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Effect of a pectin edible coating obtained from orange peels with lemon essential oil on the shelf life of table grapes (*Vitis vinifera* L. var. Red Globe)

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Abstract

The effects of a pectin edible coating (EC) made from orange peels (*Citrus sinensis*) (1.5%, w/v) and added with lemon essential oil (1%, v/v) were investigated to prolong the shelf life of Red Globe grape variety (*Vitis vinifera* L.). Coated and uncoated grapes were evaluated for their physicochemical (weight loss, water activity, total soluble solids, pH, titratable acidity, color, and proximate composition), microbiological, and sensory properties during 35 d of storage at 4°C. Pectin extraction yield from orange peel was 21.83 ± 2.38%. The results showed that EC reduced 1.5% the weight loss, and kept pH lower at the end, in addition to providing an intense red color ($p < 0.01$) without affecting their proximate composition. Despite EC was not a contamination source of mesophilic aerobes or coliforms during its application, it induced yeast and mould growth at the end of the storage ($p < 0.05$). Moreover, consumers preferred grapes with EC at the beginning ($p < 0.01$), and the acceptance was maintained during storage ($p < 0.01$). The assessed EC had beneficial effects mainly on weight loss and colour of Red Globe grapes without affecting their sensory characteristics, preserving them for 35 d at 4°C. Therefore, pectin EC with lemon essential oil has potential to reduce post-harvest losses of Red Globe grapes; thus, it should be more widely studied.

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Introduction

Grape (*Vitis vinifera* L.) is a product of remarkable economic importance. The worldwide production of grape in 2018 was around 78 million tons, of which 36% corresponded to table grapes (OIV, 2019). Among varieties of table grapes, the Red Globe variety is one of the most important for export (Sortino *et al.,* 2017). Grapes are usually consumed fresh due to their sensory and nutritional characteristics (Oh *et al.,* 2017). Grapes, and even their seeds, have antioxidant and free radical scavenging properties due to their high content of phenolic compounds, such as anthocyanins and proanthocyanidins. Grapes may even provide protective effect against degenerative and cardiovascular diseases (Aghamirzaei *et al.,* 2015; Guerra *et al.,* 2016).

The table grape is a non-climacteric fruit. Therefore, following harvest, it is very perishable and begins to deteriorate by water loss, oxidation, and fungal decay. These processes lead to the rachis darkening and turgor losses of the berries, thus negatively affecting the sensory properties of grapes, and as a consequence, the product becomes unmarketable (Sortino *et al.,* 2017).

Several methods have been developed in order to prolong post-harvest life of grapes, such as fumigation and refrigeration. Fumigation with sulphur dioxide (SO_2) is used to prevent fungal formation, but an excessive addition of SO_2 can cause damages, such as rachis browning, cracking, and bleaching of the berry surface (Sortino *et al.,* 2017). In contrast, storage under refrigeration needs a strict control around 0°C because higher temperatures may have deleterious effects on the product like weight loss, stem browning, rachis desiccation, and rot caused by *Botrytis cinerea* (Pereira *et al.,* 2018).

The use of edible coatings (EC) has been widely studied and considered to preserve the quality of fresh fruits as well as prolonging their post-harvest life (Arnon *et al.,* 2015; Gomes *et al.,* 2017; Mannozzi *et al.,* 2017; Rahmawati *et al.,* 2017; Takma and Korel, 2017). Edible coatings are thin layers of natural macromolecules that are directly applied on the food surface (Nottagh *et al.,* 2020). They reduce the fruit respiration and weight loss because they act as semi-permeable barrier for gases and water vapour. Similarly, EC preserve fruit firmness and provide gloss to the coated product (Karaca *et al.,* 2014; Mannozzi *et al.,* 2017). Moreover, EC are capable to carry bioactive

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compounds, like antimicrobial agents, antioxidants, organic acids, flavours, and pigments (Nottagh *et al.,* 2018). Thus, EC mixtures with improved properties can be produced to extend the shelf life of food and enhance its organoleptic properties (Atarés and Chiralt, 2016; Nottagh et al., 2018).

Several polysaccharide-based EC, mainly from chitosan, have been reported to effectively preserve quality of fresh grapes during post-harvest life. For instance, weight loss reduction and maintenance of the physicochemical properties of grapes have been documented (Castelo Branco Melo *et al.,* 2018; Sun *et al.,* 2018; Sabir *et al.,* 2019). Besides, some authors pointed out that the phenolic content and antioxidant capacity of grapes (Punia *et al.,* 2019; Sabir *et al.,* 2019), as well the skin colour of the berries, were preserved during shelf life evaluation (Castelo Branco Melo *et al.,* 2018; Sabir *et al.,* 2019). Moreover, bactericidal effects against Gram-positive and slightly in Gram-negative bacteria (Castelo Branco Melo *et al.,* 2018; Sun *et al.,* 2018), in addition to a mild effectiveness against mould and yeast (Sun *et al.,* 2018) have also been observed in coated grapes. Furthermore, a moderately acceptance of grapes with EC was registered (Castelo Branco Melo *et al.,* 2018; Sun *et al.,* 2018; Punia et al., 2019). However, Castelo Branco Melo *et al.* (2018) reported that consumers still preferred the flavour of uncoated grapes.

Another polysaccharide widely used in the fabrication of EC is pectin (Moreira *et al.,* 2017; Sanchís *et al.,* 2017; Muñoz-Labrador *et al.,* 2018; Sakooei-Vayghan *et al.,* 2020). However, this is rarely employed in grape coating. The use of pectin in grapes has been documented as pectin-based oligosaccharides form, and it was focused on the ripening stage to increase colour and anthocyanin content (Ochoa-Villarreal *et al.,* 2011; Villegas *et al.,* 2016). However, pectin EC have not been explored for the extending of the post-harvest life of grapes.

Orange peel is a pectin-rich waste from orange juice industry that nowadays is used for industrial pectin extraction (Hosseini *et al.,* 2016). These citrus by-products correspond approximately to 45% of the total mass of the orange; thus, orange peel could cause environmental problems if they are not well disposed (Hilali *et al.,* 2019). Hence, pectin EC could provide an alternative for the use of this polysaccharide. However, pectin EC need to improve their water vapour barrier properties because of their hydrophilic nature. The incorporation of essential oils has been a good option to overcome this drawback in EC from polysaccharides due to their hydrophobic properties, which reduce moisture loss caused by transpiration and respiration (Guerra *et al.,* 2016). Moreover, the antimicrobial and antioxidant activities of EC can be improved with the addition of essential oils (Gomes *et al.,* 2017), like lemon essential oil (LEO). Major components of LEO are D-limonene, citral, and linalool, which have inhibitory effects against several types of microorganisms (Rahmawati *et al.,* 2017). The objective of the present work was therefore to evaluate the effect of pectin EC made from orange peels, added with lemon essential oil, on the physicochemical, microbiological, and sensory properties of Red Globe grapes during storage at 4°C.

Materials and methods

Plant material

Oranges (*Citrus sinensis*) were obtained from local market "Molina" (Ciudad Juarez, Chihuahua, Mexico). Afterwards, orange pulp was removed from the peels, and the peels were washed, stored in plastic bags, and frozen until use. Red Globe grapes were purchased from Plaza del Sol (Ciudad Juarez, Chihuahua, Mexico), and the bunches were selected based on their size, ripeness, and without visible defects. Subsequently, the samples were placed in perforated bags, and refrigerated (2 - 4°C) for 1 d until their sanitisation treatment. Then, samples were transported to the Food Science Laboratory of the UACJ where they were washed with soap water and rinsed. Sanitisation of the grapes was carried out in water with sodium hypochlorite (0.003 ppm) for 5 min, and they were air-dried at ambient temperature following Norma Oficial Mexicana, method NOM-251-SSA1 (NOM, 2010).

Pectin extraction

The orange peels were thawed, chopped, and put for 1 min in microwave blanching. Then distilled water was added to them in a proportion of 1:3, and they were liquefied to obtain a homogeneous paste. After that, the prepared mixture was poured into an Erlenmeyer flask with distilled water in a ratio of 1:9 (peels:water), and the pH was adjusted to 2 by adding 1 N HCl (Hycel®). The mixtures were heated at 85 ± 1°C for 1 h with constant agitation. Subsequently, the extracts were filtered through six layers of cheese cloth, and 1.5 volumes of cold absolute ethanol (96%, AZ®) were added to the filtrate in order to precipitate the pectin. After its storage for 24 h at 2 - 4°C, the alcohol of the mixture was vacuum filtered, and the obtained pectin was washed with 1.5 volumes of 60% (v/v) ethanol. The purified pectin was dried at 50°C in an oven (Thermo Scientific®, Precision model) for 24 h, milled, and stored at room temperature in sealed jars until use. Pectin extraction and purification was performed in multiple batches to obtain enough pectin

for the coating. Pectin yield (PY, %) was calculated as follows (Eq. 1):

$$PY = \frac{m_1}{m} \times 100\% \quad \text{(Eq. 1)}$$

where, m = weight of the dried obtained pectin, and m_1 = dry weight of the orange peels used for the extraction.

The degree of esterification (DE) of the extracted pectin was evaluated by the titrimetric method, according to Lira-Ortiz et al. (2014).

Preparation and application of edible coating

The coating solution was prepared in a ratio of 1.5% (w/v) of the extracted pectin with distilled water, and it was heated between 80 to 85°C until complete solubilisation. Afterwards, the pectin solution was left to cool to 25°C, and lemon essential oil (Productos del Roble®) was added in a proportion of 1% (v/v). This solution was homogenised in a blender until an emulsion was obtained, and it was reheated for 15 min at 85°C. Finally, coating solution was transferred to sterile bottles, sealed, and stored overnight at room temperature (Guerra-Rosas *et al.,* 2017). The grapes were coated by immersion for 2 min (Oh *et al.,* 2017), and left to dry at room temperature (Figure 1), while control fruits did not receive any treatment. Lastly, they were stored in sanitised perforated bags, which were kept under refrigeration (2 - 4°C) until their analysis.

Weight loss

Table grapes were separated in bunches of five berries, and placed in small perforated plastic bags. For each group evaluated (control and coated grapes), there were 15 bags which were sealed and stored in refrigeration (2 to 4°C). The bags of each group were weighed two times per week on an analytical scale (AD®, mod. 250A), and results were expressed in percentage of weight loss considering the initial weight of samples.

Physicochemical analysis

Approximately 30 g of homogenised grapes without seeds, were used to evaluate the pH, titratable acidity (TA), water activity (a_w), total soluble solid (TSS), and colour. These experimental processes were performed at room temperature (25°C) in triplicate, twice a week (Takma and Korel, 2017).

The pH determination was made with a previously calibrated potentiometer (Accumet® basics, model AB15 Plus). For TA, samples were titrated with sodium hydroxide (99%, DEQ®) 0.1 N until pH 7, and the results were expressed as a percentage of tartaric acid. The determination of a_w was carried out by adding the grape homogenates in sample cups and putting them into a water activity meter (AQUA LAB®, mod. Series 3), meanwhile the TSS was measured with a refractometer (Sper Scientific, mod 300001). Colour of coated and uncoated grapes was evaluated with a colorimeter (Konica Minolta®, model CR-400) based on the CIELab system. These measurements were made directly on the grape surface (GS) and in grape homogenate (GH). The color measurement for GS was at the berry equator for ten fruits of each group evaluated (Takma and Korel, 2017). The GH of each sample was put in a Petri dish, and the colorimeter was placed on the samples to collect the color parameters (L*, a*, and b*). The obtained data were used to calculate the chroma (C*), the hue degrees (h*), and the saturation (S*) by using Eqs. 2, 3, and 4, respectively.

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad \text{(Eq. 1)}$$

$$h^* = \tan\left(\frac{b^*}{a^*}\right) \quad \text{(Eq. 1)}$$

$$S^* = \frac{C^*}{L^*} \quad \text{(Eq. 1)}$$

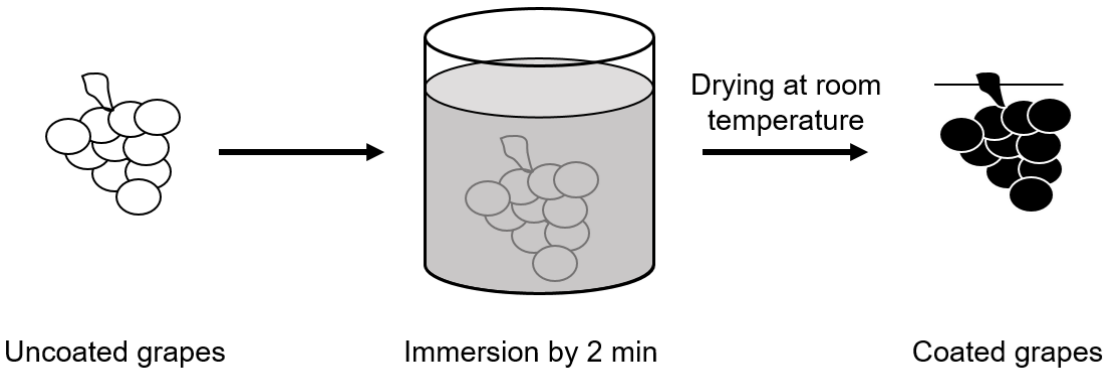

Figure 1. Schematic representation of the edible coating application on grapes.

Proximate analysis

Proximate analysis was made for moisture, ash, crude protein, total fat, and total carbohydrate following the procedures of AOAC (2002), employing 400 g of GH. These experimental processes were done once a week, and each determination were made by triplicate in both samples evaluated.

Microbiological analysis

The food safety indicators determined were mesophilic aerobes, total coliforms, and moulds and yeasts. These analyses were made once per week in duplicate. Briefly, a sample of grape was aggregated in a sterile bag (Whirl-Pak®, Nasco) with sterile physiological water at a 1:10 ratio. Thereafter, the bag was closed and homogenised, and 1 mL of the solution was transferred to each culture media (3M™, Petrifilm™). The mesophilic aerobes and total coliforms plates were placed in an incubator at 37°C for 24 h, meanwhile mould and yeast medium was incubated at 25°C for 48 h.

Sensory evaluation

The sensory tests were carried out at the beginning and at the end of the shelf life with a group of 120 consumers, under a randomised, balanced, and counterbalanced design. The evaluated samples were placed in 1 oz plastic cups, coded with random 3-digit numbers. First, a paired preference test to compare coated and uncoated grapes (Lawless and Heymann, 2010) was applied, and then, an affective test of both samples using a hedonic scale of 9 categories from "like extremely" to "dislike extremely" was done.

Statistical analysis

The obtained data in physicochemical and microbiological analysis were analysed by a Student's *t*-test (O'Mahony, 2017). The preference test data were analysed using the chi square test (χ^2), and the affective test data were analysed using a one-way ANOVA. The statistical analyses were carried out with the XLSTAT v. 2015 software (Addinsoft, Paris, France), considering an $\alpha < 0.05$.

Results and discussions

Pectin extraction

The average yield of multiple pectin extractions was 21.83 ± 2.38% with a degree of esterification (DE) of 55.2 ± 1.6%. Hosseini *et al.* (2016) obtained similar results for *Citrus aurantium* L. peel, which had an extraction yield of 17.95 ± 0.3% with a DE range of 57 to 83% under optimal conditions (T: 95°C, t: 90 min, liquid/solid ratio: 25 v/w). The similarity of both yields might be due to the extraction conditions used, because in both cases, pectin extraction increased when pH of media decreased and temperature increased (Andersen *et al.,* 2017). A pH value between 1 and 3 allows the hydrolysation of insoluble pectin, meanwhile high temperatures enhances diffusivity and solubility of pectin from plants (Maran *et al.,* 2015). However, differences in DE could be due to the extraction conditions, like high temperature and extraction period, and low liquid/solid ratio, which may increase degradation of polygalacturonic chains (Hosseini *et al.,* 2016).

Weight loss

The weight loss of Red Globe grapes during shelf life is presented in Table 1. The grapes with the edible coating had a lower weight loss from day 5 ($p < 0.01$) until day 30 ($p = 0.04$). At the end of shelf life (35 d), control grapes (C) lost 12.7% of weight as compared to 11.25% of the coated grapes. The pronounced decrease of weight in C and EC was due to water migration from fruit to the environment through its transpiration (Fakhouri *et al.,* 2015). Nonetheless, in the present work, EC acted as a barrier by retaining 1.5% more moisture, probably for its hygroscopic properties that enabled the formation of a water barrier between the fruit and its environment, thus reducing moisture loss. Moreover, the water vapour permeability diminished due to the hydrophobic phase formed with essential oil addition, which reduced water diffusion through the EC (Galus and Kadzińska, 2016). In other study, Red Globe grapes were coated with 1% chitosan and 0.5% commercial grapefruit seed extract, and they preserved 2.1% more moisture for 63 d due to the hydrophilic nature of chitosan, which might have had reduced water loss by absorbing water molecules through hydrogen bonds (Nottagh *et al.,* 2020).

Physicochemical analysis

The physicochemical parameters presented some differences between coated and control grapes (Table 1). The a_w of C and EC ranged from 0.973 to 0.980, and significant differences were observed on days 0, 16, and 35 [$F(17,36) = 2.8$, $p < 0.01$)]. The a_w of grapes during shelf life was in zone III. Therefore, table grapes are highly perishable fruits with severe post-harvest problems (Guerra *et al.,* 2016). Similar results were obtained by other authors in Crimson seedless grape with a mean of 0.977 (Pereira *et al.,* 2018).

The content of TSS varied throughout experiment. Although there were differences between C and EC [$F(17,36) = 189.2$, $p < 0.01$] in some days, a specific trend was not observed. This behaviour could be related to preharvest composition of grape, since veraison (i.e., onset of ripening) until grapevine is harvested, the components will not be uniformly distributed and

Table 1. Physicochemical parameters of the Red Globe grapes with and without pectin edible coating during their storage at 4°C.

t	T	Weight loss (%)	a_w	TSS (°Brix)	pH	TA (%)*
0	C	0.00 ± 0.00[a]	0.973 ± 0.002[a]	18.07 ± 0.12[a]	3.71 ± 0.03[a]	0.55 ± 0.00[a]
	EC	0.00 ± 0.00[a]	0.978 ± 0.004[b]	20.33 ± 0.12[a]	3.97 ± 0.00[b]	0.44 ± 0.01[b]
4	C	2.04 ± 0.38[a]	0.980 ± 0.001[a]	16.77 ± 0.06[a]	3.56 ± 0.08[a]	0.54 ± 0.00[a]
	EC	1.59 ± 0.23[b]	0.980 ± 0.004[a]	17.13 ± 0.06[b]	3.49 ± 0.01[a]	0.54 ± 0.03[a]
8	C	3.18 ± 0.49[a]	0.977 ± 0.001[a]	17.53 ± 0.12[a]	3.52 ± 0.03[a]	0.54 ± 0.00[a]
	EC	2.62 ± 0.30[b]	0.978 ± 0.002[a]	18.87 ± 0.12[b]	3.53 ± 0.02[a]	0.55 ± 0.01[a]
13	C	4.77 ± 0.70[a]	0.978 ± 0.002[a]	17.73 ± 0.12[a]	3.71 ± 0.00[a]	0.46 ± 0.00[a]
	EC	4.09 ± 0.43[b]	0.977 ± 0.002[a]	18.27 ± 0.12[b]	3.54 ± 0.03[b]	0.55 ± 0.01[b]
16	C	6.08 ± 0.89[a]	0.975 ± 0.002[a]	19.13 ± 0.12[a]	3.53 ± 0.03[a]	0.61 ± 0.01[a]
	EC	5.30 ± 0.56[a]	0.979 ± 0.002[b]	17.29 ± 0.10[b]	3.53 ± 0.02[a]	0.59 ± 0.00[a]
20	C	7.77 ± 1.04[a]	0.976 ± 0.001[a]	17.73 ± 0.12[a]	3.74 ± 0.03[a]	0.52 ± 0.00[a]
	EC	6.71 ± 0.71[b]	0.978 ± 0.003[a]	18.27 ± 0.12[b]	3.56 ± 0.03[b]	0.61 ± 0.00[b]
23	C	8.83 ± 1.28[a]	0.976 ± 0.001[a]	18.53 ± 0.12[a]	3.55 ± 0.01[a]	0.52 ± 0.00[a]
	EC	7.61 ± 0.75[b]	0.979 ± 0.002[a]	17.17 ± 0.06[b]	3.43 ± 0.08[a]	0.62 ± 0.01[b]
27	C	10.26 ± 1.58[a]	0.976 ± 0.001[a]	17.73 ± 0.12[a]	3.71 ± 0.03[a]	0.57 ± 0.01[a]
	EC	8.88 ± 0.86[a]	0.978 ± 0.002[a]	18.17 ± 0.06[b]	3.39 ± 0.02[b]	0.69 ± 0.01[b]
30	C	11.50 ± 1.79[a]	NA	NA	NA	NA
	EC	9.92 ± 0.97[b]	NA	NA	NA	NA
35	C	12.87 ± 1.93[a]	0.973 ± 0.002[a]	18.73 ± 0.12[a]	3.56 ± 0.03[a]	0.66 ± 0.01[a]
	EC	11.35 ± 1.29[a]	0.978 ± 0.004[b]	18.37 ± 0.15[b]	3.44 ± 0.02[b]	0.67 ± 0.01[a]

t: time in days; T: treatment; C: control grapes; EC: grapes with edible coating; a_w: water activity; TSS: total soluble solids; TA: titratable acidity; NA: not available. *Data are reported as percent of tartaric acid. Results are presented as mean ± SD. Different superscript letters indicate significant differences at $p < 0.05$ between both groups for each period of time.

this may generate diverse characteristics on grape berries (Sortino *et al.,* 2017). In fact, it has been reported that grape berries exposed to sun contains higher amount of anthocyanins, sugars, and phenolic compounds in comparison to grapes that are shaded (Edo-Roca *et al.,* 2013). Moreover, grape composition and ripeness are influenced by different environmental factors, such as soil composition, water availability, light conditions, and temperature (Kuhn *et al.,* 2014).

The pH of samples was in the range of 3.4 - 3.9, with significant differences on day 13 ($p = 0.01$), 20 ($p < 0.01$), 27 ($p < 0.01$), and 35 ($p < 0.01$). EC maintained lower pH values from day 27 in comparison with C. TA showed differences on days 13, 20, 23, and 27 ($p < 0.01$), where EC presented a higher acidity from day 20 to 27 as compared to C, but no identified effects of the coating on the grapes were detected.

The pH in EC was lower as compared to C, which increased over time, while TA in EC did not decrease in time as compared to C. Possibly, the edible coating decreased the respiration rate as well as the tartaric acid conversion into simple sugars (Mannozzi *et al.,* 2017). Similar effects were found in Alphonse Lavalleé grape with an edible coating of 2% alginate added with 1% vanillin, where the preservation was higher in coated fruit because the coating reduced respiration (Takma and Korel, 2017).

The results of colour evaluation of the grapes are shown in Table 2. The measurement of the GS showed significant differences in C* from day 23 to 35, where EC had the greatest chromaticity [$F(17,162) = 3.9$, $p < 0.01$)]. The saturation (S*) in GS also presented significant differences at the beginning (0, 4, and 8 d) and at the end (23, 27, and 35 d) of the shelf life [$F(17,162) = 6.7$, $p < 0.01$)], which means that EC generated an intensification of the red colour, probably due to the essential oil presence, which dispersed the light and increased colour perception (Atarés and Chiralt, 2016).

A similar behaviour was found for a coating of 8% soy protein with cinnamon oil in a protein:oil ratio of

Table 2. Colour parameters (CIELab) of the Red Globe grapes with and without pectin edible coating during their storage at 4°C.

t	T	L*	a*	b*	C*	h*	S*
Grape surface							
0	C	30.61 ± 1.99	3.48 ± 1.26	1.91 ± 0.71	3.99 ± 1.38^a	29.82 ± 8.06^a	0.13 ± 0.04^a
	EC	27.38 ± 1.53	4.36 ± 1.29	3.59 ± 0.86	5.68 ± 1.45^a	40.15 ± 5.73^b	0.21 ± 0.05^b
4	C	27.68 ± 1.78	3.35 ± 1.34	3.26 ± 0.89	4.72 ± 1.45^a	45.88 ± 8.97^a	0.17 ± 0.05^a
	EC	23.40 ± 2.95	4.41 ± 1.76	3.86 ± 1.44	5.88 ± 2.22^a	41.94 ± 5.19^a	0.25 ± 0.10^b
8	C	30.59 ± 1.83	2.90 ± 1.72	2.42 ± 1.53	3.80 ± 2.25^a	40.69 ± 8.78^a	0.12 ± 0.07^a
	EC	25.96 ± 1.19	4.02 ± 1.58	3.77 ± 1.05	5.54 ± 1.82^a	44.13 ± 6.00^a	0.21 ± 0.07^b
13	C	23.88 ± 1.97	4.98 ± 1.64	4.58 ± 2.17	6.83 ± 2.53^a	40.95 ± 7.61^a	0.28 ± 0.09^a
	EC	24.92 ± 2.25	6.06 ± 2.54	4.51 ± 1.45	7.61 ± 2.78^a	37.37 ± 6.26^a	0.30 ± 0.10^a
16	C	25.89 ± 2.00	4.86 ± 1.31	3.82 ± 1.08	6.21 ± 1.55^a	38.26 ± 6.08^a	0.24 ± 0.05^a
	EC	25.65 ± 1.19	5.51 ± 1.74	4.34 ± 1.40	7.05 ± 2.13^a	38.32 ± 5.23^a	0.27 ± 0.08^a
20	C	28.55 ± 1.70	3.43 ± 1.85	2.66 ± 1.56	4.47 ± 2.13^a	40.50 ± 14.03^a	0.16 ± 0.07^a
	EC	27.59 ± 2.34	4.21 ± 1.57	4.35 ± 1.34	6.12 ± 1.81^a	46.69 ± 9.63^a	0.22 ± 0.07^a
23	C	27.75 ± 1.88	3.21 ± 1.27	3.48 ± 1.37	4.79 ± 1.70^a	48.00 ± 10.21^a	0.17 ± 0.06^a
	EC	26.38 ± 1.71	5.00 ± 1.70	4.59 ± 1.41	6.81 ± 2.13^b	43.50 ± 6.40^a	0.26 ± 0.08^b
27	C	25.46 ± 1.30	3.04 ± 1.34	2.62 ± 1.26	4.03 ± 1.78^a	40.99 ± 5.81^a	0.16 ± 0.06^a
	EC	26.64 ± 2.65	5.48 ± 1.96	4.34 ± 1.56	7.07 ± 2.24^b	38.39 ± 9.85^a	0.27 ± 0.09^b
35	C	23.30 ± 1.45	4.26 ± 1.65	3.33 ± 1.10	5.44 ± 1.87^a	38.75 ± 6.66^a	0.23 ± 0.08^a
	EC	23.35 ± 3.08	5.38 ± 1.16	4.92 ± 1.91	7.40 ± 1.80^b	41.31 ± 9.73^a	0.31 ± 0.05^b
Grape homogenate							
0	C	31.63 ± 1.83	12.37 ± 1.78	6.15 ± 2.02	13.26 ± 0.51^a	16.29 ± 0.33^a	0.45 ± 0.02^a
	EC	32.84 ± 0.99	11.74 ± 2.01	7.43 ± 1.35	15.33 ± 0.13^b	22.42 ± 0.26^b	0.48 ± 0.00^a
4	C	32.91 ± 1.15	10.89 ± 1.35	7.89 ± 0.94	14.25 ± 1.07^a	36.55 ± 0.43^a	0.43 ± 0.03^a
	EC	32.80 ± 1.35	10.42 ± 1.34	7.58 ± 0.97	12.51 ± 1.49^a	40.49 ± 1.10^b	0.37 ± 0.04^a
8	C	32.15 ± 1.28	11.18 ± 1.35	7.33 ± 0.88	13.97 ± 0.37^a	29.64 ± 0.30^a	0.45 ± 0.02^a
	EC	32.23 ± 1.25	10.74 ± 1.21	7.46 ± 0.87	12.57 ± 0.18^b	40.81 ± 0.40^b	0.37 ± 0.01^b
13	C	31.89 ± 0.81	11.15 ± 0.90	7.42 ± 0.84	13.39 ± 1.75^a	29.88 ± 1.07^a	0.43 ± 0.05^a
	EC	32.33 ± 0.91	11.00 ± 0.83	7.58 ± 0.65	13.43 ± 1.07^a	33.14 ± 0.02^b	0.43 ± 0.03^a
16	C	32.59 ± 0.92	11.13 ± 1.00	7.39 ± 1.14	13.21 ± 0.27^a	36.78 ± 0.67^a	0.41 ± 0.01^a
	EC	32.76 ± 0.99	11.65 ± 1.08	7.55 ± 1.27	13.31 ± 0.97^a	36.51 ± 0.14^a	0.40 ± 0.03^a
20	C	32.65 ± 1.01	11.44 ± 1.31	7.39 ± 1.23	13.00 ± 1.38^a	26.98 ± 0.25^a	0.41 ± 0.04^a
	EC	33.42 ± 0.78	11.29 ± 1.33	7.93 ± 0.88	15.43 ± 0.18^a	34.45 ± 0.12^b	0.46 ± 0.01^a
23	C	33.52 ± 0.83	11.28 ± 1.35	7.56 ± 0.91	13.07 ± 0.89^a	34.50 ± 0.23^a	0.40 ± 0.03^a
	EC	33.74 ± 1.14	11.64 ± 1.33	7.73 ± 1.05	12.92 ± 0.78^a	39.61 ± 0.61^b	0.38 ± 0.02^a
27	C	33.35 ± 2.05	12.08 ± 0.88	7.73 ± 1.30	14.30 ± 0.75^a	27.52 ± 1.22^a	0.43 ± 0.02^a
	EC	34.24 ± 2.89	11.25 ± 1.38	8.41 ± 1.19	15.23 ± 0.30^a	34.97 ± 0.22^b	0.44 ± 0.01^a
35	C	34.13 ± 3.64	10.63 ± 1.29	8.25 ± 1.47	13.66 ± 0.71^a	31.02 ± 0.63^a	0.44 ± 0.02^a
	EC	37.45 ± 0.34	9.56 ± 0.65	9.45 ± 0.91	13.44 ± 1.09^a	44.63 ± 1.08^b	0.36 ± 0.03^b

t: time in days; T: treatment; C: control grapes; EC: grapes with edible coating; L*: lightness; a* and b*: colour axis components; C*: chroma; h*: hue angle; S*: saturation. Results are presented as mean ± SD. Different superscript letters indicate significant differences at $p < 0.05$ between both groups for each period of time.

1:0.075, but this effect depends on the type of oil used and its concentration (Atarés and Chiralt, 2016). This is because C* and S* parameters and luminosity tend to decrease when oil concentration increases (Galus and Kadzińska, 2016; Dehghan Manshad *et al.,* 2019). Besides, colour changes in grape skin can be attributed to ripening during storage (Oh *et al.,* 2017) and to an unequal distribution of anthocyanins in the berries' skin. This is related to anthocyanin accumulation during veraison phase, which is influenced by environmental and management conditions (Barnuud *et al.,* 2014). The high content of anthocyanins is due to sunlight exposure in berries (Edo-Roca *et al.,* 2013), and these compounds are responsible for grape berries' colour (Kuhn *et al.,* 2014). However, for hue angle (h*), EC did not have a significant effect on the appearance of the grapes' colour.

Significant differences were observed in GH at days 0 ($p = 0.01$) and 8 ($p = 0.01$) in C*, but in h*, all samples with the EC, except on day 16, had an increase in the appearance of colour towards the yellow tone as compared to C ($p < 0.05$). These results could be attributed to pectin coating, which had a slightly yellow color due to carotenes present in the orange peel (Nasirifar *et al.,* 2018). A similar result was obtained with Alphonse Lavallée table grapes, which had an alginate and vanillin coating, and showed an orientation to white because the vanillin has that characteristic color (Takma and Korel, 2017).

Proximate analysis

The moisture contents of C and EC was in the range of 82.3 to 85.0%, where significant differences were observed in weeks 3 and 4, with higher moisture content for C and EC, respectively [$F(9,20) = 36.4$, $p < 0.01$]. Due to these discrepancies in moisture content, the proximate compositions of the grapes are presented in dry matter (Table 3). The content of ashes was between 2.0 and 2.9%, protein between 2.3 and 3.9%, fat between 0.20 and 0.74%, and the total carbohydrate content between 75.6 and 79.2%. There was no specific trend or effect observed by the edible coating on the composition of the evaluated samples. The results obtained are attributed to each cluster composition, because it was found that 'floral differentiation' and 'timing of harvest' are crucial stages in achieving a harvest of homogeneous berry size and composition (Edo-Roca *et al.,* 2013). Therefore, several factors like climate, genotype, management, and soil type affect the concentration of grape berries' components (Barnuud *et al.,* 2014). Grape berries' distribution in the grapevine, even in the bunch, and its unequal exposure to solar radiation also affect berry ripening and composition (Kuhn *et al.,* 2014). Besides, an excess in preharvest heat increases protein proportion in grape, which uses to protect itself from adverse weather conditions and pathogenic microorganisms, and this can cause a decrease in its nutrients (Wu *et al.,* 2015).

Microbiological analysis

The counts of mesophilic aerobes and total coliforms where null for both samples during all the storage time. This shows that the sanitisation process was properly carried out and that the storage conditions throughout the study were also correct.

Table 3. Proximate analysis* of the Red Globe grapes with and without pectin edible coating during their storage at 4°C.

t	T	Dry matter	Ash	Protein	Fat	Total carbohydrate
0	C	15.97 ± 0.10^{a}	2.58 ± 0.10^{a}	2.74 ± 0.07^{a}	0.24 ± 0.01^{a}	78.46 ± 0.06^{a}
	EC	15.93 ± 0.10^{a}	2.02 ± 0.04^{b}	3.06 ± 0.05^{b}	0.24 ± 0.01^{a}	78.75 ± 0.02^{a}
1	C	16.71 ± 0.27^{a}	2.25 ± 0.10^{a}	2.72 ± 0.08^{a}	0.20 ± 0.01^{a}	78.13 ± 0.11^{a}
	EC	16.55 ± 0.20^{a}	2.10 ± 0.05^{b}	2.81 ± 0.01^{a}	0.21 ± 0.01^{a}	78.31 ± 0.23^{a}
2	C	17.64 ± 0.10^{a}	2.58 ± 0.03^{a}	2.99 ± 0.13^{a}	0.30 ± 0.00^{a}	76.48 ± 0.17^{a}
	EC	17.55 ± 0.15^{a}	2.59 ± 0.02^{a}	2.79 ± 0.06^{b}	0.37 ± 0.02^{b}	76.71 ± 0.13^{a}
3	C	15.69 ± 0.03^{a}	2.97 ± 0.07^{a}	3.19 ± 0.12^{a}	0.74 ± 0.01^{a}	77.41 ± 0.16^{a}
	EC	16.61 ± 0.28^{b}	2.72 ± 0.09^{b}	3.03 ± 0.11^{b}	0.63 ± 0.02^{b}	77.00 ± 0.21^{b}
4	C	16.06 ± 0.49^{a}	2.64 ± 0.07^{a}	2.30 ± 0.08^{a}	0.35 ± 0.01^{a}	78.66 ± 0.40^{a}
	EC	14.97 ± 0.28^{b}	2.83 ± 0.09^{b}	2.42 ± 0.06^{b}	0.57 ± 0.01^{b}	79.21 ± 0.21^{b}

t: time in weeks; T: treatment; C: control grapes; EC: grapes with edible coating. * Data are expressed as percentage of the dry matter. Results are presented as mean ± SD. Different superscript letters indicate significant differences at $p < 0.05$ between both groups for each period of time.

However, important changes in moulds and yeasts analysis were observed (Figure 2). Particularly, the CFU of moulds and yeasts in grapes with the EC increased after week 2, meanwhile decreased in C, so the coated grapes had greater growth of these microorganisms in weeks 3 (p = 0.03) and 4 (p = 0.02). Previous studies indicate that grape surface is colonised by microorganisms, mainly yeasts, moulds, lactic acid bacteria, and acetic acid bacteria (Panda, 2017). Some authors reported that Red Globe grape is principally colonised by *Kloeckera apiculata* and *Saccharomyces cerevisiae* (Kántor and Kačániová, 2015). Nevertheless, the microbial population was reduced with sodium hypochlorite sanitisation due to its antibacterial and antifungal capacity (Kwolek-Mirek *et al.,* 2011). This takes effect when sodium hypochlorite is water-solubilised, the HOCl formed induces cyto- and genotoxic effects against *S. cerevisiae* (Carmona-Gutierrez *et al.,* 2013), and for this reason, yeast population decreased in both treatments in weeks 1 and 2. However, grape yeasts can grow in the cuticular wax of grapes, since *S. cerevisiae* is able to use oleanolic acid of epicuticular wax as growth substrate (Jackson, 2014). Therefore, it could be that a fraction of moulds and yeasts were not eliminated during sanitisation because they were protected by this waxy layer (Panda, 2017). Also, the utilisation of lemon essential oil (LEO) in the EC probably had an effect against microorganisms at the beginning of shelf life, possibly for D-limonene presence that possesses antimicrobial activity (Guerra-Rosas *et al.,* 2017). In a study of strawberry with a coating of sodium alginate and starch added with LEO, it had an inhibitory effect against bacteria at a concentration of 0.6%, while at 1% of LEO, it had the same effect on fungi such as *Rhizopus stolonifer* and *Botrytis* sp. (Rahmawati *et al.,* 2017). Essential oils' effect against microorganisms is not completely understood, but most studies suggest that cell wall of microorganisms is negatively affected (Gomes *et al.,* 2017). In the third week, CFU's of moulds and yeasts started to increase in grapes with pectin coating. This could be due to certain grape yeasts such as S. cerevisiae, which is able to use galacturonic acid as growth substrate (Biz *et al.*, 2016), so the pectin edible coating became a source of food for the yeasts instead. In contrast, a study on Red Globe grapes, where they used a coating of 1% chitosan with 0.1% grapefruit seed extract, moulds and yeasts decreased. This is because both components have antifungal properties, which only allowed a smaller CFU's development in grape.

Sensory evaluation

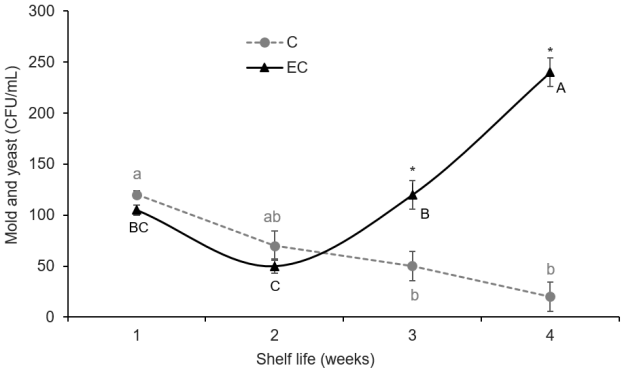

Figure 2. Effect of pectin edible coating on mould and yeast development (CFU/mL) in Red Globe grapes during their storage at 4°C. C: control grapes; EC: grapes with edible coating. Different lowercase and uppercase letters indicate significant differences between periods of time at $p < 0.05$ for C and EC, respectively. *Significant differences at $p < 0.05$ between both groups for each period of time.

The paired preference test is used to determine if panellist perceives specific differences between two samples (Yang and May, 2017). This leads the consumers to be more selective in their decision. For this reason, this test has been considered more sensitive than affective test (Lawless and Heymann, 2010). The initial evaluation through this test indicated that consumers preferred coated grapes with respect to C (z = -3.031, $p < 0.01$) at the beginning, possibly because it possessed a bright appearance (Figure 3A) and a slight citrus flavour provided by the pectin coating. However, the results had the opposite behaviour in the final test (z = 2.220, p = 0.03), this could be related to the detachment of EC from the grape surface (Figure 3A) from day 16. Consequently, grape berries' visual aspect could have had influenced the consumers' preference. The deterioration of the EC could be caused by endemic yeast activity, which use galacturonic acid as growth substrate (Biz *et al.,* 2016). Additionally, the absence of plasticisers possibly caused brittleness in EC, since they increase molecular flexibility and mobility by reducing intermolecular hydrogen bond strength (Sanyang *et al.,* 2016).

On the other hand, affective hedonic tests evaluate how much a consumer likes a product (Meilgaard *et al.,* 2015), and also provide some information about if a product has the total acceptance of consumer (Lawless and Heymann, 2010). To determine the degree of acceptance of the grapes by the consumer, data were analysed by areas of liking, neutrality, and dislike (Figure 3B, left axis). EC did not affect the acceptance of the fruit by the consumers as compared to C ($\chi 2$ = 668.5, $p < 0.0001$). EC were mainly in the area of liking (> 74% of the consumers), even panellists did not perceive the difference between the

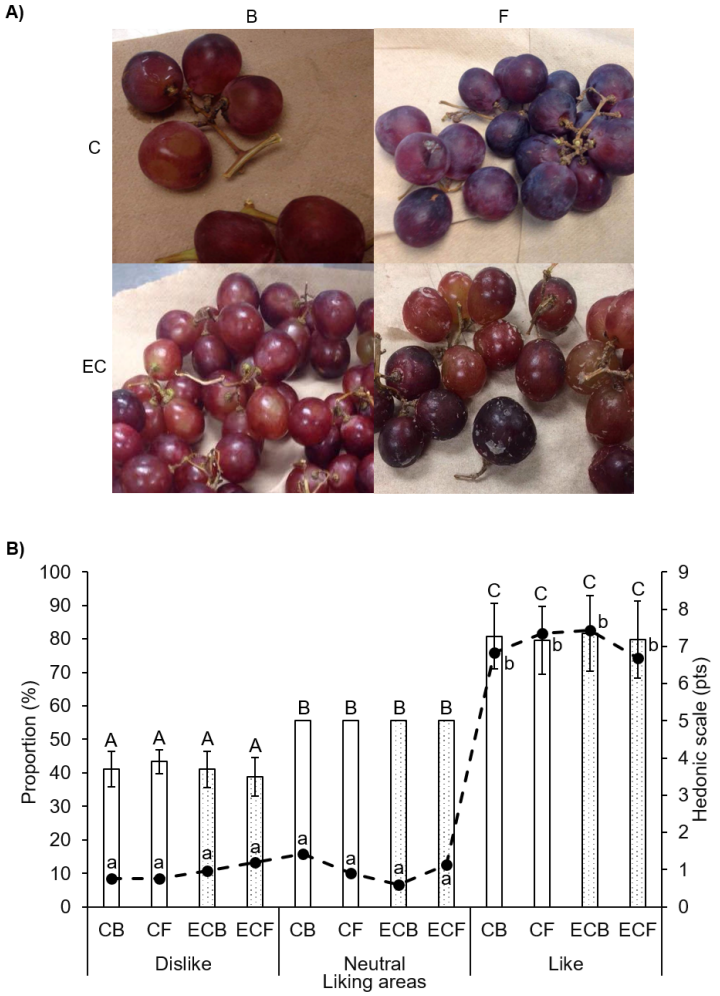

Figure 3. Visual appearance (A), and consumers' acceptance (B) of Red Globe grapes by consumer with EC (grapes with edible coating) and without C (control grapes) coating at the beginning (B) and at the end (F) of their storage at 4°C. The line shows results by areas of liking (left axis), and the bars represents the affective test by satisfaction level (right axis). Different lowercase and uppercase letters indicate significant differences at $p < 0.05$ by liking areas and satisfaction level, respectively.

coated grapes and those that were not, which implies that the edible coating did not change the taste characteristics of grapes during storage. In fact, at the end of the storage, LEO presence did not diminish the grapes' taste, although undesirable changes in flavour, appearance, and nutritional quality occurred due to lipid oxidation over time (Dehghan Manshadi *et al.,* 2018). Similar results were obtained by Guerra *et al.* (2016) in a study with cv. Isabella table grapes (*Vitis labrusca* L.), coated with chitosan EC combined with *Mentha (piperita* L. *or* × *villosa* Huds) essential oil, where panellists did not notice differences between coated grapes and control.

The hedonic scale in acceptance test showed the level of panellist satisfaction by categories (Figure 3, right axis). At the beginning of the shelf life, EC were particularly ranked from "like slightly" to "like very much" (values from 6 to 8 in the hedonic scale), while C were in the neutral category (value of 5 in the hedonic scale) ($z = 2.06$, $p = 0.04$). A comparison of the initial and final test indicated that, in general, the edible coating did not affect the acceptance of the Red Globe grape and its acceptance was maintained for 35 d of storage at 4°C, with an average acceptance of "like moderately" [$F(11,468) = 85.464$, $p < 0.0001$].

Conclusion

The edible coating with pectin had favourable effects on Red Globe grapes because it prolonged the grapes' shelf life by 35 d. Besides, it prevented fungal decay and moisture loss without affecting the grape composition. Even grape berries' appearance was improved in brightness and colour at the beginning of shelf life. Although edible coating controlled bacterial growth, it led to mould and yeast growth from the second week of storage at 4°C. Also, from day sixteen, the coating began to detach from the surface of the grape, which had a negative impact on the appearance of the fruit. However, the edible coating

did not affect the consumers' acceptance during four weeks of shelf life, and this thus represents an option for further and better coating development.

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