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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
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
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 focused 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...
coating composition and formulation SC of hydroxypropyl methylcellulose (HPMC)-
lipid edible coatings on the physicochemical, sensory and nutritional quality of
‘Valencia’ oranges.

2. Material and methods

2.1 Materials

HPMC (Methocel E15) was purchased from Dow Chemical Co. (Midland, MI, USA). Shellac and beeswax (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•), potassium dihydrogen phosphate (KH₂PO₄), meta-phosphoric acid (MPA), phosphoric acid (H₃PO₄), folin-ciocalteu’s phenol reagent, sodium carbonate (Na₂CO₃), 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, 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.

2.2 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. 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. BW and shellac content was 60% (db). Ammonia (15%, w/w, shellac/ammonia) was added to dissolve shellac. Formulations were prepared at two different BW:shellac ratios (1:3 and 3:1) and two SC (4% and 8%). Table 1 shows the composition of the HPMC-based coatings (T3 to T6).

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

2.3 Fruit preparation–coating application

‘Valencia’ oranges (Citrus sinensis) were hand-harvested with an average maturity index of 8.7 from a local grove in Valencia (Spain) and transferred to the IVIA postharvest facilities where they were selected, randomised, washed with tap water, and 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\textsubscript{2} and O\textsubscript{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
transferred to 10 mL vials with crimp-top caps and TFE/silicone septum seals and frozen until analysis. EC was analysed 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 °C, respectively. Helium was used as the carrier gas at a flow rate of 28 mL/min. A 1 mL sample of the head-space was withdrawn from each vial previously equilibrated in the autosampler incubation chamber for 10 min at 40 °C. EC concentration was calculated using peak areas of the samples relative to the peak area of standard solution. Results were expressed as mg/L juice.

2.5 Sensory quality

Sensory evaluation was conducted by 10 trained judges (5 females and 5 males), 25 to 50 years old, at the end of each storage period. Judges evaluated overall flavour and off-flavour of mandarins. Overall flavour was rated on a 9-point scale, where 1 to 3 represented a range of non-acceptable quality with the presence of off-flavour, 4 to 6 represented a range of acceptable quality, and 7 to 9 represented a range of excellent quality. Off-flavour presence was evaluated using a 6-point intensity scale where 0= absence of off-flavour and 5= high presence of off-flavour. Six fruit per treatment were peeled and separated into individual segments. Two segments from two different fruit were presented to judges in trays labelled with 3-digit random codes and served at room temperature (25±1 °C). The judges 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 panellist. A 3-point scale was
used, in which the aspect was classified as 1= bad, 2= acceptable, and 3= good. Panellists were also asked to rank visually the treatments from highest to lowest gloss. Sum of rankings were calculated (AENOR, 1997). 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 labelled with 3-digit random codes and presented to the judges under the same conditions (light intensity and temperature) to minimize variations in human perception.

2.6 Nutritional quality

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

Total ascorbic acid (TAA). TAA was determined by the sum of AA plus L-dehydroascorbic acid (DHA), by using the reducing agent DTT (Sánchez-Mata et al., 2000). One mL of orange juice was diluted to 10 mL with 2.5% (w/v) MPA. Two mL of this solution were mixed with 0.4 mL of DTT (20 mg mL$^{-1}$) for 2 h in darkness.
Afterwards, the extracts were filtered through a 0.45 µm Millipore filter before being HPLC analysed. The HPLC analyses were performed on a Lachrom Elite HPLC (Merck Hitachi, Germany) equipped with an autosampler (Model L-2200), quaternary pump (Model L-2130), column oven (Model L-2300), and diode array detector (Model L-2450). A reversed-phase C18 LiChrospher®100 column (250 x 4 mm, 5 µm-particle, Merck, Darmstadt, Germany) preceded by a precolumn (4 x 4 mm) was used. System conditions were: injection volume 20 µL, oven temperature 25 ºC, detector wavelength 243 nm and flow rate 1 mL min⁻¹. The mobile phase was 2% KH₂PO₄ adjusted to pH 2.3 with H₃PO₄. The flow rate was fixed at 1 mL min⁻¹ and the wavelength of measurement was 243 nm. AA was identified and quantified by comparison of peak areas with external standard and results were expressed as mg/L 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. (2008) slightly modified. Two mL of orange 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 analysed 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) 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 compound 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≤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≤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 °C, followed by 1 week at 20 °C. Weight loss increased with storage time, increasing to nearly 12% after 16 weeks of storage at 5 °C plus 1 week at 20 °C 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 5°C, 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 °C followed by 7 d of shelf-life at 20 °C (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 CO₂ and decreased the O₂ 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
coatings exerted a higher gas barrier than the CW, although the effect depended on composition of the HPMC-based coatings. Up to 8 weeks of storage at 5 °C plus 1 week at 20 °C, an increase in SC of the HPMC-based coating increased the internal CO₂ level and decreased the O₂ level of the oranges. 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 (Cisneros-Zevallos and Krochta, 2003; Navarro-Tarazaga and Pérez-Gago, 2006).

For similar SC, coatings containing more shellac (BW:shellac ratio 1:3) induced a higher modification of the orange internal atmosphere, which can be explained by the higher gas barrier than shellac provides compared to waxes such as BW (Hagenmaier, 2000). In general, when comparing all the HPMC-based coatings, T4 was the treatment that induced the highest CO₂ and the lower O₂ accumulation in the fruit, since this coating had the highest SC and shellac content (8% SC and BW:shellac ratio 1:3), whereas, oranges coated with T5 (4% SC and BW:shellac ratio 3:1) did not show differences in internal atmosphere with those coated with the CW.

Among the different ingredients incorporated to coating formulations, shellac has been known to reduce gas exchange in a greater extend than waxes, creating in many cases an anaerobic/fermentative environment in the fruit (Hagenmaier, 2000). Although the HPMC-based coatings and the CW contained shellac in their formulations, the concentration of internal CO₂ and O₂ on coated oranges at the end of the storage reached values around 7-11 and 5-11 kPa, respectively. In general, these levels of internal O₂ could be considered not low enough to create anaerobic conditions inside the 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).
3.2 Sensory quality

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

The appearance of the oranges 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 citrus appearance by conferring gloss. Panellists were asked to rank the five treatments on the basis of perceived gloss (1= the most glossy and 6= the least glossy) and the sum of the rank values was calculated (Table 2). Therefore, treatments with low 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\textsubscript{50} values of coated and uncoated ‘Valencia’ oranges stored
at 5 °C for 6, 8 and 16 weeks plus 1 week at 20 °C. As mentioned earlier, the DPPH•
radical decreases by reacting with antioxidants present in the sample; therefore, the
highest the EC\textsubscript{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\textsubscript{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
content of the different flavonoids, were not affected by storage length. Similarly, these FGs were not affected after 3 months of storage at 5 ºC in ‘Fortune’ mandarin (Palma et al., 2005) or 24 days of storage at cold-quarantine temperature at 1 ºC in ‘Valencia’ oranges (Contreras-Oliva et al., 2010). In general, coating application had not an important effect on the level of the different flavonoids, although some significant differences were found among treatments for NAT after 16 weeks of storage at 5 ºC plus 1 week at 20 ºC.

In addition to flavanones, the 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 total phenolic concentration (Gil-Izquierdo et al. 2002). TPC of ‘Valencia’ oranges ranged from 625 to 887 mg/L juice (GAE) (Table 3). TPC of ‘Valencia’ oranges was not affected by storage time at 5 ºC. Other works have shown that cold storage at quarantine temperatures of 1 ºC increased TPC of ‘Valencia’ oranges (Contreras-Oliva et al., 2010). However, Rapisarda et al. (2008) found a decrease in TPC of ‘Valencia’ oranges after 40 days of storage at 6 ºC attributed to senescence phenomena during storage. Other works have shown either an increase during storage, attributed to an increase in the PAL activity during low temperature storage of citrus fruit (Patil et al., 2004) or no effect, such as in ‘Fortune’ mandarins after 90 d of storage at 5 ºC (Palma et al., 2005). Although some significant differences were found among treatments after 4 and 8 weeks of storage at 5 ºC, no tendency was found due to coating application, which makes difficult to withdraw any conclusion regarding the effect of coating composition.

4. Conclusion
Coating application had little effect controlling weight loss of ‘Valencia’ oranges. However, after 16 weeks of storage at 5 °C plus 1 week at 20 °C, 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.

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References


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

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

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

BW= beeswax, CW= commercial wax (polyethylene-shellac), HPMC= hydroxypropyl methylcellulose, Sh= shellac, SC= solid content.
Table 2. Physico-chemical and sensory quality of coated and uncoated ‘Valencia’ oranges after storage at 5 ºC followed by 1 week at 20 ºC.

<table>
<thead>
<tr>
<th>Storage period</th>
<th>Treatment</th>
<th>Weight loss (%)</th>
<th>Internal CO₂ (KPa)</th>
<th>Internal O₂ (KPa)</th>
<th>Ethanol content (mg/L juice)</th>
<th>Off-Flavour</th>
<th>Flavour</th>
<th>Gloss visual rank (sum of ranks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At harvest</td>
<td>T1</td>
<td>2.99 cd</td>
<td>2.81</td>
<td>18.93</td>
<td>39.8</td>
<td>0.08</td>
<td>7.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>2.70 b</td>
<td>5.04 bc</td>
<td>14.50 c</td>
<td>386.3 a</td>
<td>0.45 a</td>
<td>6.35 c</td>
<td>101 d</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>2.21 a</td>
<td>5.93 cd</td>
<td>11.39 b</td>
<td>791.5 b</td>
<td>0.65 ab</td>
<td>6.30 c</td>
<td>76 bcd</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>2.79 bc</td>
<td>8.90 e</td>
<td>7.64 a</td>
<td>1280.5 c</td>
<td>2.05 c</td>
<td>4.15 a</td>
<td>36 a</td>
</tr>
<tr>
<td></td>
<td>T5</td>
<td>3.03 d</td>
<td>4.19 b</td>
<td>16.29 cd</td>
<td>727.1 b</td>
<td>1.35 bc</td>
<td>5.25 b</td>
<td>80 cd</td>
</tr>
<tr>
<td>4 wk 5 ºC +</td>
<td>T6</td>
<td>2.74 b</td>
<td>6.22 d</td>
<td>7.91 a</td>
<td>2172.2 d</td>
<td>1.95 c</td>
<td>3.90 c</td>
<td>59 abc</td>
</tr>
<tr>
<td>1 wk 20 ºC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>6.30 c</td>
<td>2.94 a</td>
<td>18.42 e</td>
<td>832.0 a</td>
<td>0.48 a</td>
<td>5.19 c</td>
<td>115 c</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>5.29 ab</td>
<td>5.14 b</td>
<td>15.10 d</td>
<td>976.4 a</td>
<td>1.19 ab</td>
<td>4.81 bc</td>
<td>52 a</td>
</tr>
<tr>
<td>8 wk 5 ºC +</td>
<td>T3</td>
<td>5.01 a</td>
<td>8.06 c</td>
<td>7.24 b</td>
<td>1503.3 b</td>
<td>1.90 bc</td>
<td>3.90 ab</td>
<td>76 ab</td>
</tr>
<tr>
<td>1 wk 20 ºC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>5.14 a</td>
<td>11.64 d</td>
<td>3.67 a</td>
<td>3318.4 d</td>
<td>2.24 c</td>
<td>4.05 ab</td>
<td>43 a</td>
</tr>
<tr>
<td></td>
<td>T5</td>
<td>5.63 b</td>
<td>6.82 bc</td>
<td>10.10 c</td>
<td>1379.1 b</td>
<td>1.52 bc</td>
<td>4.43 abc</td>
<td>97 bc</td>
</tr>
<tr>
<td></td>
<td>T6</td>
<td>6.11 c</td>
<td>7.30 c</td>
<td>5.77 b</td>
<td>2104.4 c</td>
<td>2.33 c</td>
<td>3.57 a</td>
<td>56 a</td>
</tr>
<tr>
<td>16 wk 5 ºC+</td>
<td>T1</td>
<td>11.33 c</td>
<td>5.10 a</td>
<td>15.97 c</td>
<td>1085.9 a</td>
<td>1.41 a</td>
<td>4.41 a</td>
<td>81 b</td>
</tr>
<tr>
<td>1 wk 20 ºC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>11.50 c</td>
<td>7.31 b</td>
<td>11.38 b</td>
<td>1745.4 b</td>
<td>1.18 a</td>
<td>4.71 a</td>
<td>36 a</td>
</tr>
<tr>
<td>20 wk 5 ºC+</td>
<td>T3</td>
<td>9.29 a</td>
<td>10.56 cd</td>
<td>7.11 a</td>
<td>2192.6 b</td>
<td>2.06 a</td>
<td>4.35 a</td>
<td>45 a</td>
</tr>
<tr>
<td>1 wk 20 ºC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>11.59 c</td>
<td>10.71 d</td>
<td>4.59 a</td>
<td>5465.1 d</td>
<td>1.82 a</td>
<td>3.82 a</td>
<td>50 ab</td>
</tr>
<tr>
<td></td>
<td>T5</td>
<td>10.36 b</td>
<td>8.08 bc</td>
<td>10.98 b</td>
<td>1779.3 b</td>
<td>1.24 a</td>
<td>4.53 a</td>
<td>62 ab</td>
</tr>
<tr>
<td></td>
<td>T6</td>
<td>9.84 ab</td>
<td>8.47 bcd</td>
<td>10.93 b</td>
<td>3271.1 c</td>
<td>1.24 a</td>
<td>4.24 a</td>
<td>61 a</td>
</tr>
</tbody>
</table>

**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.

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

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

Means within each storage period with the same letter are not different (p ≤ 0.05).
Table 3. Antioxidant activity (EC₅₀), total ascorbic acid (TAA), flavonoids and total phenolics contents of coated and uncoated ‘Valencia’ oranges after storage.

<table>
<thead>
<tr>
<th>Storage period</th>
<th>Treatment</th>
<th>EC₅₀ (L juice/Kg DPPH)</th>
<th>TAA (mg/L juice)</th>
<th>Narirutin (mg/L juice)</th>
<th>Hesperidin (mg/L juice)</th>
<th>Didymin (mg/L juice)</th>
<th>Total phenolics (mg GAE/L juice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>T1</td>
<td>233 ± 14</td>
<td>337 ± 17</td>
<td>28.3 ± 2.3</td>
<td>217 ± 11</td>
<td>9.1 ± 0.0</td>
<td>743 ± 80</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>345 ± 22 a</td>
<td>445 ± 32 a</td>
<td>34.9 ± 3.8</td>
<td>253 ± 4 a</td>
<td>10.8 ± 1.0</td>
<td>787 ± 23 b</td>
</tr>
<tr>
<td>4 wk 5 ºC +</td>
<td>T3</td>
<td>339 ± 22 a</td>
<td>463 ± 21 a</td>
<td>35.9 ± 2.2</td>
<td>270 ± 17 a</td>
<td>12.1 ± 1.1</td>
<td>811 ± 32 bc</td>
</tr>
<tr>
<td>1 wk 20 ºC</td>
<td>T4</td>
<td>360 ± 23 a</td>
<td>420 ± 30 a</td>
<td>37.0 ± 0.9</td>
<td>250 ± 11 a</td>
<td>11.6 ± 0.1</td>
<td>784 ± 33 b</td>
</tr>
<tr>
<td></td>
<td>T5</td>
<td>354 ± 23 a</td>
<td>434 ± 24 a</td>
<td>36.8 ± 2.1</td>
<td>288 ± 23 a</td>
<td>11.9 ± 0.4</td>
<td>783 ± 22 b</td>
</tr>
<tr>
<td></td>
<td>T6</td>
<td>350 ± 18 a</td>
<td>417 ± 21 a</td>
<td>34.2 ± 2.0</td>
<td>276 ± 17 a</td>
<td>11.5 ± 0.2</td>
<td>835 ± 22 c</td>
</tr>
<tr>
<td>8 wk 5 ºC +</td>
<td>T1</td>
<td>368 ± 15 a</td>
<td>366 ± 24 a</td>
<td>37.2 ± 1.1</td>
<td>220 ± 75 a</td>
<td>10.4 ± 0.1</td>
<td>866 ± 14 d</td>
</tr>
<tr>
<td>1 wk 20 ºC</td>
<td>T2</td>
<td>363 ± 25 a</td>
<td>342 ± 18 a</td>
<td>39.7 ± 1.0</td>
<td>262 ± 11 a</td>
<td>10.7 ± 0.2</td>
<td>794 ± 8 ab</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>342 ± 17 a</td>
<td>358 ± 15 a</td>
<td>37.0 ± 1.8</td>
<td>263 ± 6 a</td>
<td>9.6 ± 1.1</td>
<td>768 ± 27 a</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>338 ± 2 a</td>
<td>355 ± 7 a</td>
<td>37.9 ± 4.0</td>
<td>274 ± 4 a</td>
<td>10.4 ± 0.2</td>
<td>838 ± 29 cd</td>
</tr>
<tr>
<td>16 wk 5 ºC +</td>
<td>T5</td>
<td>325 ± 31 a</td>
<td>379 ± 13 a</td>
<td>38.4 ± 1.0</td>
<td>270 ± 14 a</td>
<td>10.2 ± 0.1</td>
<td>827 ± 30 bcd</td>
</tr>
<tr>
<td>1 wk 20 ºC</td>
<td>T6</td>
<td>365 ± 15 a</td>
<td>355 ± 19 a</td>
<td>39.2 ± 2.1</td>
<td>271 ± 12 a</td>
<td>10.3 ± 0.1</td>
<td>824 ± 11 bc</td>
</tr>
</tbody>
</table>

T1=uncoated, T2=CW, T3= 1:3 BW:Shá4% SC, T4= 1:3 BW:Shá8% SC, T5= 3:1 BW:Shá4% SC, T6= 3:1 BW:Shá8% 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 \( p \leq 0.05 \).