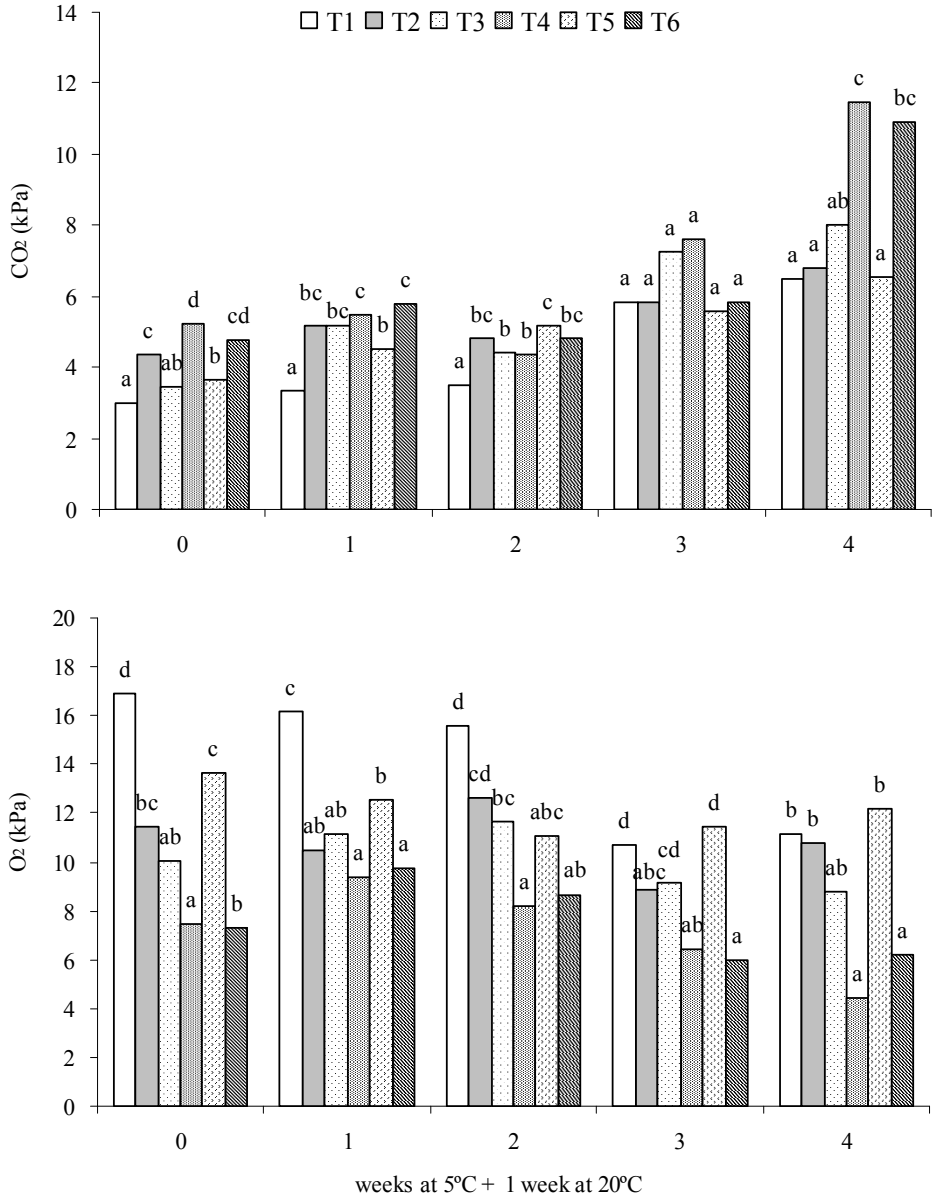

583

584 Figure 3. Internal CO_2 and O_2 contents of coated and uncoated 'Oronules' mandarins

585 during storage.

586 T1=uncoated, T2=CW, T3= 1:3 BW:Sh-4 g Kg^{-1} SC, T4=1:3 BW:Sh-8 g Kg^{-1} SC,

587 T5=3:1 BW:Sh-4 g Kg^{-1} SC, T6=3:1 BW:Sh-8 g Kg^{-1} SC.

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

589 At harvest, internal CO_2 and O_2 were 1.2 and 20.0 kPa, respectively.

590 Means within each storage with the same letter are not different by the least significant

591 difference (LSD) test ($p \leq 0.05$).

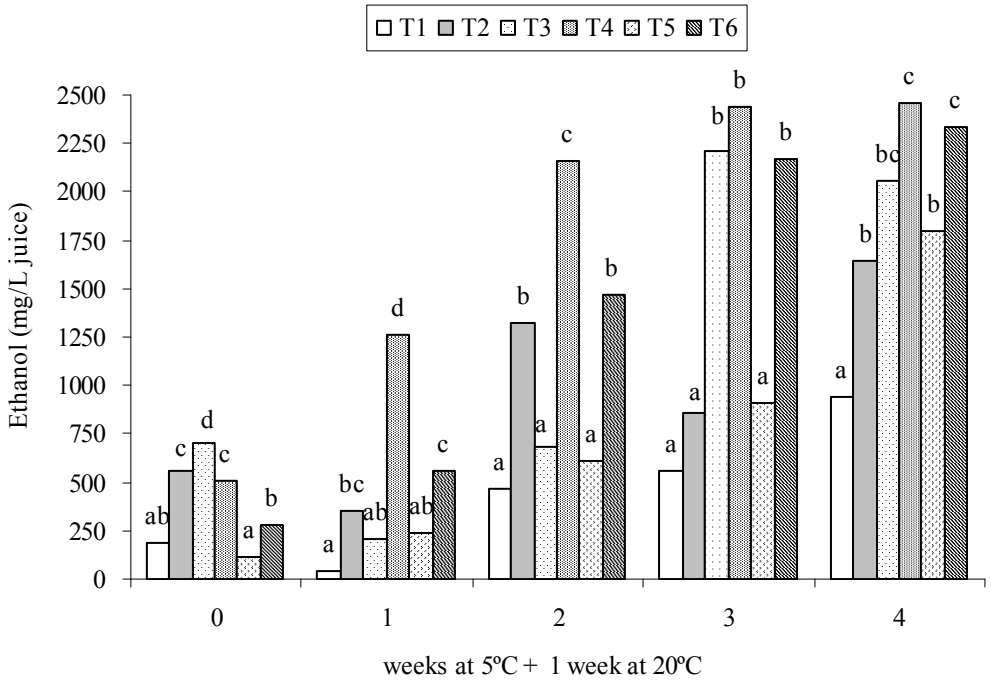

Figure 4. Ethanol content of coated and uncoated 'Oronules' mandarins during storage.

T1=uncoated, T2=CW, T3= 1:3 BW:Sh-4 g Kg^{-1} SC, T4=1:3 BW:Sh-8 g Kg^{-1} SC, T5=3:1 BW:Sh-4 g Kg^{-1} SC, T6=3:1 BW:Sh-8 g Kg^{-1} SC.

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

At harvest, ethanol content was 18 mg/L.

Means within each storage with the same letter are not different ($p \leq 0.05$) by the least significant difference (LSD) test ($p \leq 0.05$).

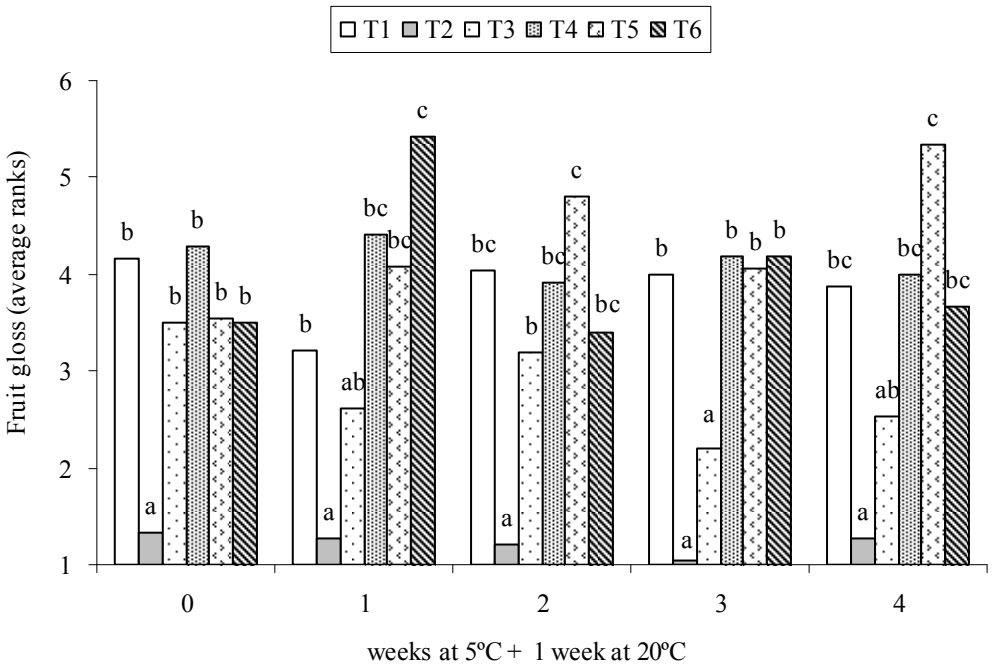

Figure 5. Gloss (average ranks) of coated and uncoated 'Oronules' mandarins during storage. Panelists ranked visually the treatments from highest (1) to lowest gloss (6) and the sum of ranks is presented.

T1=uncoated, T2=CW, T3= 1:3 BW:Sh-4 g Kg^{-1} SC, T4=1:3 BW:Sh-8 g Kg^{-1} SC, T5=3:1 BW:Sh-4 g Kg^{-1} SC, T6=3:1 BW:Sh-8 g Kg^{-1} SC.

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

Means within each storage with the same letter are not different ($p \leq 0.05$).

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621 Table 1. Treatments and composition of the HPMC-based coatings (g Kg^{-1}, wet basis)
622 applied to 'Valencia' oranges.

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

623 T3, T4, T5 and T6 correspond to the HMPC-based edible coatings.
624 BW= beeswax, CW= commercial wax (polyethylene-shellac), HPMC= hydroxypropyl
625 methylcellulose, Sh= shellac, SC= solid content.

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Table 2. Flavor and off-flavor of coated and uncoated 'Oronules' mandarins after storage at 5 ºC followed by 1 week at 20 ºC.

Treatments	Initial (At harvest)		0 wk 5ºC + 1 wk 20ºC		1 wk 5ºC + 1 wk 20ºC		2 wk 5ºC + 1 wk 20ºC		3 wk 5ºC + 1 wk 20ºC		4 wk 5ºC + 1 wk 20ºC	
	Flavor	Off-flavor	Flavor	Off-flavor	Flavor	Off-flavor	Flavor	Off-flavor	Flavor	Off-flavor	Flavor	Off-flavor
T1	7.00	0.00	6.13 a	0.46 c	5.43 a	0.61 a	5.28 a	0.96 b	4.57 a	1.57 abc	4.80 a	1.60 bc
T2	7.00	0.00	5.21 ab	0.83 c	4.22 a	1.74 a	5.20 a	1.24 b	4.43 a	1.52 abc	4.67 ab	1.40 c
T3	7.00	0.00	5.21 ab	1.04 bc	4.96 a	1.26 a	5.20 a	0.92 b	3.71 a	2.43 a	4.20 ab	1.93 bc
T4	7.00	0.00	4.04 c	2.08 a	4.13 a	1.78 a	3.40 b	2.72 a	3.19 a	2.33 ab	3.40 bc	2.73 ab
T5	7.00	0.00	5.88 a	0.83 c	4.52 a	1.57 a	5.16 a	1.16 b	4.76 a	0.86 c	4.00 ab	2.33 bc
T6	7.00	0.00	4.38 bc	1.83 ab	4.22 a	1.83 a	3.96 b	2.44 a	4.67 a	1.38 bc	2.47 c	3.80 a

T1=uncoated, T2=CW, T3=1:3 BW:Sh-4 g Kg^{-1} SC, T4=1:3 BW:Sh-8 g Kg^{-1} SC, T5=3:1 BW:Sh-4 g Kg^{-1} SC, T6=3:1 BW:Sh-8 g Kg^{-1} SC.

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

Flavor was rated from 1-9 and off-flavor from 0-5.

Means within each storage with the same letter are not different by the least significant difference (LSD) test ($p \leq 0.05$).

639 Table 3. Antioxidant activity (EC_{50}), total ascorbic acid (TAA), flavonoids and total phenolics contents of coated and uncoated 'Oronules'
640 mandarins after storage at 5 ºC followed by 1 week at 20 ºC.

Storage time	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		340±11	451±19	8.7±1.5	189±10	0.8±0.1	618±6
0 wk 5ºC + 1wk 20ºC	T1	301± 8 a A	382± 31 a A	10.0±1.2 a BC	186±11 a A	0.9±0.1 a B	672±40 a A
	T2	294± 8 a A	452± 44 ab A	10.3±1.9 a A	192±16 a A	0.9±0.2 a A	775±65 a B
	T3	291±37 a A	519± 28 bc A	8.8±1.2 a A	191±10 a A	0.8±0.1 a A	798±35 a C
	T4	279±20 a A	589± 86 c AB	9.7±0.5 a A	225±10 c BC	0.9±0.1 a C	741±31 a C
	T5	283±17 a A	434± 29 a A	10.1±0.7 a A	217± 8 bc BC	1.0±0.1 a B	691±95 a A
	T6	260±17 a A	401± 37 a A	10.1±0.2 a A	201± 2 ab A	1.0±0.0 a A	684±26 a B
1 wk 5ºC + 1wk 20ºC	T1	265±25 a A	839± 21 c B	10.5±0.1 a C	212± 9 ab C	1.0±0.0 a B	765±69 a B
	T2	283± 5 a A	641±136 b AB	10.3±1.2 a A	201± 6 a A	0.9±0.0 a A	781±25 a B
	T3	298±16 a A	759± 68 bc B	10.6±1.5 a A	205±14 a A	1.0±0.1 a A	816±64 a C
	T4	291± 3 a A	746±139 bc BC	12.6±1.0 a B	221± 3 bc BC	1.2±0.1 a D	833±30 a D
	T5	273±29 a A	682± 50 bc B	10.9±1.8 a A	211±11 ab AB	1.1±0.2 a B	799±67 a B
	T6	271±26 a A	375± 59 a A	12.1±1.6 a A	234± 6 c A	1.1±0.2 a A	844±23 a C
2 wk 5ºC + 1wk 20ºC	T1	272±23 a A	767±117 a B	8.9±0.5 a AB	204± 9 a BC	1.1±0.0 b C	627±21 a A
	T2	261±21 a A	599±228 a AB	9.6±1.7 a A	199±20 a A	0.9±0.2 b A	622±29 a A
	T3	270± 6 a A	563±172 a AB	10.0±2.5 a A	194±21 a A	0.9±0.2 b A	603±33 a A
	T4	274±17 a A	476±107 a A	11.4±1.2 a B	236±15 a C	0.6±0.1 a A	608±17 a A
	T5	258±47 a A	533±187 a AB	11.1±1.7 a A	237±22 a C	1.1±0.1 b B	615±19 a A
	T6	246±33 a A	1288±311 b C	10.5±1.3 a A	232±27 a A	0.9±0.1 b A	632± 9 a A
3 wk 5ºC + 1wk 20ºC	T1	232±11 a A	575± 29 a AB	10.2±0.4 a BC	232±10 c D	1.1±0.0 a C	685±29 b A
	T2	280±35 a A	785± 146 b B	9.2±1.2 a A	184±19 ab A	0.8±0.1 ab A	619± 5 a A
	T3	298±50 a A	1193± 149 c C	9.6±1.0 a A	195±10 ab A	0.9±0.0 b A	656±24 b AB
	T4	284± 4 a A	642± 95 ab AB	8.7±0.3 a A	174± 5 a A	0.8±0.0 a B	617±10 a A
	T5	250±34 a A	620± 14 ab B	9.9±1.3 a A	196±15 ab AB	1.0±0.1 bc AB	670± 8 b A
	T6	252±16 a A	606± 13 a A	9.8±0.5 a A	206±10 b A	0.9±0.1 ab A	674±24 b B
4 wk 5ºC + 1wk 20ºC	T1	272±36 a A	1155±304 bc C	8.4±0.9 a A	193± 5 abc AB	0.8±0.1 a A	668±15 bc A
	T2	283±42 a A	1151 ±167 abc C	9.2±1.1 a A	179±13 a A	0.8±0.1 a A	662±22 bc A
	T3	286± 5 a A	1380± 45 c C	10.5±0.3 a A	208± 5 bc A	0.7±0.2 a A	681± 7 c B
	T4	263±28 a A	929±142 ab C	11.2±0.2 a B	211± 7 c B	0.9±0.0 a C	671±13 bc B
	T5	251±19 a A	928± 58 ab C	8.7±0.6 a A	189± 5 ab A	0.8±0.0 a A	648±19 ab A
	T6	260±16 a A	872± 61 a B	9.6±2.1 a A	203±21 bc A	0.9±0.1 a A	629±24 a A

641 T1=uncoated, T2=CW, T3=1:3 BW:Sh-4 g Kg^{-1} SC, T4=1:3 BW:Sh-8 g Kg^{-1} SC, T5=3:1 BW:Sh-4 g Kg^{-1} SC, T6=3:1 BW:Sh-8 g Kg^{-1} SC.

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

643 GAE= gallic acid equivalents
644 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
645 different storage period, means with the same capital letter are not different by the least significant difference (LSD) test ($p \leq 0.05$).