



Article Improving the Storage Quality of Ready-to-Eat Clementine Fruits Using Lemon By-Products

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Abstract: In this study, the effect of the antioxidant extract from lemon by-products (*Citrus* × *Limon* L.) integrated into an edible alginate-based coating was evaluated to preserve the storage quality of ready-to-eat Clementine (*Citrus* × *Clementina*) fruits. The effects of different coatings (1.5% of alginate and 1.5% of alginate + 2–4% of lemon by-product extract) were assessed by the physical, chemical, microbiological, sensorial, and structural analyses of ready-to-eat Clementine fruits stored for 21 d at 4 °C. Ready-to-eat Clementine fruits coated with alginate and extract from lemon by-products showed greater levels of polyphenols, flavonoids, antioxidant activity, and organic acids. A microbiological analysis revealed the dose-dependent effect of the extract to contrast the growth of mesophilic bacteria, yeast, and molds during storage. A sensory analysis confirmed that the enriched coating improved the visual, structural, and olfactory parameters until the end of storage. The evidence in this study proves that an antioxidant extract from lemon by-products is a great sustainable treatment to preserve the visual.

Keywords: antioxidant extract; *Citrus* × *Clementina*; edible coating; lemon by-product; ready-to-eat fruits

1. Introduction

Seasonal fruits have always been considered beneficial for human health. Many studies have confirmed that the different mechanisms of action of the bioactive compounds present in fruits (polyphenols, flavonoids, vitamins, fibers, etc.) promote human health and counteract the onset of various human diseases [1].

Clementine (*Citrus* \times *Clementina*) is a typical autumn citrus cultivated in Italian temperate areas, especially in the Calabria region (South of Italy), where it finds the appropriate conditions for optimal ripeness. Though they are very appreciated for their sweetness and the absence of seeds, Clementine fruits have also been recognized as a source of innumerable healthy compounds, such as flavonoids and ascorbic acid (vitamin C) [2]. Still, the habits of modern consumers often hinder consumption because the washing and peeling operations require a length of time that is not always available, especially in cases of meals that are consumed outside the home and in a short time.

Currently, the main challenge for the fruit industries is to meet the needs of consumers, both in terms of nutritional quality and high-service content regarding user-friendliness. For these reasons, in recent decades, the demand and the sale of ready-to-eat fruits have increased [3]. The main problem of ready-to-eat fruits is related to the operations they undergo before packaging (peeling, cutting, etc.) that predispose them to rapid physical, chemical, sensory, and microbiological decay (including a loss of color, smell, taste, and texture; reduced health compounds; and faster growth of pathogenic and spoilage microorganisms) compared to their entire fruit [4–6]. Currently, to slow these phenomena, "obstacle strategies" are used, which consist of the simultaneous use of synthetic



Citation: Boninsegna, M.A.; De Bruno, A.; Piscopo, A. Improving the Storage Quality of Ready-to-Eat Clementine Fruits Using Lemon By-Products. *Agriculture* 2024, *14*, 1488. https:// doi.org/10.3390/agriculture14091488

Academic Editor: Shixiang Yao

Received: 23 July 2024 Revised: 26 August 2024 Accepted: 28 August 2024 Published: 1 September 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). phenolic compounds, such as butylated hydroxyanisole (BHA), modified atmosphere packaging (MAP), and low temperatures [7,8], which lead to a substantial increase in the unit cost of food. Modern consumers perceive the conventional conservation methods negatively: a prolonged intake of synthetic preservatives leads to collateral effects on human health [8–10], while conventional packaging strategies can increase pollution and be unsafe for improper sales/distribution conditions (mechanical damage to trays, high temperatures, etc.), causing a faster decrease in quality and excessive food loss [11–13]. In recent times, polymer-based edible coatings, such as sodium alginate with the addition of natural preservative compounds [14–19], have been evaluated as effective eco-friendly treatments in finding a solution to meet actual consumer, environmental, and industry needs [20].

The extracts of citrus by-products have been recognized for being rich in bioactive compounds (polyphenols, flavonoids, and ascorbic acids), with high antioxidant and antimicrobial activity [21–25], making them suitable for improving the shelf life and the presence of health-beneficial compounds in ready-to-eat fruits [26].

Sodium alginate is a natural, edible, and biodegradable anionic polysaccharide derived from brown algae that has already been used to improve the chemical and physical characteristics and to prolong the storage of various fruits such as apples, sweet cherries, mangos, avocados, and peaches [27–31]. The induction to cross-linking sodium alginate in the presence of bivalent ions, such as Ca⁺⁺, determines the modification of its chemical structure and the formation of an impermeable barrier around fruits that improves its structural characteristics and slows down the physiological, chemical, physical, and microbial processes that are the basis of the qualitative decay of ready-to-eat fruits [32–34].

Our previous studies found that the alginate-based coating is an excellent alternative to modified atmosphere packaging in ready-to-eat Clementine fruits [35]. However, there are no scientific studies on the application of edible coatings on Clementine segments enriched with antioxidant by-product extracts. This study aimed to evaluate the efficiency of different edible alginate-based coatings to maintain the chemical, physical, sensory, and microbiological qualities of ready-to-eat Clementine fruits.

Therefore, the purpose of the study was to (i) test the influence of the lemon by-product extract on improving the characteristics of the edible alginate-based coating, (ii) test the effectiveness of the lemon by-product extract incorporated in edible alginate-based coatings to preserve the quality and safety of ready-to-eat Clementine fruits during storage, and (iii) offer a natural and sustainable alternative to preserve ready-to-eat fruits.

2. Materials and Methods

2.1. Extraction of Antioxidant Compounds from Lemon Pomace (LP)

Lemon pomace, the by-product of the industrial processing of lemons (peel, pulp, and seeds), was supplied by a local citrus cooperative (Citrus Juices SRL, Reggio Calabria, Italy). Immediately after receipt at the FoodTec laboratory of the University Mediterranean of Reggio Calabria, the lemon pomace was dried for 3 h at 50 °C \pm 5 °C until reaching a moisture content of 10%, and it was then ground and homogenized. The obtained sample (LP) was then used to prepare the extracts. The solid–liquid extraction was performed by mixing 100 g of LP with 400 mL of hydroalcoholic solvent (EtOH 50%) on a stirring plate at 60 °C for 60 min. The extract obtained (LPE) was centrifuged (6000 rpm) for 10 min at 4 °C (NF 1200R, Nüve, Ankara, Turkey). Subsequently, the supernatant was recovered, filtered through a Buchner funnel with a 0.45 mm filter paper, refiltered with a 0.45 mm PTFE filter, and then stored at -21 °C until further analyses.

2.2. Clementine Fruit Sample Preparation

The Clementine fruits (*Citrus* \times *Clementina*) were bought at a local market situated in Reggio di Calabria (Italy) in February 2023, transported to the FoodTec laboratory of the University Mediterranean of Reggio Calabria, and then picked for similar sizes (weight: 80–90 g, height: >50 mm, width: >60 mm) and color (completely orange 'flavedo') [35]. The whole fruits were then sanitized by immersing them in a sodium hypochlorite solution (200 ppm) for 2 min, which were then rinsed with distilled water and dried on stainlesssteel grids in a vertical laminar flow hood (UV lamp 30 W, mod. ASALAIR 1200 FLV, Asal Srl, Milan, Italy) at room temperature and with forced air [35]. Clementine fruits were peeled, reduced manually into segments, and then divided into 4 groups to apply the treatments provided in the experimental plan.

To prepare the coating solution, 1.5% (w/v) of sodium alginate powder (Sigma-Aldrich, Merk Life Science S.r.l., Milan, Italy) was dissolved in distilled water under stirring (70 °C, 60 min). Then, the temperature was reduced by 30 °C, and 1.5% (w/v) of glycerol (Carlo Erba reagents, Comaredo (Milan) Italy) was added under continuous stirring (30 °C, 30 min) [35]. The obtained solution was named AL. For the other coating solutions, 2% and 4% (v/v) of LPE were added to the formulation of AL, respectively, with the names AL-LPE 2% and AL-LPE 4%. Concomitantly, a CaCl₂ solution (2% w/v) was prepared by dissolving calcium chloride (Labochimica s.r.l., Campodarsego (Padova), Italy) in distilled water under stirring for 30 min (25 °C).

The coating solutions (AL, AL-LPE 2%, and AL-LPE 4%) were thus prepared and used to realize the edible coatings on ready-to-eat Clementine fruits.

The segments of the Clementine fruits were dipped in the AL, AL-LPE2%, and AL-LPE 4% solutions for 2 min, recovered, and left for 1 min at room temperature on stainless-steel grids to remove the excess solution. Therefore, the segments were dipped in the CaCl₂ solution for 2 min to induce a cross-linked reaction of sodium alginate, recovered, and placed on stainless-steel grids at room temperature up until complete drying (for about 3 h) [35]. The operations of preparation, coating, and drying of the Clementine fruits were conducted in a vertical laminar flow hood (UV lamp 30 W, mod. ASALAIR 1200 FLV, Asal Srl, Milan, Italy) to avoid microbiological contaminations. The uncoated samples were used as a control (CTR).

The Clementine samples (about 100 g) were packaged in a PP tray that was heat-sealed with PP/PE film using a packaging machine (Orved, VGP 25N, Italy) and stored at 4 °C for 21 d under the light to recreate the real sale conditions. The Clementine juice was obtained by homogenizing 70 g of the sample (Ultra-Turrax, T 25 digital, IKA, Staufen, Germany) and centrifuging (NF 1200R, Nüve, Ankara, Turkey) for 10 min at 10,000 rpm and 4 °C. The supernatant was recovered, filtered by PTFE 0.45 μ m (diameter 15 mm), and used for the chemical determinations.

Physical, chemical, and microbiological analyses were performed at 0, 3, 7, 14, and 21 days of storage. Sensory analyses were conducted at the beginning and end of storage.

2.3. Chemical Analyses of LPE and Clementine Fruits

The pH of Clementine juice and the LPE were determined by using a digital pH meter (Crison Basic 20, Spain).

The Clementine fruit moisture was quantified by the AOAC standard method [36] and expressed in percentage. The total soluble solids were quantified in Clementine juice by digital refractometer (DBR 047 SALT) and expressed in degrees Brix (°Bx) at 25 °C [35].

The diluted Clementine juice (1:10) was titrated with 0.1 M NaOH up to pH 8.1 (digital pH meter Crison Basic 20, Spain) to measure the titratable acidity (TA), and the results were expressed as citric acid % [37].

The trend of organic acids (oxalic, malic, ascorbic, and citric acids) during the storage of Clementine fruits was identified with high-performance liquid chromatography (HPLC), following the procedure suggested by Jurić et al., with some modifications [38]. Briefly, 20 μ L of juice was injected into a Knauer HPLC Smartline Pump 1000, equipped with a Knauer Smartline UV Detector 2600 and SYNERGY HYDRO-RP (250 mm × 4.6 mm i.d., 4 μ m). The thermostat was set at 22 °C, and the separation was carried out in an isocratic condition with potassium phosphate 20 mM at pH 2.9 at a flow rate of 0.7 mL/min. The ascorbic acid was recorded at 254 nm, and the other organic acids at 210 nm; their concentrations were reported as mg of acid 100 g⁻¹.

The total phenolic content (TPC) of the LPE was carried out with the methods described by Imeneo et al. [39], opportunely modified. A total of 1 mL of diluted LPE (1:5) and 1 mL of Folin–Ciocalteu reagent were mixed and, after a short incubation (8 min) at room temperature, 10 mL of Na₂CO₃ 20% (w/v) was added to the solution. The reaction mixture was made up to volume (25 mL) with deionized water and incubated in darkness and at room temperature (25 °C) for 2 h. The absorbance was recorded at 765 nm against a blank (the reaction mixture without a sample) by a double-beam ultraviolet–visible spectrophotometer (Perkin-Elmer UV–Vis k2, Waltham, MA, USA). The TPC of Clementine fruits was instead quantified following the method of Boninsegna et al. [35]. The results were compared with a gallic acid calibration curve and expressed as the mg of gallic acid equivalent (GAE) g⁻¹ of the LPE dry weight (d.w.) and mg of gallic acid equivalents (GAE) kg⁻¹ of the Clementine fruits.

To determine the total flavonoid content (TFC), 1.2 mL of the diluted LPE (1:1) and 0.15 mL of the 5% (w/v) NaNO₂ were mixed and left at room temperature for 6 min, then 0.15 mL of the 10% AlCl₃ (w/v) was added. After 6 min, 2 mL of NaOH 1M was added and finally, distilled water was used up to a volume of 5 mL. The reaction mixture was left for 15 min in darkness at room temperature (25 °C). Subsequently, the absorbance was registered at 515 nm versus a blank (the reaction mixture without a sample) by a spectrophotometer (Perkin-Elmer UV–Vis k2, Waltham, MA, USA) [39]. The TFC of the Clementine fruits was performed using the colorimetric methods described by Boninsegna et al. [35]. The results were expressed as mg of the catechin equivalents (CE) g^{-1} of LPE d.w. and mg of the catechin equivalents (CE) kg^{-1} of the Clementine fruits, using a catechin calibration curve.

The identification of individual flavonoids in LPE and Clementine fruits was performed according to Romeo et al. (2019) [40]. Briefly, 5 μ L of the sample was injected into a UHPLC PLATIN blue system (Knauer, Berlin, Germany) equipped with a binary pump, coupled with a PDA-1 (photodiode array detector) PLATINblue (Knauer, Germany), Knauer blue orchid C18 column (1.8 mm, 100 × 2 mm), and Clarity 6.2 software. Individual flavonoids were detected at 280 nm using water acidified with formic acid to pH 3.10 (A) and acetonitrile (B) as the elution solvents. The elution program is reported in Table 1. External standards were used to quantify the principal flavonoids (hesperidin, eriocitrin, narirutin, naringin, and neoeriocitrin). The obtained results were expressed as mg g⁻¹ of LPE d.w. and mg kg⁻¹ of Clementine fruits.

Time (min)	Eluent A (%)	Eluent B (%)	Flow (mL/min)
Initial	95.00	5.00	0.40
3.00	95.00	5.00	0.40
17.00	60.00	40.00	0.40
17.50	0.00	100.00	0.40
20.00	95.00	5.00	0.40
21.00	95.00	5.00	0.40

Table 1. Elution program for principal flavonoid quantification in LPE.

The total antioxidant activity (TAA) of both the LPE and Clementine fruits was tested using the DPPH and ABTS assays. The radical solutions of the DPPH and ABTS were formulated according to earlier reports [35,41], and then the analysis was conducted by mixing 20 μ L of the sample with 2980 μ L of the methanolic DPPH or ethanolic ABTS radical solutions in a cuvette, with a reaction time of 30 min and 6 min, respectively. The decrease in the absorbance value due to the interaction between antioxidants and free radicals was recorded at 734 nm for the ABTS assay and 515 nm for the DPPH assay using a spectrophotometer (Perkin-Elmer UV-Vis k2, Waltham, MA, USA) versus a blank (ethanol for the ABTS assay and methanol for the DPPH assay). The obtained absorbances were elaborated using a Trolox calibration curve, and the results were expressed as mM Trolox equivalent g^{-1} of the LPE (d.w.) and mM Trolox equivalent kg^{-1} of the Clementine fruits.

2.4. Physical Analyses of Clementine Fruits

The Clementine segments' color was recorded with a tristimulus colorimeter (Minolta CM-700d Spectrophotometer, Osaka, Japan) and acquired on 12 segments for the storage times and treatments. The determinations were made in triplicate according to the CIE L* a* b* parameters (L* for lightness, a* for red/green, and b* for yellow/blue tones) [35].

The weight loss was estimated by the AOAC standard method [42] and expressed in percentage.

Changes in the oxygen and carbon dioxide levels in the trays' headspace were checked using a gas analyzer (PBI, DANSENSOR, CPO_2/CO_2) provided with a thin needle to take a representative gas sample from the headspace of the packages. The determinations were made in triplicate, and the results were expressed as O_2 and CO_2 percentages.

The penetration test was carried out on ready-to-eat Clementine fruits to find the effect of alginate-based coatings on firmness [17]. The TA-XT Plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK) equipped with Exponent software 6.1.4.0 (Stable Micro Systems Ltd., Godalming, UK) was used for the data acquisition and integration. The analysis was conducted by a 5 mm diameter stainless-steel probe (P/5). The following conditions were used: penetration distance of 3 mm, test speed of 1.0 mm/s, and posttest speed of 3.0 mm/s [43]. The results of firmness (N) were expressed as a means of 20 replicates for each treatment for each storage time.

2.5. Microbiological Analyses of Clementine Fruits

Mesophilic bacteria (TBC) and yeasts and molds (Y&M) were verified following the protocols of Boninsegna et al. and Glicerina et al. [35,42]. Serial dilutions were prepared as described in earlier studies and plated on Dichloran Rose Bengal Chloramphenicol (DRBC) and Plate Count Agar (PCA) for the enumeration of Y&M and TBC, respectively. The plates were incubated at 25 °C, and the enumeration was performed after incubation for 5 d for the Y&M and 2 d for the TBC. The results were reported as log10 CFU g⁻¹.

2.6. Sensory Analyses of Clementine Fruits

The sensory characteristics were evaluated through a quantitative descriptive sensory analysis (QDA) attended by 10 trained people aged between 21 and 42. The visual (color intensity, shape, gloss, and surface uniformity), olfactory (intensity, fruity, citrus, and spicy), structural (consistency, chewiness, humidity, crunchiness, and turgidity), and taste characteristics (sweet, salty, acidic, bitter, citrus, fruity, astringent, and aftertaste) were evaluated on a scale from 0 to 9, where 0 indicated no perception and 9 the maximum perception of the considered attribute. Concerning total acceptability, each participant issued a judgment from 0 to 9 on the total acceptability of the ready-to-eat Clementine fruits, where the limit score was 4.5. The results were elaborated as a mean of the scores obtained for each sensorial attribute [35].

2.7. Statistical Analysis

The analytical data were reported as the mean value \pm standard error. The analysis of variance (one-way ANOVA) was conducted by SPSS software (Version 15.0, SPSS Inc., Chicago, IL, USA), applying the Tukey post hoc test at *p* < 0.05.

3. Results and Discussion

3.1. Chemical Characterization of LPE

The chemical characteristics of the extracts play an important role in the formulation of edible coatings, their structure, the enrichment of bioactive compounds, and finally, the antioxidant and antimicrobial activities related to them. The retention of the antioxidant extract within the polysaccharide matrix depends on multiple factors such as pH, ionic interactions, and the chemical compositions of the polysaccharide and the extract [44–46]. Table 2 reports the chemical characterization of the LPE.

Parameter Results 3.95 ± 0.02 pН TPC (mg GAE $g^{-1} d.w.$) 12.67 ± 0.17 TFC (mg CE g^{-1} d.w.) 2.10 ± 0.11 Hesperidin (mg g^{-1} d.w.) 3.88 ± 0.35 Eriocitrin (mg g^{-1} d.w.) 1.51 ± 0.39 Narirutin (mg g^{-1} d.w.) 0.03 ± 0.00 Naringin (mg g^{-1} d.w.) 0.01 ± 0.05 Neoeriocitrin (mg g^{-1} d.w.) 0.01 ± 0.04 DPPH ($\mu M TE g^{-1} d.w.$) 22.97 ± 0.53 ABTS ($\mu M TE g^{-1} d.w.$) 18.90 ± 0.29

Table 2. Chemical characterization of lemon pomace extract (LPE).

The resulting pH value (3.95) was optimal for the enrichment of the edible alginatebased coating, as indicated in previous studies (ranging from 3 to 5) on the physicalchemical properties of alginate [47–49]. The extract pH has been considered a major factor responsible for the thickness and consistency of the edible alginate-based coating since, in acidic environments (pH < 3), the ionic interactions determine the partial precipitation of alginate, which results in a contraction of the coating and a decrease in its thickness [47,48].

The LPE exhibited a high content of TPC (12.67 mg GAE g⁻¹) and TFC (2.10 mg CE g⁻¹), with hesperidin and eriocitrin as the main abundant phenolic compounds, according to other studies on green extractions of bioactive compounds from lemon by-products [39]. These results are also related to the measured antioxidant activity (22.97 μ M TE g⁻¹ for the DPPH assay and 18.90 μ M TE g⁻¹ for the ABTS assay). The retention of these compounds within the alginate-based coating increased the functional characteristics of the fruit and provided an efficient natural barrier to counteract the proliferation of spoilage and pathogenic microorganisms, as already proven in the experiments carried out on edible alginate-based coatings enriched with the vegetable extracts of *Ficus hirta*, pomegranate peel, and grape seed extracts [50–52].

3.2. Chemical, Physical, Microbiological, and Sensory Analyses Results of Clementine Fruits

In citrus fruits, the variations in pH, TA, and TSS are normal physiological processes that determine the qualitative decay and can be assisted by the wrong practices of management, post-harvest operations, and the distribution/sale of fruits [53]. The decrease in TSS (mainly sugars) and the progressive increase in TA—due to intense metabolic activities after harvesting and peeling—result in a considerable variation in the typical taste of Clementines, which compromises storage quality and acceptability by the final consumer [45–56].

The initial levels of TA and TSS in the Clementine fruits were within the range of 0.51-0.58% and 11.75-12.13 °Bx, respectively, with significant differences (p > 0.01) in the pH and TA values for AL-LPE 2% and AL-LPE 4% with respect to the CTR and AL due to the different concentrations of extract used in the formulation of edible coatings, as also confirmed by the pH values (Table 3). This trend has been maintained throughout storage, with important variations from 7 d for the CTR and from 14 to 21 d for the AL and AL-LPE 4%, respectively, while for the AL-LPE 2%, no difference was recorded up to 21 d at 4 °C. The CTR also showed important variations in TSS during storage, unlike all of the samples treated with the edible alginate-based coatings.

Parameter Sa	C 1	Time (Days)					
	Sample	0	3	7	14	21	Sig.
	CTRL	$12.10\pm0.05~^{\rm AB}$	$12.15\pm0.17~^{\rm AB}$	$10.25 \pm 0.72 \ ^{\mathrm{bC}}$	12.18 ± 0.10 ^A	11.47 ± 0.38 ^B	**
TSS	AL	11.88 ± 0.13	12.20 ± 0.23	12.57 ± 0.78 ^a	12.12 ± 0.07	11.85 ± 0.06	n.s.
(°Bx)	AL-LPE 2%	12.13 ± 0.61	12.90 ± 0.69	$12.08\pm0.55~^{\rm a}$	12.02 ± 0.00	11.80 ± 0.92	n.s.
	AL-LPE 4%	11.75 ± 0.40	12.30 ± 0.69	11.37 ± 0.43 $^{\rm a}$	11.60 ± 0.00	11.20 ± 0.58	n.s.
Sig.		n.s.	n.s.	**	n.s.	n.s.	
	CTRL	$0.59\pm0.03~^{\mathrm{bcAB}}$	$0.64\pm0.08~^{abA}$	$0.53\pm0.04~^{\mathrm{bAB}}$	$0.49\pm0.0~^{ m bB}$	$0.51 \pm 0.02 \ ^{\mathrm{cB}}$	**
TTA	AL	$0.51\pm0.03~^{\mathrm{bB}}$	$0.55\pm0.02~^{\mathrm{cAB}}$	$0.58\pm0.05~^{abA}$	$0.48\pm0.00~^{\mathrm{bB}}$	$0.54\pm0.01~^{abAB}$	*
(%)	AL-LPE 2%	$0.63\pm0.04~^{\mathrm{ab}}$	0.65 ± 0.03 $^{\mathrm{a}}$	0.62 ± 0.04 a	0.65 ± 0.03 $^{\mathrm{a}}$	0.63 ± 0.01 a	n.s.
	AL-LPE 4%	$0.68\pm0.08~^{\mathrm{aA}}$	$0.61\pm0.02~^{bB}$	$0.64\pm0.04~^{abAB}$	$0.69\pm0.03~^{\mathrm{aA}}$	$0.61\pm0.02~^{bB}$	*
Sign.		**	*	**	**	*	
	CTRL	$3.84\pm0.01~^{aB}$	$3.85\pm0.01~^{aB}$	$3.91\pm0.01~^{\rm bAB}$	$3.93\pm0.01~^{\mathrm{aAB}}$	$4.07\pm0.22~^{\mathrm{aA}}$	*
тЦ	AL	$3.75\pm0.01~^{\mathrm{abB}}$	$3.77\pm0.02~^{ m abB}$	3.99 ± 0.04 $^{\mathrm{aA}}$	3.78 ± 0.02 ^{bB}	$3.80 \pm 0.02 \ ^{\mathrm{cB}}$	**
pН	AL-LPE 2%	$3.59 \pm 0.07 \ ^{ m cC}$	$3.68\pm0.02~^{ m abBC}$	$3.83\pm0.01~^{\mathrm{cA}}$	3.81 ± 0.03 ^{bAB}	$3.69 \pm 0.13 \ ^{ m bcC}$	**
	AL-LPE 4%	$3.67\pm0.08~^{bc}$	$3.64\pm0.15~^{b}$	$3.74\pm0.03~^{d}$	$3.77\pm0.04~^{b}$	$3.71\pm0.13~^{bc}$	n.s.
Sign.		**	*	**	**	*	
	CTRL	$85.29\pm0.55~^{\rm AB}$	88.46 ± 2.00 ^A	$87.60\pm1.90~^{\rm AB}$	$85.59\pm1.85~^{\rm AB}$	$83.20\pm1.16^{\text{ Bb}}$	*
Moisture	AL	85.38 ± 2.00	85.56 ± 0.30	86.74 ± 1.97	85.80 ± 1.35	86.77 ± 1.23 ^a	n.s.
(g/100 g)	AL-LPE 2%	85.69 ± 1.61	86.63 ± 1.83	85.57 ± 2.21	85.17 ± 1.49	86.58 ± 0.89 ^a	n.s.
-	AL-LPE 4%	85.76 ± 1.41	86.89 ± 1.53	86.04 ± 1.32	86.42 ± 0.50	86.15 ± 0.79 $^{\rm a}$	n.s
Sign.		n.s.	n.s.	n.s.	n.s.	*	

Table 3. Chemical parameters in ready-to-eat Clementine fruits during storage.

Data are mean (n = 3) \pm s.d. Small letters within a column and capital letters within a row show significant differences as assessed by Tukey's *post-hoc* test. Abbreviations: **, significance at p < 0.01; *, significance at p < 0.05; n.s., not significant.

The total soluble solids and titratable acidity are considered among the most important indicators of citrus quality; their ratio is related to many factors, including the cultivar, harvest time, and post-harvest treatments [45–54]. In Europe, the commercial maturity of mandarin and Clementine fruits occurs when the TA and TSS levels reach values above 0.3% and 8 °Bx, respectively [56]. A reduction in these values indicates important sensory and chemical changes that affect the nutritional and qualitative characteristics determining commercial decay (a loss of freshness, low nutritional compounds, etc.) [55,56].

The moisture content after 21 d of storage was significantly (p < 0.05) lower in the CTR (83.2%) than in the AL (86.77%), AL-LPE 2% (86.58%), and AL-LPE 4% (86.15%). These trends showed that the barrier provided by alginate prevents the reduction of moisture, as already observed in ready-to-eat fruits and vegetables [57,58], such as melons [59] and strawberries [60].

The content of organic acids is considered an important quality index, both for the nutritional aspect and the typical fresh taste of citrus fruits. In Clementine fruits, the most representative acids are citric, ascorbic, and malic acids. Table 4 shows the trends of the organic acids during 21 d of refrigerated storage.

The application of edible coatings on the Clementine segments resulted in a significant variation (p > 0.01) in the malic and ascorbic acids up to 14 and 21 d of storage, respectively, while no statistically significant differences for the citric acid were found between samples up to 21 d of storage. The detected organic acid composition was in accordance with the TTA values previously shown in this study (Table 3), expressed as a % of citric acid (the most abundant acid present in citrus), but it does not include all of the organic acids that can interact with NaOH, nor indeed, does it include the ascorbic and malic ones.

Organic Acids (mg 100 g ⁻¹)	C 1	Time (Days)					
	Sample	0	3	7	14	21	Sig.
	CTRL	473.77 ± 19.74	455.9 ± 43.93	501.37 ± 2.88	444 ± 3.43	425.6 ± 66.4	n.s.
<u> </u>	AL	500.52 ± 2.07	423.52 ± 21.23	499.74 ± 53.59	463.21 ± 7.20	464.46 ± 2.75	n.s.
Citric acid	AL-LPE 2%	516.75 ± 18.34	477.42 ± 43.89	540.55 ± 66.43	482.49 ± 56.63	483.04 ± 67.26	n.s.
	AL-LPE 4%	489.48 ± 2.82	426.6 ± 21.78	422.11 ± 8.95	465.44 ± 0.00	439.97 ± 23.93	n.s.
Sign.		n.s.	n.s.	n.s.	n.s.	n.s.	
	CTRL	86.2 ± 4.28 ^a	82.57 ± 6.28 ^a	71.24 ± 5.40 ^{ab}	$63.88 \pm 14.29 \ ^{ab}$	68.13 ± 11.13	n.s.
N 6 1º · · 1	AL	62.45 ± 5.43 ^b	59.37 ± 4.90 ^b	55.38 ± 9.19 ^b	48.31 ± 0.03 ^b	62.55 ± 6.71	n.s.
Malic acid	AL-LPE 2%	57.99 ± 11.6 ^{Bb}	84.84 ± 16.66 Aa	$76.52\pm0.31~^{\rm ABa}$	$71.26\pm0.15~^{\rm ABab}$	76.07 ± 13.11 ^{AB}	*
	AL-LPE 4%	$59.43\pm5.64^{\text{ bB}}$	87.74 \pm 13.37 $^{\mathrm{Aa}}$	$52.21\pm4.34~^{\text{Bb}}$	75.8 ± 0.26 $^{\rm Aba}$	$52.63\pm23.29\ ^{\text{B}}$	*
Sign.		**	*	*	**	n.s.	
	CTRL	152.99 + 3.19 ^{aA}	104.62 + 13.14 ^{bB}	94.56 + 1.85 ^{bB}	97.19 + 1.65 ^{bB}	90.51 + 0.99 ^{cB}	**
A 1 · · · 1	AL	124.49 + 18.75 ^b	112.18 + 35.52 ^b	169.82 + 14.13 ^a	$170.78 \pm 3.11~^{\rm a}$	$124.28 \pm 1.21 \ ^{ m bc}$	n.s.
Ascorbic acid	AL-LPE 2%	205.69 + 36.80 ^{ab}	175.41 + 4.95 ^a	176.35 + 5.72 ^a	177.99 ± 2.52 ^a	202.96 ± 12.10 ^a	n.s.
	AL-LPE 4%	172.31 + 7.61 ^{ab}	180.92 + 11.50 ^a	175.31 + 6.37 ^a	180.61 ± 1.98 $^{\rm a}$	$184.37\pm8.26~^{ab}$	n.s.
Sign.		**	*	**	**	**	

Table 4. Organic acids composition in ready-to-eat Clementine fruits during storage.

Data are mean (n = 3) \pm s.d. Small and capital letters,**, *; n.s., see Table 3.

The combined action of enriched edible coatings and storage conditions favors the maintenance of citric acid levels and the increase in malic acid levels due to the regulation of the genetic expression of citrate hydrolase, citrate synthase, NADP-malic enzyme, and NAD-malate dehydrogenase enzyme, which are responsible for the changes in malic and citric acids in fruits during storage [61–65]. In agreement with them, the results obtained in this study showed that from 3 d of storage, an increased malic acid level was recorded in the AL-LPE 2% and AL-LPE 4%, while no statistically significant differences were observed throughout the storage of the CTR and AL.

The CTR showed a marked and significant (p > 0.01) ascorbic acid loss, from 152.99 mg 100 g⁻¹ to 90.51 mg 100 g⁻¹ after 21 d. It remained stable until the end of storage for AL, as already observed by authors in previous studies [35]. The enrichment with LPE improved the level of ascorbic acid in the AL-LPE 2% and AL-LPE 4% by about 35% with respect to the AL and CTR, and these quantities were maintained until 21 d. Significant differences (p > 0.01) were found with the last storage time among the samples, with the highest content in the AL-LPE 2% (202.96 mg 100 g⁻¹), followed by the AL-LPE 4% (184.37 mg 100 g⁻¹), AL (124.28 mg 100 g⁻¹), and CTR (90.51 mg 100 g⁻¹).

The degradation of ascorbic acid is a natural process that occurs during the preservation of fruits due to the oxidation and respiration rates and can be sped up by the storage conditions [65–69]. The presence of good levels of ascorbic acid in fruits gives the food excellent health characteristics. An intake of 80–100 mg of ascorbic acid per day is strictly recommended to counteract the onset of various human diseases [70]. The results in Table 4 show that the presence of LPE in the edible coating allows an increase in high levels of ascorbic acid and prevents its degradation for 21 d with cold storage, making these fruits excellent and beneficial for the final consumer.

Citrus fruits and the by-products resulting from their processing are recognized as a source of phenolic compounds, with flavonoids being the most representative class [22,71]. Immediately after the application of the edible coating enriched with the LPE, AL-LPE2%, or AL-LPE4%, the samples showed a significantly higher TFC than the CTR, while no significant differences were found among the samples for the TPC (Figure 1).

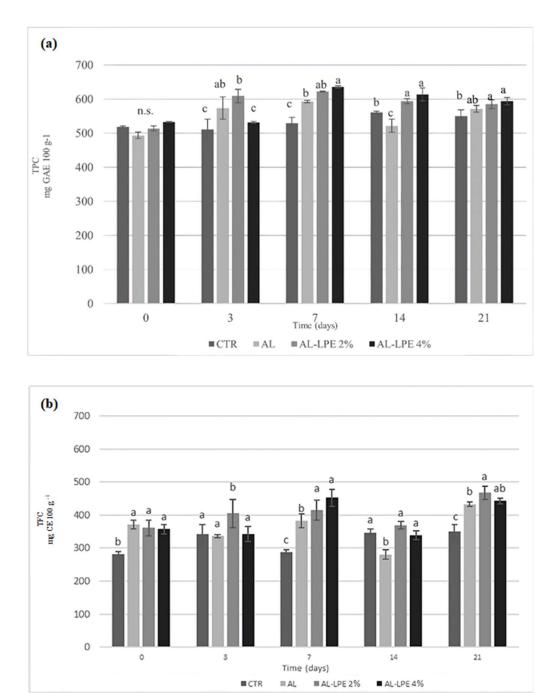


Figure 1. Total phenolic content (TPC) (**a**) and total flavonoid content (TFC) (**b**) of ready-to-eat Clementine fruits during storage. Letters show the significant differences among samples for each monitoring time by Tukey's post hoc test; n.s., see Table 3.

The TPC and TFC tended to increase during storage for the AL, AL-LPE2%, and AL-LPE4%, rising to the highest levels after 7 d. These results are according to a previous study by authors that showed a faster phenolic loss over time on coated Clementine segments [35]. The increased TPC and TFC is, therefore, a natural physiological process that occurs during fruit storage, whose main cause is the biosynthesis of new compounds catalyzed by enzymatic reactions at the expense of unavailable high molecular weight compounds, converted into available low molecular weight compounds [72–74]. Nevertheless, if the appropriate preservation strategies are not used, this observed increase is followed by a drastic decrease due to the susceptibility of these compounds to oxidation [75–78]. The data in Figure 1 clearly show that the application of the tested alginate-based edible coating (AL)

preserves the TPCs and TFCs in the segments for up to 21 d of cold storage. Clementines are naturally a source of flavonoids, with hesperidin and narirutin being among the most abundant [71–75]. The data obtained in this study showed that the addition of LPE to the formulation of the alginate-based edible coating resulted in a significant dose-dependent increase in the hesperidin and eriocitrin contents, according to the chemical characterization of the LPE mentioned above (Table 2). Regarding narirutin, no significant change was observed after the application of the edible coating (Table 5) since narirutin was present in low quantities in the LPE, whereas it is strictly related to the chemical composition of Clementine fruits [72–75].

	6 1	Time (Days)						
	Sample	0	3	7	14	21	Sign	
	CTRL	170.01 ± 3.37	190.44 ± 17.82	170.62 ± 9.56	165.35 ± 3.01 ^b	168.80 ± 3.53	n.s.	
NT ' ''	AL	175.32 ± 6.74 ^{CB}	$207.38 \pm 6.02 \ ^{\rm A}$	175.04 ± 0.42 ^B	$152.61 \pm 0.66 \ ^{ m cC}$	$173.02 \pm 4.91 \ ^{\mathrm{BC}}$	**	
Narirutin	AL-LPE 2%	187.50 ± 2.36 ^A	$213.05 \pm 6.46\ ^{\rm B}$	$181.76 \pm 4.60 \ ^{\rm AB}$	$192.56 \pm 2.01 \ ^{\mathrm{aAB}}$	$188.78 \pm 8.24 \ ^{\rm AB}$	*	
	AL-LPE 4%	191.65 ± 10.11	201.86 ± 0.09	183.02 ± 13.55	167.81 \pm 0.76 $^{\mathrm{b}}$	178.98 ± 2.12	n.s.	
Sig.		n.s.	n.s.	n.s.	**	n.s.		
Hesperidin	CTRL	$96.39 \pm 13.34 \ ^{\rm cC}$	$124.56\pm12.21~^{\rm A}$	$96.24 \pm 12.83 \ ^{\rm cC}$	102.23 ± 12.83	$116.90 \pm 13.65 \ ^{\rm AB}$	*	
	AL	$123.61\pm6.08~^{bBC}$	$128.44\pm4.95~^{\rm A}$	$125.03 \pm 18.47 {}^{bA}$	104.75 ± 18.74	$115.57\pm6.08\ ^{\text{B}}$	**	
	AL-LPE 2%	$125.54 \pm 13.68 \ ^{\mathrm{bA}}$	$138.62 \pm 12.65 \ ^{\rm B}$	$126.49 \pm 6.20 \ ^{\mathrm{bC}}$	$141.77\pm6.52~^{\mathrm{aAB}}$	$117.35 \pm 12.52^{\text{ C}}$	**	
	AL-LPE 4%	142.78 \pm 17.21 $^{\rm aA}$	$129.11\pm7.62~^{\rm AB}$	$142.92\pm8.91~^{\mathrm{aA}}$	$115.22 \pm 8.91 \ ^{\rm bBC}$	$110.90 \pm 21.21 \ ^{\rm C}$	**	
Sign.		**	n.s.	**	**	n.s.		
	CTRL	$0.39 \pm 0.01 \ ^{\rm cA}$	$0.40\pm0.00~^{\rm bA}$	$0.22 \pm 0.01 \ ^{ m cB}$	$0.07 \pm 0.00 \ ^{\rm cC}$	$0.08 \pm 0.00 \ ^{\mathrm{bC}}$	**	
T · · · ·	AL	0.38 ± 0.00 dA	0.37 ± 0.01 ^{bA}	$0.36\pm0.00~^{\mathrm{abA}}$	$0.06 \pm 0.00 \ ^{\rm cB}$	$0.08 \pm 0.00 \ ^{\mathrm{bB}}$	**	
Eriocitrin	AL-LPE 2%	0.99 ± 0.01 ^{bA}	$0.76\pm0.02~^{abB}$	$0.46\pm0.00~^{ m abC}$	$0.35 \pm 0.00 \ ^{ m bD}$	$0.49\pm0.00~^{\mathrm{aC}}$	**	
	AL-LPE 4%	$1.06\pm0.01~^{aAB}$	$1.19\pm0.02~^{aA}$	$0.65\pm0.01~^{aAB}$	$0.55\pm0.00~^{aAB}$	$0.51\pm0.00~^{aB}$	*	
Sign.		**	*	*	**	**		

Table 5. Flavonoids composition (mg kg $^{-1}$) in ready-to-eat Clementine fruits during storage.

Data are mean (n = 3) \pm s.d. Small and capital letters,**, *; n.s., see Table 3.

In recent literature, there are no studies about the application of edible coatings on ready-to-eat Clementine fruits, whereas studies on whole Clementine fruit proved that different post-harvest preservation treatments prevent loss and determine the synthesis of new compounds [79–81].

The antioxidant potential of foods depends on the synergistic action of many compounds and, particularly in citrus fruits, it is mainly due to the action of phenolic compounds and ascorbic acid [69,78]. In this study, the ABTS and DPPH assays were applied to test the antioxidant activity of molecules with different polarities [82,83].

An increment of antioxidant activity was detected immediately after the treatments with LPE on Clementine fruits until 3 d of storage (Figure 2). After 7 d of storage, a decrement was recorded using the ABTS assay and a slight rise with the DPPH assay, probably due to the synthesis of new compounds with different antioxidant actions. However, given the nature of the compounds present in citrus, the scavenging activity registered against the ABTS radical was always higher than the DPPH radical.

The coated Clementine segments (AL, AL-LPE 2%, and AL-LPE 4%) showed higher antioxidant activities than the CTR after 21 d of storage and were coherent with the discussed trends for the TPCs, TFCs, and organic acids.

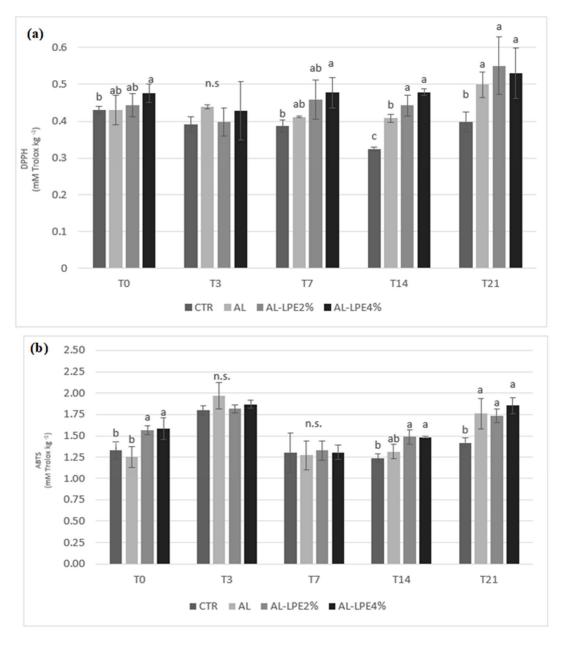


Figure 2. Antioxidant activity of ready-to-eat Clementine fruits during storage Letters show the significant differences among samples for each monitoring time by Tukey's post hoc test. n.s., see Table 3.

Regarding the color parameters, no significant (p < 0.05) differences were noted among the samples and during storage, as evidenced in Table 6. The contribution made by the LPE addition to the edible coating was more evident for the weight loss, headspace gas composition, and microbiological count. A natural decrease in weight was observed in all samples during storage, and significant differences (p < 0.05) were noted, particularly after 21 d for AL-LPE 2% and AL-LPE 4%, which both showed slower dehydration. Regarding the O₂:CO₂ ratio in the headspaces of the trays, significant changes were found from the fourteenth day of storage between the uncoated segments (CTR), coated ones with alginate (AL), and coated ones with LPE-enriched alginate. The concentration of oxygen and carbon dioxide is due to the combined action of tissue metabolic activity after peeling and microbial respiration [84]. In this study, the differences recorded between the coated and non-coated samples for weight loss and the O₂:CO₂ ratio in the headspaces of the trays were due to the simultaneous action of alginate and LPE. The application of the edible alginate-based coating on the fruit surface determined a modification of the atmosphere surrounding each coated segment, slowing down its metabolic activity. LPE, moreover, slowed or inhibited the proliferation of microorganisms and, consequently, the quality changes associated with [57,58,85,86]. The microbiological analysis of ready-to-eat Clementine fruits also confirmed its antimicrobial activity, as evidenced by the slow growth of mesophilic bacteria (TBC) and mold, with a dose-dependent effect. Yeasts were not detected in all samples and for each time of storage. The hydroalcoholic extract obtained from lemon by-products was already indicated by several authors as antimicrobial against many microorganisms that cause spoilage problems and food safety (*Bacillus* spp., *Salmonella* spp., *Staphylococcus* spp., *Listeria monocytogenes, Enterobacteraerogenes, Escherichia coli, Pseudomonas aeruginosa, Alternaria* sp., *Aspergillus*, and *Rhizopus* sp.), in accordance with the results obtained in this study [39,87].

Demons (Time (Days)										
Parameter	Sample	0	3	7	14	21	Sig				
	CTRL	50.19 ± 1.69	50.72 ± 1.42	53.25 ± 1.22	52.86 ± 2.65	51.71 ± 1.67	n.s				
T v	AL	54.80 ± 1.33	52.62 ± 1.69	53.84 ± 1.16	52.57 ± 1.54	50.92 ± 2.10	n.s				
L*	AL-LPE 2%	49.67 ± 5.70	53.15 ± 1.80	52.31 ± 1.83	51.99 ± 2.10	51.58 ± 6.11	n.s				
	AL-LPE 4%	51.90 ± 5.86	50.54 ± 2.39	50.98 ± 2.48	50.70 ± 3.19	50.40 ± 2.09	n.s				
Sign.		n.s.	n.s.	n.s.	n.s.	n.s.					
	CTRL	8.30 ± 1.21	8.41 ± 1.27	8.74 ± 1.01	8.85 ± 2.14	8.67 ± 1.32	n.s				
×	AL	8.18 ± 1.30	9.27 ± 2.01	9.42 ± 2.38	8.60 ± 1.96	9.46 ± 1.56	n.s				
a*	AL-LPE 2%	8.80 ± 3.06	8.12 ± 1.50	8.91 ± 2.29	8.39 ± 1.47	8.79 ± 2.29	n.s				
	AL-LPE 4%	8.31 ± 2.95	8.88 ± 2.10	8.54 ± 1.90	7.84 ± 1.89	8.19 ± 1.21	n.s				
Sign.		n.s.	n.s.	n.s.	n.s.	n.s.					
	CTRL	19.48 ± 1.07	20.90 ± 1.54	19.78 ± 1.43	21.71 ± 1.97	20.21 ± 1.82	n.s				
b*	AL	20.27 ± 1.50	20.48 ± 1.41	24.23 ± 2.40	20.60 ± 2.63	21.39 ± 1.58	n.s				
D	AL-LPE 2%	19.39 ± 2.16	19.66 ± 2.07	21.43 ± 1.53	20.71 ± 2.08	21.47 ± 2.96	n.s				
	AL-LPE 4%	20.83 ± 1.74	21.9 ± 1.45	21.07 ± 2.30	18.67 ± 2.75	19.21 ± 2.81	n.s				
Sign.		n.s.	n.s.	n.s.	n.s.	n.s.					
Weight loss	CTRL	$0.00\pm0.00~^{\rm D}$	$0.03\pm0.01^{\text{ C}}$	$0.02\pm0.01~^{\rm Cb}$	$0.07\pm0.02^{\text{ B}}$	$0.10\pm0.01~^{\rm Aa}$	**				
	AL	0.00 ± 0.00 ^C	0.05 ± 0.02 ^B	0.05 ± 0.01 ^{Ba}	0.07 ± 0.01 $^{ m AB}$	0.10 ± 0.01 $^{ m Aa}$	**				
$(g \ 100 \ g^{-1})$	AL-LPE 2%	$0.00\pm0.00~^{\rm D}$	0.04 ± 0.02 ^{BC}	$0.03\pm0.01~^{ m ABCab}$	0.06 ± 0.02 $^{ m AB}$	0.08 ± 0.00 $^{ m Aab}$	*				
	AL-LPE 4%	$0.00\pm0.00~^{\rm B}$	0.05 ± 0.03 $^{\rm A}$	$0.05\pm0.01~^{\rm Aa}$	$0.07\pm0.02~^{\rm A}$	$0.05\pm0.02~^{\rm Ab}$	*				
Sign.		n.s.	n.s.	*	n.s.	*					
	CTRL	$21.00\pm0.00\ ^{\rm A}$	$14.52\pm1.11~^{\rm Bb}$	$14.30\pm1.90\ ^{\text{B}}$	$7.10\pm0.99~^{\rm Cb}$	$5.6\pm0.71~^{\rm Cc}$	**				
O2	AL	21.00 ± 0.00 ^A	16.40 ± 0.43 ^{Ba}	$13.30\pm0.63~^{\rm C}$	8.25 ± 0.57 $^{ m Dab}$	8.30 ± 0.68 ^{Db}	**				
(%)	AL-LPE 2%	21.00 ± 0.00 A	17.40 ± 0.38 ^{Ba}	14.70 ± 1.86 ^C	9.40 ± 0.14 ^{Cab}	10.60 ± 0.21 ^{Ca}	**				
	AL-LPE 4%	$21.00\pm0.00\ ^{\rm A}$	$17.70\pm0.75~^{\mathrm{Ba}}$	$14.92\pm1.06\ ^{\rm C}$	$12.50\pm2.12^{\text{ Da}}$	$8.80\pm0.64~^{\rm Eb}$	**				
Sign.		n.s.	**	n.s.	**	**					
	CTRL	$0.02\pm0.00~^{\rm D}$	$9.40\pm1.37^{\text{ C}}$	$10.00\pm2.30~^{\mathrm{BC}}$	$13.00\pm1.13~^{\rm ABb}$	$19.90\pm0.14~^{\rm Aa}$	**				
CO ₂	AL	0.02 ± 0.00 ^D	9.20 ± 0.43 ^C	9.80 ± 0.81 ^C	$14.90\pm0.10~^{\rm Ba}$	18.25 ± 0.51 $^{ m Aab}$	**				
(%)	AL-LPE 2%	0.02 ± 0.00 ^C	7.75 ± 1.82 ^B	8.90 ± 1.69 ^B	16.80 ± 0.92 $^{ m Aa}$	$17.30\pm0.78~^{\rm Ab}$	**				
	AL-LPE 4%	$0.02\pm0.00~^{\rm D}$	$7.70\pm0.79\ ^{\rm C}$	$10.30\pm1.22~^{\rm C}$	$13.50\pm2.25~^{Bab}$	$17.50\pm1.82~^{\rm Aab}$	**				
Sign.		n.s.	n.s.	n.s.	*	*					
	CTRL	$0.00\pm0.00~^{\rm D}$	$0.00\pm0.00~^{\rm D}$	$1.45\pm0.26~^{abC}$	$3.00\pm0.04~^{aB}$	$5.11\pm0.08~^{\rm aA}$	**				
CBT	AL	$0.00\pm0.00~^{\rm C}$	$0.00\pm0.00~^{\rm C}$	$1.89\pm0.35~^{\mathrm{aB}}$	$1.50\pm0.28~^{\mathrm{bB}}$	$2.75\pm0.25~^{\rm bA}$	**				
$\log_{10} \text{CFU g}^{-1}$)	AL-LPE 2%	$0.00\pm0.00~^{\rm C}$	$0.00\pm0.00~^{\rm C}$	$1.10\pm0.17~^{\mathrm{bB}}$	$1.09\pm0.12~^{\rm bcB}$	$2.45\pm0.58~^{\rm bA}$	**				
	AL-LPE 4%	$0.00\pm0.00\ ^{\rm C}$	$0.00\pm0.00\ ^{\rm C}$	$0.96\pm0.24~^{bB}$	$1.00\pm0.00~^{\rm cB}$	$2.32\pm0.51~^{bA}$	**				
Sign.		n.s.	n.s.	*	**	**					
	CTRL	$0.00\pm0.00~^{\rm C}$	$0.00\pm0.00~^{\rm C}$	$0.00\pm0.00~^{\rm C}$	$1.77\pm0.10~^{\mathrm{aB}}$	$2.15\pm0.63~^{aA}$	**				
Mold	AL	0.00 ± 0.00 ^C	$0.00\pm0.00~^{\rm C}$	$0.00\pm0.00~^{\rm C}$	$1.03\pm0.10~^{\mathrm{aB}}$	1.94 ± 0.34 $^{\mathrm{aA}}$	**				
\log_{10} CFU g ⁻¹)	AL-LPE 2%	0.00 ± 0.00 ^B	0.00 ± 0.00 ^B	0.00 ± 0.00 ^B	$0.00 \pm 0.00 \ ^{\mathrm{bB}}$	$1.66\pm0.00~^{\mathrm{aA}}$	**				
- 0 /	AL-LPE 4%	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	n.s				
Sign.		n.s.	n.s.	n.s.	**	*					

 Table 6. Physical and microbiological parameters in ready-to-eat Clementine fruits during storage.

Data are mean (n = 3; n = 12 for color parameters) \pm s.d. Small and capital letters, **, *; n.s., see Table 3.

The textural analyses (Figure 3) showed that all of the tested coatings significantly (p < 0.01) improved the firmness of the Clementine segments, as reported in previous studies on similar coatings [88,89]. Predictably, the CTR showed a lower firmness than the AL, AL-LPE 2%, and AL-LPE 4% due to the natural softening process after the peel's removal and dissection in citrus segments. During the storage period, different trends were observed as follows: the AL showed a stronger increase in firmness (49.18%), from 1.83 N to 2.73 N, whereas no significant variations were noted for the AL-LPE 2% and AL-LPE 4%. The trend of AL was due both to the interactions between alginate and cell-wall pectins

and to the chemical structure of the edible coating, which causes the excessive drying and hardening of vesicles [90]. The results of our study seem coherent with the literature that reports an improvement in the physical performance of coatings by the addition of vegetable extracts ranging from 3–5 pH [48,49,91].

Several studies showed that edible alginate coatings can improve the visual and structural properties of ready-to-eat fruits over a long shelf life, avoiding structural changes in the cell wall and visual appearance due to the degradation of valuable constituents [92,93].

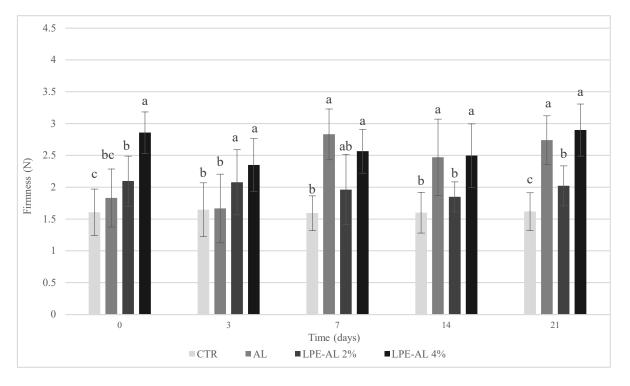


Figure 3. Firmness (N) of ready-to-eat Clementine fruits during storage. Letters show the significant differences among samples for each monitoring time by Tukey's post hoc test.

The sensory evaluation confirmed the results obtained for the physical analysis. In particular, the treatments applied to the AL, AL-LPE 2%, and AL-LPE 4% statistically improved (p < 0.01) the color and firmness of the Clementine segments compared to the CTR immediately after their application. Meanwhile, the parameters related to the olfactory and gustatory sensations were unchanged, as well as the total acceptability (Table 7). The results obtained at the beginning of storage indicated that the LPE did not negatively affect the visual characteristics and the flavor of the fruits, which is one of the main requirements that edible coatings must have to be applied to food [94].

	Days	CTR	AL	AL-LPE2%	AL-LPE4%	Sign
	0	7.00 ± 0.50 ^b	$8.67\pm0.47~^{\rm a}$	$8.33\pm0.47~^{a}$	8.50 ± 0.49 ^a	**
Color	21	$6.83\pm0.86\ ^{b}$	$8.00\pm0.82~^a$	7.5 ± 0.96 ab	8.00 ± 0.63 a	*
Visual	0	7.83 ± 0.76	8.5 ± 0.50	8.5 ± 0.76	8.33 ± 0.49	n.s.
Appearance	21	8.17 ± 0.69	8.17 ± 0.37	8.33 ± 0.94	7.67 ± 1.02	n.s.
Fruity	0	7.17 ± 1.34	6.33 ± 0.47	6.83 ± 0.69	7.00 ± 0.63	n.s.
Fully	21	6.33 ± 0.47 $^{\mathrm{ab}}$	6.00 ± 0.57 $^{\rm b}$	7.00 ± 0.58 $^{\rm a}$	$6.50\pm0.49~^{\mathrm{ab}}$	*
Citrusy	0	6.50 ± 0.76	6.67 ± 0.75	6.83 ± 0.69	6.83 ± 0.80	n.s.
	21	5.83 ± 0.68	6.50 ± 0.05	6.83 ± 0.68	6.67 ± 0.80	n.s.
Sweetness	0	6.83 ± 0.75	5.93 ± 1.05	6.33 ± 0.55	6.33 ± 0.62	n.s.
	21	4.33 ± 0.70	5.10 ± 0.55	5.00 ± 0.66	5.00 ± 0.75	n.s.
Acidity	0	3.00 ± 0.89	3.05 ± 0.57	2.33 ± 0.74	2.66 ± 0.48	n.s.
	21	5.67 ± 0.95 $^{\rm a}$	4.33 ± 0.75 a	$2.66\pm0.55~^{\rm b}$	$2.83\pm0.55~^{\rm b}$	**
	0	7.00 ± 1.45	8.16 ± 0.85	8.00 ± 1.10	7.33 ± 0.95	n.s.
Aftertaste	21	6.33 ± 0.51 $^{\rm b}$	8.00 ± 0.65 $^{\rm a}$	8.33 ± 0.45 $^{\rm a}$	7.66 ± 1.15 $^{\rm a}$	*
	0	6.66 ± 0.47 ^b	$8.16\pm0.68~^{\rm a}$	8.17 ± 0.72 $^{\rm a}$	7.70 ± 1.01 ^{ab}	*
Crunchiness	21	$4.5\pm0.89~^{\rm b}$	$7.00\pm0.67~^{\rm ab}$	8.00 ± 0.48 $^{\rm a}$	7.33 ± 0.45 a	**
г.	0	$6.83\pm1.07~^{\rm b}$	8.00 ± 0.58 $^{\rm a}$	$8.33\pm0.47~^{a}$	$8.17\pm0.63~^{a}$	**
Firmness	21	6.17 ± 0.75 $^{\rm b}$	$7.00\pm0.95~^{ab}$	8.17 ± 0.37 $^{\rm a}$	8.00 ± 0.75 a	**
Overall ac-	0	6.60 ± 0.80	6.40 ± 0.49	6.80 ± 0.75	6.80 ± 0.40	n.s.
ceptability	21	$5.33\pm0.82^{\text{ b}}$	$6.17\pm0.41~^{\mathrm{ab}}$	6.83 ± 0.41 $^{\rm a}$	7.00 ± 0.89 a	**

Table 7. Sensory evaluation of Clementine segments during the storage.

Data are mean (n = 10) \pm s.d. Small letters, **, *; n.s., see Table 3.

The data at the end of storage showed that the alginate-based coating and the addition of LPE improved the sensory acceptability for color, fruitiness, and turgidity at 21 d of storage at 4 °C, with the overall acceptability scores at the end of storage being 6.83 and 7.0 for the AL-LPE 2% and AL-LPE 4%, respectively.

4. Conclusions

The synergistic action of the alginate-based coating and lemon by-product extract has considerably improved the storage and safety of ready-to-eat Clementine fruits up to 21 d of storage at 4 °C. These parameters are intimately linked to the visual acceptability perceived by the consumer at the time of purchase regarding both the status of the trays (swelling, fog, etc.) and the appearance of the fruit (intensity, color, turgidity, wrinkling, presence of molds, etc.), as confirmed by the sensorial analysis. Among the tested treatments, the addition of both concentrations of LPE to the coating formulation allowed us to obtain a structure that favored the retention of antioxidant compounds, microbial safety, and good sensory acceptance in Clementine fruits.

In the modern consumer scenario, the challenge is to meet consumer demand for high-value fruit products: the presence of the LPE in the edible coating allowed increased high levels of ascorbic acid and prevented its degradation for 21 d of cold storage, making these ready-to-eat fruits excellent and beneficial foods for the final consumer. Eating 50 g of ready-to-eat Clementine segments AL-LPE 2% and AL-LPE 4% can support the daily requirement of ascorbic acid. Moreover, the combination of alginate and LPE is an efficient and sustainable natural treatment to preserve ready-to-eat fruits, satisfying consumer demand for natural preservatives and the environmental need for the sustainable reuse of by-products derived from food processes.

The use of LPE can, therefore, allow for the preservation of the total quality of the ready-to-eat fruits for 21 d and, at the same time, the reuse of lemon by-product could encourage the rapid transition of the citrus industry from a linear to circular economy, thus promoting the sustainability of production and the reduction of food waste with a high environmental impact. Future studies could focus on the in vivo activity of the compounds recovered from lemon by-products.

Author Contributions: Conceptualization, A.P. and M.A.B.; methodology, M.A.B. and A.D.B.; software, M.A.B.; validation, A.P.; formal analysis, M.A.B.; investigation, M.A.B.; data curation, M.A.B. and A.P.; writing—original draft preparation, M.A.B.; writing—review and editing, A.P. and A.D.B.; supervision, A.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data will be made available upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

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