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Assessment of Chitosan-Rue (*Ruta graveolens* L.) Essential Oil-Based Coatings on Refrigerated Cape Gooseberry (*Physalis peruviana* L.) Quality

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Abstract: Cape gooseberry (*Physalis peruviana* L.) is one of the main exotic fruits in demand throughout the world market. However, this fruit has problems with physical and microbial decay causing losses up to thirty percent during post-harvest stage and market storage. As an alternative for conservation, technologies based on edible coatings of biopolymers incorporating essential oils have been developed. In this paper we studied the effect of edible coatings based on chitosan (CS) and *Ruta graveolens* L. essential oil (RGEO) at different concentrations applied on the surface gooseberries at 18 ± 2 °C. The emulsions exhibited a reduction in the viscosity and the particle size with the increasing in the RGEO amount (from 124.7 cP to 26.0 cP for CS + RGEO 0.5% and CS + RGEO 1.5%, respectively). A lower weight loss was obtained for fruits coated with CS + RGEO 0.5% (12.7%) as compared to the uncoated (15%), while the maturity index increased in a lower amount for CS + RGEO coated than the uncoated fruits. The mesophyll growth was delayed three days after the coating applications for CS + RGEO 1.0% and 1.5%. At day twelve of the coating process, fruits with CS + RGEO 1.5% presented only 3.1 Log UFC/g of aerobic mesophylls and 2.9 Log UFC/g of molds and yeasts, while the uncoated fruits presented 4.2 Log UFC/g of aerobic mesophylls and 4.0 Log UFC/g of molds and yeasts, demonstrating a microbial barrier of the coatings incorporating RGEO in a concentration dependent manner. The CS + RGEO coating also preserve the antioxidant property of case gooseberries after twelve days of treatment under storage according to the 2,2′-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid) (ABTS) results. It was demonstrated by the ABTS method that T5 antioxidant capacity from day one to day twelve only decreases from 55% to 44%, while in the uncoated fruits (T1) the antioxidant capacity decreased from 65% to 18%. On the other hand, using the DPPH method the reduction was from 73% to 24% for the uncoated samples and 55% to 43% for T5. From the sensorial analysis, we recommend the use of CS + RGEO 0.5% that was still accepted by the panelists after the sixth day of application. These results show the potential application of these coatings as postharvest treatment under storage and low temperature conditions during twelve days of treatment for cape gooseberry fruits.

1. Introduction

Cape gooseberry or goldenberry (*Physalis peruviana L.*) is one of the leading exotic fruits appreciated in the world market due to its high content of vitamins, fiber, antioxidants, therapeutics, and organoleptic compounds [1]. It is from the Solanaceae family, a fruit native of Peru considered a climacteric fruit, which forms its fruit in an inflated calyx. Usually, calyx and skin color can be used to indicate maturity [2]. Cape gooseberry is highly appreciated for its texture, flavor, and aroma, introducing low calorie content in a rich fiber diet [3].

Colombia is considered one of the leading producers of cape gooseberries in the world, annually exporting 7.2×10^3 tons of cape gooseberries, the second most exported fruit after banana fruit, mainly to European countries [4].

The cape gooseberry fruit is acid-sweet in taste, with a pleasant flavor and typically consumed fresh, whole, or sliced, without the calyx, but with the skin [2]. The fruit is also significant for the preparation of salads, desserts, and different puddings. However, the most considerable consumption is fresh. Nowadays, the goldenberry crops are considered very promising for subtropical areas of the USA [5].

In 2009, the primary consumers of Colombian cape gooseberries were The Netherlands (3200 tons), Germany (1600 tons), Belgium (966 tons), Sweden (214 tons), and France (130 tons). However, Colombia only exported 18.4 tons to the United States as a result of the demanding quarantine cold treatment, where edible coatings to preserve fruits and increase the storage stability under refrigerated conditions could be very useful [6]. Usually, for distribution to the world markets, cape gooseberries are packed in plastic or wood boxes with a capacity of 8 kg for fruit with a calyx and 10 kg without a calyx, which means that the present treatment will favor more fruit per box, avoiding a higher plastic consumption around the world [7].

When the calyx is removed from the cape gooseberries, the fruits have higher respiration rates, generating higher levels of ethylene and accelerating their decay, it is not adequately stored [8]. With the ripening process, the fruit colors change from green (chlorophyll) to yellowish-orange (carotenoids) and a softening of the surface, with several cell-wall enzymatic changes [9].

In addition to some contamination during the production stage, goldenberry suffers post-harvest diseases caused by several fungi. For example, in the Maharashtra region of India, Rao, and Subramoniam described a post-harvest disease caused by *Fusarium equiseti* (Corda) Sacc., entering at points of injuries and bruises on stored berries and subsequently inciting a soft rot [8,10]. In addition, in the fruit market of Aligarh (India), Sharma and Khan isolated on goldenberry the fungi *Alternaria alternata* (Fr.) Keissler, *Cladosporium cladosporioides* (Fres.) de Vries, and *Penicillium italicum* Wehmer, directly affected in storing markets [11].

Usually, for commercialization purposes, the fruits must accomplish quality, nutritional, and healthy characteristics, for client acceptance in the international markets [12]. To meet those requirements, they are treated using chemical products (synthetic fungicides such as iprodione, fludioxonil, and fenhexamid), which due to their excessive use, have generated problems of pollution to the environment, complications in human health, and resistant in microorganisms [13,14].

The natural polymer-based coatings to preserve quality and freshness of fruits should have consumer acceptation without altering the original flavor, appearance, aroma, etc., and it should have biocompatibility, gas barrier properties, non-toxic, non-polluting, good physical appearance, and low average cost [15]. Edible coatings are sprayed or brushed directly on the fruit surface to crate modify atmospheres and a barrier effect without generating anaerobiosis or negatively affect the quality of the fruit. Since the materials will be humanly consumed, they should be based on raw materials, generally referred to as safe (GRAS) [16].

Chitosan is a world-wide abundant and safe polysaccharide, with excellent antimicrobial and biocompatibility properties broadly incorporated in edible coatings and applied on fruit surfaces for preservation purposes [14,17]. However, its hydrophilic nature is a weak moisture barrier that needs to be improved by the development of composites incorporating lipids such as essential oils and fatty

acids [18]. Therefore, alternatives incorporating hydrophobic compounds to strengthen moisture barrier such as some essential oils, having antioxidant, antibacterial, and antifungal properties that preserve and protect food during the post-harvest of the fruit is an added-value strategy [19].

Carvalho et al. [6] investigate the effect of alginate coating and cold storage on the bioactive compounds present in cape gooseberries [6]. Licodiedoff et al. [20] studied the effect of apply gelatin-calcium chloride on cape gooseberry without Calyx, which provided a reduction of weight loss and fungal deterioration [20]. However, no reports using chitosan-*Ruta graveolens* essential oil have been investigated for cape gooseberry storage under refrigeration conditions.

The incorporation of Rue essential oil (*Ruta graveolens* L.) could be an excellent option to complement the antimicrobial activity of chitosan. *Ruta graveolens* L. essential oil (RGEO) components, such as the 2-undecanone, 2-nonanone, and monoterpenes, have been recognized as antifungal components [21,22]. The incorporation of the RGEO components in the chitosan (CS) matrix improves barrier, antimicrobial, antifungal, and antioxidant properties, as previously demonstrated, especially to control *Colletotrichum gloesporioides* fungi, which allowed to prolong the stability in guavas harvested in Colombia [23].

In this research, our interest is to evaluate the effect on the physicochemical, antioxidant, and quality properties of cape gooseberries after the CS + RGEO coatings application. To the best of our knowledge, this is the first time that the application of CS + RGEO coatings is reported in cape gooseberries to evaluate the effect in quality aspects of cape gooseberry fruits.

2. Materials and Methods

2.1. Fruit Samples

Cape gooseberry (*P. peruviana* L.) fruit with the calyx, Colombian ecotype were selected from a local market of the Colombian city (Tunja), and protected with their natural calyx in a maturation stage four, according to the Colombian Technical Standard (NTC) 4580 [24]. Seven hundred fruits were selected according to the standard, healthy, and with uniform size and color. Subsequently, they were washed with a 100 mg/L solution of sodium hypochlorite, dried, and stored at a temperature of 18 ± 2 °C until their use.

2.2. Preparation of Edible Coatings

Emulsions were prepared following a previously reported methodology [23]. Briefly, one liter of a solution of a specific amount of chitosan was prepared (degree of deacetylation = 85%, Mw = 190.000–310.000 Da, Sigma-Aldrich, Palo Alto, California, USA), using a 0.1 M acetic acid a solution (pH 5.6) were glycerol (2.5% *v/v*) was added dropwise under magnetic stirring during 30 min. After that, Tween 80 was added (1% *v/v*) concerning the final volume of RGEO (Krauter, Colombia). RGEO was previously characterized and added to reach final concentrations of 0.5%, 1.0%, and 1.5% v/v, with respect to the chitosan solution [23]. The final mixture was mixed using an IKA T25-Digital Ultraturrax (Staufen) (USA) at 7000 rpm for two minutes. All the bubbles were removed by vacuum from the emulsions.

2.3. Application of Edible Coatings to Cape Gooseberries

Cape gooseberries were coated by direct immersion in the different emulsions. The treatments consisted of one control (T1) and four different treatments (T2 = CS, T3 = CS + RGEO 0.5%, T4 = CS + RGEO 1.0%, T5 = CS + RGEO 1.5%), in glass cells of 15 cm × 9 cm × 12 cm high with the help of a metal support for five minutes. Subsequently, they were dried for 60 min at 16 °C and stored in boxes of ethylene poly terephthalate (PET) under refrigerated conditions (18 ± 2 °C) for 12 days.

2.4. Physicochemical Characterizations of Emulsions

The particle size, density, viscosity, and non-volatile compounds of the emulsions were determined according to the methodologies reported previously [23,25]. Briefly, the particle size of the emulsions was tested using a laser diffractometer was used (AIMSIZER 2011, Dandong Liaoning, China). The viscosity was determined using a Brookfield LVF (Toronto, Canada) viscometer. Density determination was made based on the parameters established in ISO 8655-2 [26]. The liquid was weighed on an analytical balance (Adventurer AR2140, USA), and the results were expressed according to Equation (1).

$$d \ = \ P_1/V_1 \tag{1}$$

where P_1 is the weight of the dosed volume (g), V_1 the volume of dosage (1 mL), and d the density of the sample at 25 °C (g/mL).

The non-volatile compounds were calculated from Equation (2):

$$S\ (\%) = ((P_s - P_d)/(P_m - P_d)) \times 100 \tag{2}$$

where, % S is the percentage of non-volatile solids in the sample (g/g), P_d, is the weight of the dry and clean aluminum disk (g), P_m, is the weight of the sample plus the aluminum disk (g), and P_s is the weight of the dried sample plus the aluminum disk (g).

2.5. Physical-Chemical Analysis of Cape Gooseberries

For the physical-chemical tests of the fruits (pH, soluble solids, titratable acidity, and maturity index analysis), we used the Colombian technical standard (NTC) 4580 parameters [24], for 75 cape gooseberries on days 0, 3, 6, 9, and 12 according to the next methodologies:

2.5.1. pH and Total Soluble Solids (TSS)

The pH was determined with a potentiometer Thermo Fisher Scientific Orion (USA) calibrated at three points: 4, 7, and 10. Samples used consisted of five grams of fruit homogenized in 50 mL of distilled water [27]. The total soluble solids (TSS) (°Brix) were measured with a BRIXCO 0%–90% brand refractometer, previously calibrated with deionized water at a temperature of 20 ± 1 °C.

2.5.2. Titratable Acidity

The acidity was determined using a potentiometric titration [23], until neutral pH. Five grams of fruit were homogenized, with 50 mL of distilled water. The titration was carried out with 0.1 N NaOH. The results were expressed in Citric acid percentage, as shown in Equation (3):

$$\text{Citric Acid } (\%) = \left(\frac{V1 \times N}{V2} \right) \times K \times 100 \tag{3}$$

where, V_1 is the volume of NaOH consumed (mL), V_2 is the volume of sample (mL), k is the equivalent weight of citric acid (0.064 g/meq), and N is the normality of NaOH (0.1 meq/mL).

2.5.3. Maturity Index

It was calculated as the ratio of SST and titratable acidity [28] as shown in Equation (4):

$$\text{Maturity index } = \frac{\text{SST } (\%)}{\text{Acidity } (\%)} \tag{4}$$

2.5.4. Weight Loss

Weight loss was determined gravimetrically on days 3, 6, 9, and 12 [29], using the Equation (5):

$$Wl\ (\%) = \frac{(Wi - Wf)}{Wi} \times 100 \tag{5}$$

where % *Wl* is the percentage of weight loss, and *Wi* and *Wf*, are the initial and final weight of each sample (g).

2.5.5. Damage Index

It was evaluated visually according to the methodology proposed by Chicaiza Vélez [30] following the visual scale of Figure 1. The scale for decay index consisted in: (1) there is no damage (0% mold growth), (2) mild damage (10%–15% mold growth), (3) moderate damage (25%–50% mold growth), and (4) Severe damage (>50% mold growth). The results were expressed according to damage levels, using Equation (6):

$$\text{Decay index} = \frac{1n + 2n + 3n + 4n}{N} \tag{6}$$

where n = number of fruits classified in each level of the damage scale and N = number of total fruits analyzed in each treatment per day. The damage index of fruits was evaluated on days 0, 3, 6, 9, and 12.

No damage　　　　Mild damage　　　Moderate damage　　Severe damage
1　　　　　　　　　2　　　　　　　　　3　　　　　　　　　4

Figure 1. The damage scale of the cape gooseberries.

2.5.6. Color Index

The color index (CI) was calculated from CIELab system parameters (L * a * b *) determined through a digital imaging system using the professional photo editing software Adobe Photoshop® CS5 test version (Adobe System Inc. 2015 Edition) and measured from Equation (7) [31].

$$\text{CI}\ (\%) = \left(\frac{1000 \times \text{a} *}{\text{L} * \times \text{b} *} \right) \times 100 \tag{7}$$

2.6. Microbiological Activity

The microorganism count was performed by a duplicate for days 0, 3, 6, 9, and 12. Briefly, ten grams of cape gooseberries were weighed and homogenized in 90 mL of buffered peptone water for 15 min. Then, dilutions of 10^{-1} to 10^{-5} were prepared, and one milliliter of each dilution was taken for the broths. For the mold and yeast count, NTC 4132 [32] was followed using Potato-glucose agar (PDA) as media with 10% tartaric acid (*w/v*), and the plates were incubated at a temperature 25 ± 0.2 °C for five days. For the mesophilic aerobic count, the NTC 4092 [33] and ISO 4833-2 [34] standards were followed, using as a culture method Agar Plate Count (PCA); the plates were incubated at a temperature of 30 ± 1 °C for three days.

2.7. Sensorial Activity

The test was carried out based on the NTC 3932 [35]. The experiments were performed in the nutrition and dietetics laboratory of the Universidad del Atlántico, with 50 people non-trained panelists. Five groups of ten panelists were used to perform the tests independently, during days 0, 3, and 6 of storage. The groups were informed of the test methodology, additionally signed an informed consent that contained data on the reagents used in the preparation of the emulsions and the risks of exposition.

2.8. Antioxidant Activity

Two methods based on radical scavenging (DPPH• and ABTS•+) were used to evaluate the antioxidant activity changes during the storage time of the cape gooseberries.

For the preparation of the methanolic extract, two grams of cape gooseberries treated with CS + RGEO coatings were taken and mixed with 9 mL of 96% analytical methanol. Subsequently, the mixture was homogenized in an ultrasound bath (Branson 2800 ultrasound, USA) for 30 min at 4 °C and centrifuged (HERMLE Z326K Centrifuge, Germany) for 30 min at 25 °C and 6000 rpm. Finally, the supernatant was filtered until the five dilutions were obtained at concentrations of 500 µL, 600 µL, 700 µL, and 800 µL, which were settled in Eppendorf tubes with 96% methanol up to one milliliter. The antioxidant capacity obtained by the ABTS•+ method was obtained by interpolation as the equivalent Trolox amount (TEAC µmol Trolox/g extract). In the DPPH• method, we reported the EC_{50} with each treatment.

2.9. Statistical Analysis

The analysis of variance (ANOVA) and the LSD method (minimum significant difference) of multiple comparisons, with a 95% confidence level (α = 0.05), were used to assess the effect of edible coatings on the response variables described previously. The Stat graphics Centurion XVI program was used for these statistical analyzes.

3. Results and Discussion

3.1. Characterization of Emulsions

The main reasons of using chitosan of medium molecular weight with moderate deacetylation degree (DD) were that it had been proven to have excellent film-forming properties, presenting higher mechanical properties (higher tensile strength) and better barrier properties (better weight loss control, lower water vapor permeability, lower respiration rate, lesser loss of flavor volatiles, and delay of the microbial growth) than those of lower molecular weight [14,36,37].

On the other hand, it is generally accepted that chitosan antimicrobial properties vary depending on the molecular weight and deacetylation degree. Usually, chitosan with lower molecular weight has higher antimicrobial activity than chitosan with high molecular weight [38,39], supposedly due to an increased water solubility that stimulates the reaction with the active sites of the microorganisms [40].

When the antimicrobial activity depends on the polycation water-soluble structure, the increase in the deacetylation degree will improve this activity due to the electrostatic interaction between NH^+ groups of chitosan and the phosphoryl groups of the phospholipid component of the cell membrane of the microorganisms [41–43].

However, depending on the microorganism and the composition of its cells, the antimicrobial activity of chitosan can also be due to adsorption on the cell surface, which is also favored by a higher molecular weight (more hydrophobic interactions), especially for Gram-positive bacteria and fungi [44,45].

Thus, we decided to combine the use of a polymer matrix and Rue essential oil, reinforcing the antimicrobial properties, which also allows a reduction of the diffusion rate and higher amounts of the antimicrobial agent on the surface of the fruit. In addition, chitosan in several investigations has been proved to inhibit microbial spoilage in fresh and minimally processed fruits and vegetables [36].

The potential of coatings to protect the fruit depends on the properties and stability of their forming emulsions [46]. The properties of the coating will depend on the molecular structure rather than the molecular size or the composition. Good elasticity and flexibility are essential in layers to prevent cracking during handling and storage. Usually, adding plasticizers like glycerol and sorbitol to the emulsion will accomplish this requirement. On the other hand, emulsifiers like tween 80 are added to improve the homogeneity of the hydrocolloid-forming emulsions, improving wettability also suppressing the ripening process without negatively affect the fruit quality [47].

Coatings should be prepared from materials than can solubilize in water and some additives such as plasticizers, antimicrobials, antioxidants, colors, flavors, that improve the quality and the perception of the fruits can be added to the emulsions. The pH and temperature can be adjusted to facilitate the dispersion of the polymer. For that reason, the pH of the emulsions was acid in all cases (Table 1). Acid pH will support chitosan dissolution. However, it increased with the essential oil content due probably to the adsorption of the CS chains on the surface of the essential oil drops, decreasing the amount of availability of some hydrogen ions and increasing the pH of the solutions. It has been recognized that the introduction of essential oil amounts to chitosan emulsions will decrease the overall availability of CS chains and a net charge change as a consequence, which also affects the hydrodynamic volume and zeta potential of the emulsions [48,49].

Table 1. Physical-chemical properties of the chitosan (CS) + *Ruta graveolens* L. essential oil (RGEO) emulsions.

Essential Oil Content (%)	pH	Density (g/mL)	Viscosity (cP)	% Total Solids	Particle Size (μm)
0.0	4.36 ± 0.01 [a]	1.0018 ± 0.01 [a]	124.7 ± 0.1 [d]	2.96 ± 0.02 [a]	N.D.
0.5	4.40 ± 0.01 [b]	1.0080 ± 0.01 [b]	97.3 ± 0.1 [c]	3.40 ± 0.01 [b]	1.14 ± 0.25 [b]
1.0	4.40 ± 0.01 [b]	1.0082 ± 0.01 [b]	80.3 ± 0.1 [b]	3.51 ± 0.02 [b]	1.69 ± 0.32 [c]
1.5	4.44 ± 0.01 [c]	1.0088 ± 0.01 [c]	26.0 ± 0.2 [a]	3.53 ± 0.02 [b]	0.86 ± 0.12 [b]

Note: The superscripts (a–d) in the same column refer to significant differences ($p < 0.05$) between each treatment.

The results obtained from the density analysis of the different emulsions show typical values of diluted emulsions. However, the values significantly increased ($p < 0.05$) with the RGEO content, probably due to the increased concentration of essential oil in the dispersed phase of the emulsion. Similarly, the incorporation of RGEO had a significant effect ($p < 0.05$) on the apparent viscosity. The lower value was obtained in the emulsion with the highest concentration of RGEO (26.0 cP) and the higher value in the chitosan solution (124.7 cP). Emulsion viscosity reduction results from the decrease in the oil-phase agglomeration, increasing the stability of the emulsion. In addition, the lower availability of CS chains, which are adsorbed on the surface of RGEO surfaces, decreases the viscosity of the emulsions [50]. It is expected that the emulsions from essential oils have low viscosities and be economical for a wide-spread application [51].

CS + RGEO emulsions showed higher values in the percentage of soluble solids due to the retention of the oil in the polymer matrix. The oil is retained in the hydrophobic region of the chitosan; therefore, CS chains must be acting as a colloidal protector through van der Waals interactions and hydrogen bonds between hydroxides and amines of the CS and ketones present in the oil. Typically, it is considered that analyzing the particle size of the emulsions allows determining the stability of the emulsion [25]. On the other hand, a smaller particle size could influence the physical and chemical properties such as the viscosity and density of the emulsions, because a higher contact surface would be available that would improve the features [23].

3.2. Physical-Chemical Analysis of Fruits

After the analysis of the hydrocolloid-based coating emulsions, it is necessary to analyze the behavior of the coating on the fruit surface. Different studies are performed for that goal: changes in color and texture, weight loss, changes in titratable acidity, soluble solids, pH, and sensory properties.

3.2.1. pH

The pH indicates the total acidity of a solution and often increase during postharvest storage as organic acids are consumed in the respiration and metabolic processes. The pH increased significantly ($p < 0.05$) during the storage time, because of the organic acid consumption during metabolic reactions due to the activity of dehydrogenase enzymes (Table 2) [52]. The statistical analysis indicated significant differences ($p < 0.05$) between the two factors (Days/Treatments).

It can be evidenced that fruits coated with CS (T2) and with CS + *R. graveolens* L., (T3, T4, and T5) presented a significant difference ($p < 0.05$) concerning the control. In that sense, the T3 treatment produced the most significant delay in pH increasing during the twelve days of treatment, thus decreasing the ripening effect on the cape gooseberry. This delay in the pH increasing could be due to a change of respiration of the fruit generating an anaerobic environment because the coatings with essential oils affect the respiration rate [37].

3.2.2. Total Soluble Solids

The increase in total soluble solids (TSS) during the fruit ripening period is due to starch hydrolysis or sucrose synthesis [52]. Edible coatings have been reported to reduce increases in TSS content during storage as a result of suppressed respiration [53]. Between 80% and 90% of the TSS present in cape gooseberries correspond to sugars (glucose, sucrose, and fructose) [54]. The result can be observed in Table 2. However, increasing TSS also depends on water loss and must be kept in mind.

The multi-range test between the treatments shows that there was a statistically significant difference ($p < 0.05$) for the control, with all the procedures in the cape gooseberries coated with chitosan and Rue essential oil (*R.graveolens* L.). This result indicates that the use of coatings delays the increase in TSS in the fruit. T3 demonstrates the delay as it was the treatment that maintained higher acidity percentage values, but it was the treatment that obtained lower values of °Brix. Accumulation of sugars in the fruit explains the decrease in the acidity percentage.

3.2.3. Titratable Acidity

The acidity index is directly related to pH, due to the natural fruit ripening where organic acids are consumed. The decomposition of these acids is used as a respiratory substrate for the synthesis of new compounds, which causes the fruit to become less acidic over time, as shown in Table 2. The barrier properties of the coatings limit the amount of oxygen absorbed by the fruit, consequently reducing respiration and thus decreasing the consumption of organic acids [55].

The multifactorial statistical analysis showed that the factors (Days/Treatment) have an incidence concerning the response variable with a significant difference at a 95% confidence level. The acidity decreased from day 6, but it could be a result of the concentration of the organic acids in the media as a result of the water loss. The coated samples had a significantly higher amount of organic acids inside the fruit, as can be seen from the acidity measurements. The multiple test ranges between the treatments show that there was a statistically significant difference ($p < 0.05$) in all the treatments concerning the control. This result demonstrated that the incorporation of the procedures preserves the organic acids inside the fruit, delaying the ripening process, possibly due to the different atmosphere inside the fruit [55].

Table 2. Physical-chemical characteristic in *P. peruviana* with CS + RGEO coatings stored for 12 days at 18 ± 2 °C. T1 = Control; T2 = CS; T3 = CS + RGEO 0.5%; T4 = CS + RGEO 1.0%; T5 = CS + RGEO1.5%.

Time	Treatment	pH			TA (% Citric Acid)			TSS (°Brix)			Mature Index (%)			Damage Index (%)		
0	Control	3.65	±	0.07 [a]	2.12	±	0.09 [a]	13.74	±	0.29 [a]	6.50	±	0.32 [a]	1.00	±	0.00 [a]
	CS	3.61	±	0.05 [a]	2.08	±	0.07 [a]	13.57	±	0.29 [a]	6.53	±	0.16 [a]	1.00	±	0.00 [a]
	CS + 0.5%RGEO	3.64	±	0.01 [a]	2.12	±	0.01 [a]	13.58	±	0.29 [a]	6.40	±	0.10 [a]	1.00	±	0.00 [a]
	CS + 1.0%RGEO	3.67	±	0.03 [a]	2.11	±	0.09 [a]	13.74	±	0.29 [a]	6.53	±	0.17 [a]	1.00	±	0.00 [a]
	CS + 1.5%RGEO	3.67	±	0.02 [a]	2.12	±	0.03 [a]	13.58	±	0.29 [a]	6.40	±	0.07 [a]	1.00	±	0.00 [a]
3	Control	3.91	±	0.04 [c]	1.73	±	0.02 [a]	14.17	±	0.29 [a]	8.19	±	0.17 [c]	1.00	±	0.00 [a]
	CS	3.78	±	0.02 [b]	1.92	±	0.01 [b]	13.71	±	0.29 [a]	7.12	±	0.14 [b]	1.00	±	0.00 [a]
	CS + 0.5%RGEO	3.70	±	0.05 [a]	2.15	±	0.02 [d]	13.75	±	0.29 [a]	6.39	±	0.19 [a]	1.00	±	0.00 [a]
	CS + 1.0%RGEO	3.73	±	0.03 [ab]	2.06	±	0.03 [c]	14.07	±	0.29 [a]	6.84	±	0.21 [b]	1.00	±	0.00 [a]
	CS + 1.5%RGEO	3.76	±	0.04 [ab]	2.03	±	0.00 [c]	13.89	±	0.00 [a]	6.84	±	0.00 [b]	1.00	±	0.00 [a]
6	Control	3.96	±	0.04 [c]	1.69	±	0.00 [a]	14.66	±	0.29 [b]	8.67	±	0.18 [c]	1.33	±	0.58 [a]
	CS	3.76	±	0.02 [b]	1.86	±	0.03 [b]	14.20	±	0.29 [ab]	7.61	±	0.11 [b]	1.00	±	0.00 [a]
	CS + 0.5%RGEO	3.76	±	0.02 [a]	2.01	±	0.00 [d]	14.06	±	0.29 [a]	7.00	±	0.15 [a]	1.00	±	0.00 [a]
	CS + 1.0%RGEO	3.90	±	0.04 [ab]	1.92	±	0.03 [c]	14.37	±	0.00 [ab]	7.50	±	0.10 [b]	1.00	±	0.00 [a]
	CS + 1.5%RGEO	3.86	±	0.03 [b]	1.92	±	0.05 [c]	14.20	±	0.29 [ab]	7.42	±	0.12 [b]	1.00	±	0.00 [a]
9	Control	3.97	±	0.04 [c]	1.52	±	0.00 [a]	14.96	±	0.29 [ab]	9.83	±	0.20 [d]	2.00	±	1.00 [a]
	CS	3.91	±	0.03 [bc]	1.61	±	0.02 [b]	14.49	±	0.29 [ab]	8.74	±	0.27 [c]	1.33	±	0.58 [a]
	CS + 0.5%RGEO	3.83	±	0.04 [a]	1.67	±	0.01 [d]	14.19	±	0.29 [ab]	7.64	±	0.19 [a]	1.67	±	1.15 [a]
	CS + 1.0%RGEO	3.90	±	0.06 [bc]	1.56	±	0.02 [b]	14.66	±	0.29 [a]	8.67	±	0.06 [bc]	1.00	±	0.00 [a]
	CS + 1.5%RGEO	3.84	±	0.02 [ab]	1.66	±	0.03 [c]	14.67	±	0.29 [b]	8.35	±	0.05 [b]	1.33	±	0.58 [a]
12	Control	4.04	±	0.05 [b]	1.51	±	0.03 [a]	15.13	±	0.29 [ab]	9.99	±	0.33 [d]	2.33	±	1.53 [a]
	CS	4.00	±	0.01 [ab]	1.61	±	0.09 [abc]	14.98	±	0.29 [ab]	9.34	±	0.54 [bc]	2.00	±	1.73 [a]
	CS + 0.5%RGEO	3.99	±	0.01 [ab]	1.67	±	0.01 [c]	14.66	±	0.29 [ab]	8.76	±	0.20 [a]	2.00	±	1.00 [a]
	CS + 1.0%RGEO	3.96	±	0.01 [a]	1.56	±	0.00 [ab]	14.97	±	0.29 [a]	9.61	±	0.20 [cd]	1.33	±	0.58 [a]
	CS + 1.5%RGEO	3.98	±	0.01 [a]	1.66	±	0.09 [bc]	14.82	±	0.50 [b]	8.94	±	0.22 [ab]	1.67	±	1.15 [a]

Note: The superscript letter in the same column (a–d) refers to the significant differences ($p < 0.05$) between each treatment.

3.2.4. Maturity Index

For the mature index (Table 2), it is observed that in a tropical fruit such as cape gooseberry, at a maturity stage of six shows, the higher amount of carbohydrates and the lowest acidity level [28]. Statistical analysis indicates that the maturity index shows significant differences ($p < 0.05$) in the days of storage, and between the treatments. This result suggests that the fruits coated by CS (T2) and CS + RGEO (T3, T4, T5) retarded the ripening process in an RGEO content-dependent manner. The climatic respiratory pattern of cape gooseberry could be the reason, as cape gooseberries quickly unfold their reserves (organic acids) in response to the increase in their metabolism, increasing total soluble solids, and, consequently, the maturity index [56].

3.2.5. Damage Index

It is well known that fungal growth on the guavas fruit surface under ambient conditions decreased the quality of the fruits, increasing consumer rejection [23]. The knowledge in the damage caused directly on the fruits allows measuring the impact on production as a preventive measurement. The behavior during the days evaluated is shown in Table 2.

Despite the multi-range test that did not demonstrate significant ($p < 0.05$) differences between the treatments after twelve days, the microbial analysis showed a higher fungal decay for uncoated fruits as compared to CS + RGEO coated nuts. However, this might be due to the qualitative analysis of the assay and the absence of severe physical damage after twelve days. Nevertheless, Sinning et al. [57] and Grande et al. [23] previously reported that chitosan-based coatings and Rue essential oil in papayas decreased the growth of spoilage molds and improved the damage index.

3.2.6. Weight Loss Percentage

Monitoring the weight change during the storage period indicates the effectiveness against moisture of the coating. It is considered that the weight changes in the fruits depend almost exclusively on the water loss since the loss of volatile compounds from the fruits like aroma and flavor molecules and metabolic gas residues are practically undetectable in weight [58]. The results in Figure 2 show weight loss in the fruit. However, the nuts that were coated had a lower weight loss compared to the control fruit (Figure 2).
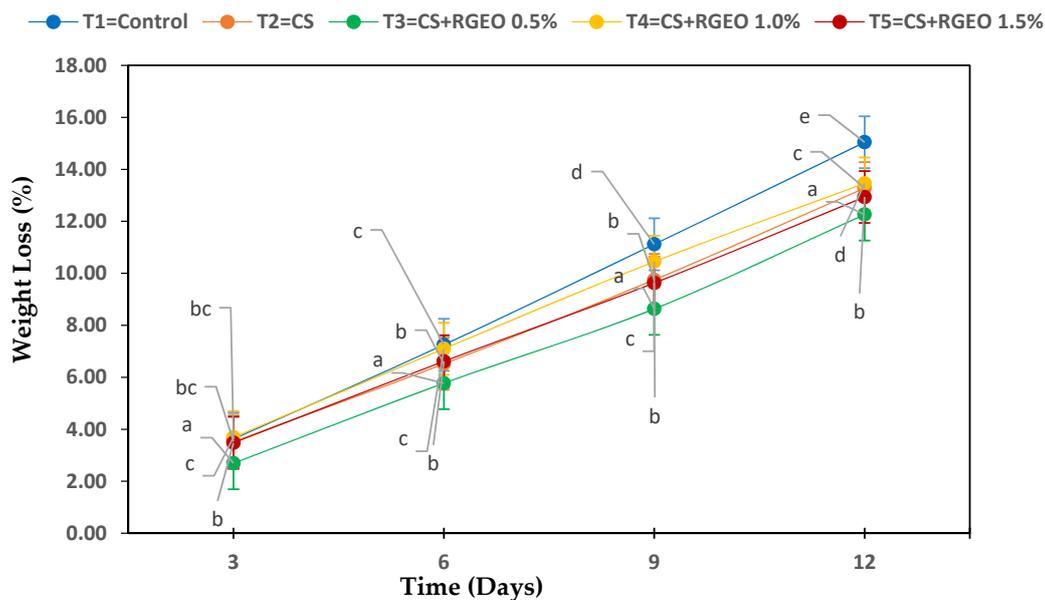

Figure 2. The behavior of the weight loss percentage during the 12 days of evaluation in cape gooseberries coated with the treatments. Note: The superscript letter (a–d) refers to the significant differences ($p < 0.05$) between each treatment.

The lower weight loss could be explained by the barrier action exerted by chitosan and RGEO against the diffusion of water vapor. When pressure steam of the external environment is lower than that of the internal fruit, it results in a decrease in weight over time, thus causing a reduction in the appearance, firmness, and nutritional quality of the fruit [59]. The multifactorial statistical analysis of type III showed that the factors Days/Treatment ($p < 0.05$), have a statistically significant effect concerning the response variable at a 95% confidence level. T3 had a considerable difference compared to the control indicating that the incorporation of RGEO has a positive effect concerning this parameter.

3.2.7. Color Index

The color of the epidermis fruit is a benchmark of the maturity index [6]. This is due to the chlorophyll degradation starting with the phytol dissociation residue and the porphyrin ring of the chlorophyll molecule. This reaction is catalyzed by an enzyme called chlorophyllase [60]. This reaction allows the color of the fruit to increase its intensity, as shown in Figure 3. We determined the color changes using a colorimeter in the CIE L * a * b * scale, where L * indicates lightness and a * and b * are the chromaticity coordinates (rectangular coordinates). On the other hand, +a * is the red direction, −a * is the green direction, +b * is the yellow direction, and −b * is the blue direction [58].
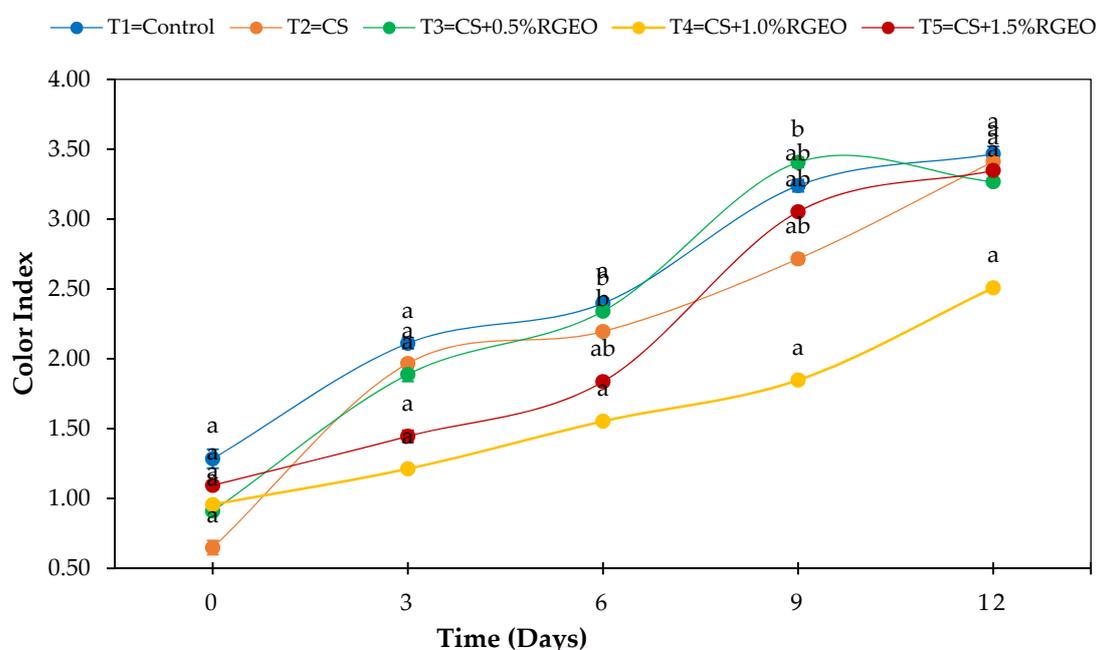

Figure 3. The color index in the fruits of cape gooseberries during the 12 days of storage covered with each treatment. The superscript letter (a–d) refers to the significant differences ($p < 0.05$) between each treatment.

Figure 3 shows an ascent of this parameter from day 0 to day 12 in all treatments and control, indicating that during the days evaluated the effect of chlorophyll degradation affects the red/green coordinates (a *) and the yellow/blue coordinates (b *) intensifying their value. These results depend on the postharvest characteristics, and the state of maturity (3–4) due to this it is expected that the color index and the evolution of the carotenoid content are high in this phase [6].

Statistical data show that there was a significant difference ($p < 0.05$) concerning days and treatments. The multiple range test showed that the most significant difference concerning the procedures was for the fruits coated with chitosan and 1.0%RGEO. An additional analysis of the coatings of CS + RGEO (0.5%, 1.0%, and 1.5%) showed that in the evaluated color scale, a lower presence of yellow and red tones was observed in comparison with the uncoated samples. Similar results were previously obtained in other researches with chitosan-essential oil coatings [23].

3.3. Microbiological Analysis

Microbial decay in cape gooseberries fruit is a result of the fungal attack of *Penicillium expansum*, *Botrytis cinerea*, *Monilinia fructicola*, and *Rhizopus stolonifer*, causing green/blue mold rot, grey mold rot, brown rot, and soft rot, respectively [10,11]. Edible coatings have been reported to be very active controlling microbial attack on the fruit surfaces, especially against fungal attack [61–63].

3.3.1. Aerobic Mesophylls

The aerobic mesophylls bacterial describe a general group, i.e., the population of bacterial colonies produced an agar-based medium under defined temperature and time of incubation, using organic nutrients for growth [64]. The effect of the application of coatings based on CS + RGEO on cape gooseberry can be studied in Figure 4, with a reduction of aerobic mesophylls on the surface of CS + RGEO coated fruits.
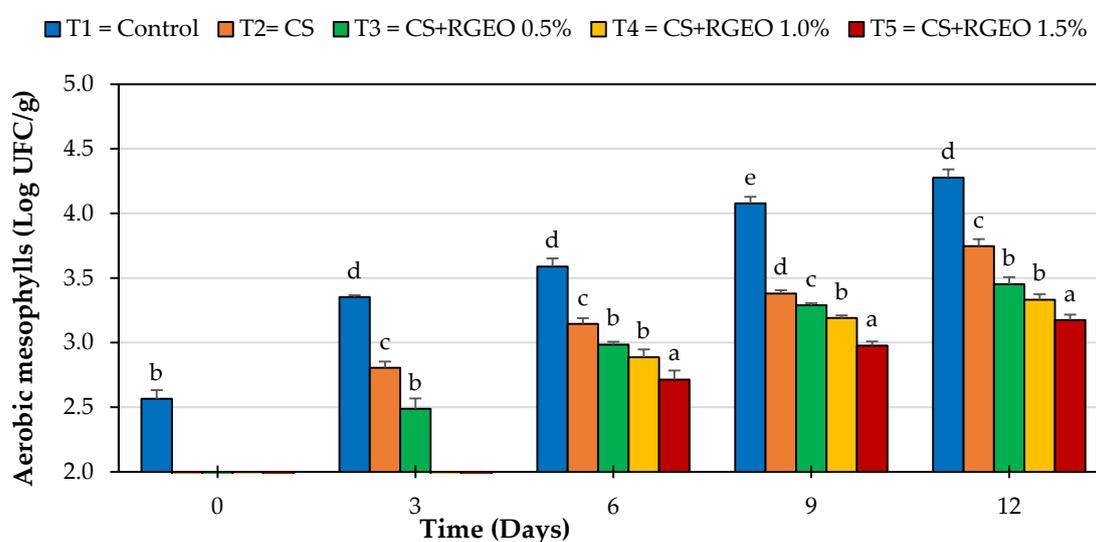

Figure 4. Aerobic mesophylls count for the treatments applied to cape gooseberry. The superscript letter (a–d) refers to the significant differences ($p < 0.05$) between each treatment.

The statistical analysis showed that there are significant differences ($p < 0.05$) in the days of storage and the treatments concerning the population growth of aerobic mesophylls at a 95% confidence level. The multi-range test between the treatments shows that there was a statistically significant difference ($p < 0.05$) for the control with all the procedures in the cape gooseberries coated with CS+RGEO. This result indicates that the antibacterial activity of chitosan and RGEO components such as ketones and sesquiterpenoids, support the decreasing of the population by almost two logarithmic units, but did not inhibit bacterial growth, indicating that the coatings only have a retarding activity for bacterial spoilage growth since after day 6, all the treatments had bacterial growth. The effect was directly proportional to the oil content, indicating a synergistic effect as compared to the T2 treatment.

3.3.2. Molds and Yeasts

Figure 5 shows the effect of the CS + RGEO coatings on the growth of fungi and yeasts on the surface of cape gooseberries during the days of storage.

Significant reduction ($p < 0.05$) in the Log CFU/g is observed in the cape gooseberries coated concerning the control. The antifungal activity of CS + RGEO delayed the growth of molds and yeasts during storage. Still, it did not inhibit the growth, because after three days, all the case gooseberries allow fungal and yeast growth (*Botrytis cinerea*, *Cladosporium sp*, and some species of *Fusarium* and *Penicillium*), probably due to the nature of these fungi [65]. After twelve days of storage, the growth for

T4 and T5 was lower than 3 log CFU/g. However, uncoated fruits had 4 Log CFU/g, which demonstrates a control effect on the growth of fungi on the case gooseberry surface.
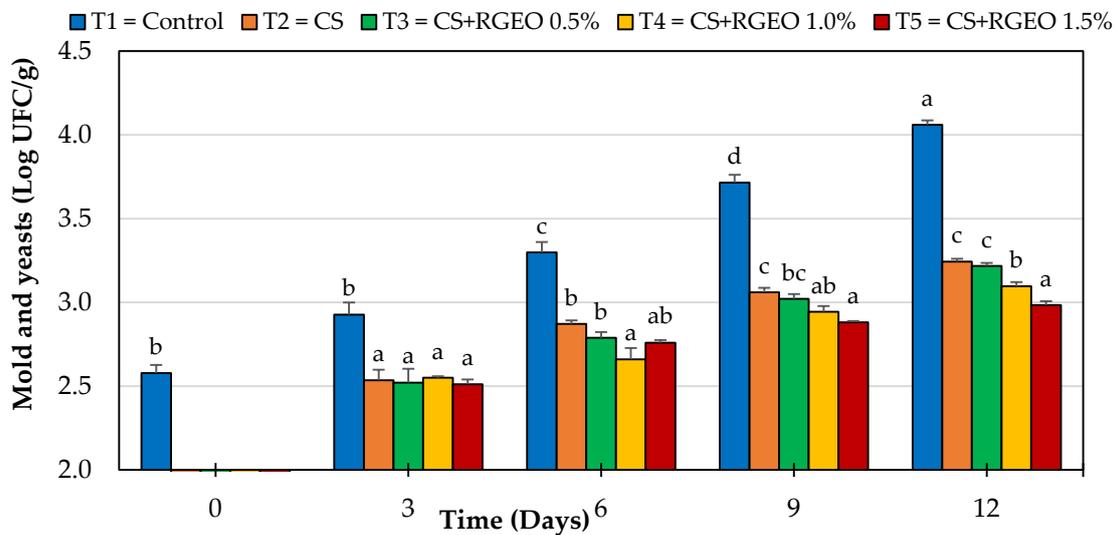

Figure 5. Mold and yeasts count for the treatments applied to cape gooseberry. The superscript letter (a–d) refers to the significant differences ($p < 0.05$) between each treatment.

Significant differences ($p < 0.05$) between days and treatments concerning the growth of molds and yeasts at a confidence level of 95% were observed.

The initial count in control cape gooseberries was 2.6 Log UFC/g up to 4.1 Log UFC/g, higher compared to cape goats treated with CS + RGEO that obtained values between 2.9 and 3.2 Log UFC/g. Previously, it has been demonstrated that chitosan-based coatings are effective in inhibiting cell growth in plums, both when plums were inoculated pre-coating as well as post-coating. Therefore, coatings may be useful in reducing microbial growth regardless of when infection occurred [66].

On the other hand, the sensory analysis allows measuring the quality of the fruit, knowing the acceptance of the products by the consumer. Sensory aspects may include appearance, color, flavor, and texture, which probably remains the most required attribute strongly affecting consumer decision to purchase the product [12]. The results of the sensory analysis represented in a hedonic curve, as seen in Figure 6.

Figure 6 shows the overall acceptability of attributes by days of storage. The treatments that showed lower effects on sensory characteristics were T1 and T2. In contrast, treatments T3, T4, and T5 were affected in the flavor and aroma attributes by a bitter and robust flavor caused by the essential oil, presenting significant differences in each treatment ($p < 0.05$). However, in the texture and flavor attributes, there were no significant differences after the storage days, which indicates that the characteristics were preserved between days 0 and 6. It can be determined that the treatments with higher acceptability in the sensory tests were T1 and T2, while low acceptability were treatments with RGEO content. Despite the enormous potential of essential oils, some authors have reported changes in the organoleptic properties of foods when these oils are used, due to their intense aroma and toxicity problems [14]. Based on the sensorial analysis, we recommend the use of T3 (CS + RGEO 0.5%) for cape gooseberries protection, which does not affect consumer acceptance drastically but prevents physical-chemical and microbiological decay of fruits.
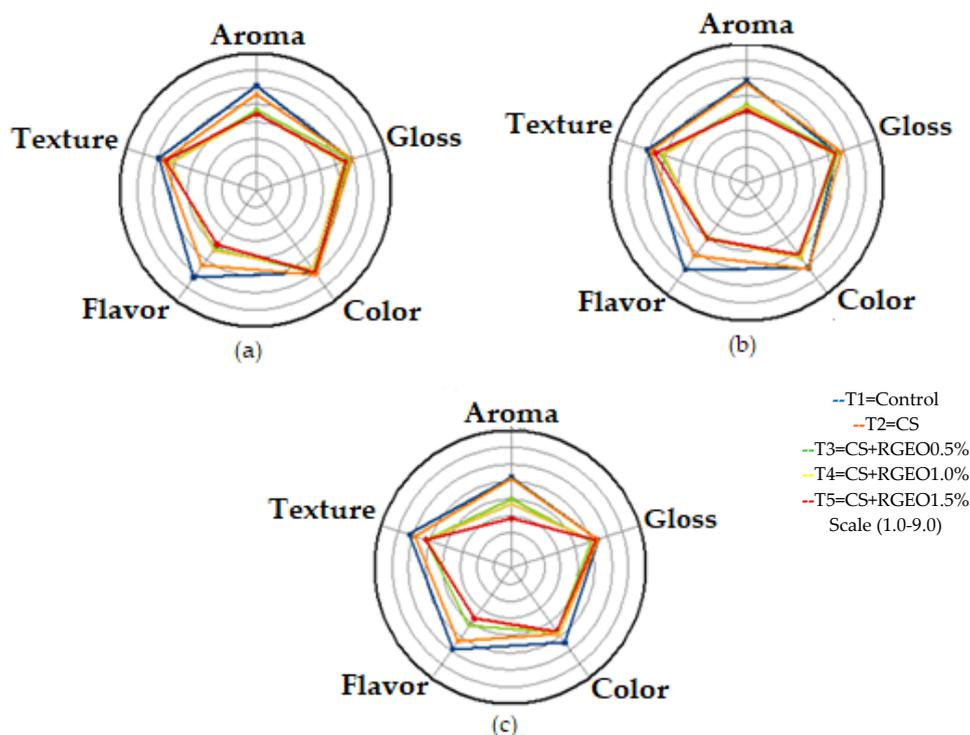
Figure 6. The hedonistic scale of the sensory analysis on days 0 (**a**), 3 (**b**), and 6 (**c**).

3.4. Antioxidant Activity

The incorporation of antioxidants is widespread in coatings for fresh-cut produce, as discoloration is often a significant challenge [62]. Chitosan-essential oil coatings have been probed to control antioxidant losses in several fruits [14,25].

3.4.1. DPPH

2,2′-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method evaluated the antioxidant potential of a compound, an extract, or another biological source, based on the spectrophotometric measurement of DPPH concentration changes resulting from the DPPH reaction with an antioxidant. The amount of remaining DPPH in the examined system is a measure of the antioxidative activity of compounds [66,67]. The results reported in Table 3 were determined based on the best inhibitory concentration, corresponding to 0.22 g of fruit/mL MeOH in each treatment.

The statistical analysis showed that there are significant differences ($p < 0.05$) in the days, but not in the treatments ($p \geq 0.05$). On day 12, T4 and T5 maintain a higher antioxidant activity (20% more) as compared to the control. It is observed that until day 6, the control samples have good antioxidant activity. However, antioxidant activity decreases with fruit attribute losses and antioxidant consumption, such as phenols and flavonoids. The exposed results are due to a post-harvest treatment in a state of maturation between 3 and 4. Cape goose after harvest is exposed to weight loss and carotenoids and secondary metabolites volatilization or degradation. During day zero, the heterogeneity of the antioxidant activity could be a result of the fruit heterogeneity. However, during the days of treatment, it is possible to see the positive preservation of the antioxidant action on the coated fruits.

Table 3. Antioxidant capacity expressed as a percentage of inhibition by the 2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid) (ABTS) and DPPH methods.

Day	Treatment	ABTS (%)			DPPH (%)		
0	Control	63.79	±	0.036 [a]	73.43	±	0.023 [ab]
	CS	64.71	±	0.051 [a]	81.21	±	0.029 [a]
	CS + 0.5%RGEO	61.43	±	0.018 [a]	64.00	±	0.113 [ab]
	CS + 1.0%RGEO	58.57	±	0.003 [a]	67.93	±	0.035 [ab]
	CS + 1.5%RGEO	62.57	±	0.040 [a]	54.79	±	0.023 [b]
3	Control	51.71	±	0.010 [a]	51.57	±	0.081 [a]
	CS	47.50	±	0.008 [a]	52.07	±	0.018 [a]
	CS + 0.5%RGEO	56.29	±	0.007 [a]	55.07	±	0.090 [a]
	CS + 1.0%RGEO	44.07	±	0.054 [a]	56.57	±	0.014 [a]
	CS + 1.5%RGEO	51.71	±	0.059 [a]	61.64	±	0.009 [a]
6	Control	50.07	±	0.015 [a]	60.86	±	0.055 [a]
	CS	52.36	±	0.025 [a]	53.86	±	0.057 [a]
	CS + 0.5%RGEO	54.86	±	0.018 [a]	50.93	±	0.076 [a]
	CS + 1.0%RGEO	51.79	±	0.011 [a]	59.07	±	0.015 [a]
	CS + 1.5%RGEO	53.21	±	0.026 [a]	55.64	±	0.080 [a]
9	Control	54.36	±	0.004 [a]	54.07	±	0.002 [bc]
	CS	47.79	±	0.052 [a]	57.07	±	0.017 [b]
	CS + 0.5%RGEO	58.00	±	0.004 [a]	62.50	±	0.001 [a]
	CS + 1.0%RGEO	49.14	±	0.023 [a]	50.43	±	0.004 [c]
	CS + 1.5%RGEO	43.79	±	0.094 [a]	55.79	±	0.012 [b]
12	Control	17.79	±	0.002 [d]	23.86	±	0.003 [d]
	CS	46.36	±	0.008 [a]	51.79	±	0.019 [b]
	CS + 0.5%RGEO	34.64	±	0.008 [c]	57.71	±	0.007 [a]
	CS + 1.0%RGEO	41.71	±	0.010 [b]	49.29	±	0.010 [b]
	CS + 1.5%RGEO	44.86	±	0.021 [ab]	43.36	±	0.001 [c]

Note: the superscript letter in the same column (a–d) refers to the significant differences ($p < 0.05$) between each treatment.

3.4.2. ABTS

The methods that we used here (DPPH and ABTS) to evaluate the antioxidant activity are typically used in the antioxidant evaluation of different foods. Still, it cannot be forgotten that it is more related to radical scavenging. In many cases, this activity does not correspond to the antioxidant activity since different molecules based on their antioxidant activity interfering in the lipid's oxidation or enzymatic browning. ABTS method is based on the reaction between 2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid) (ABTS) with a peroxidase (such as metmyoglobin and H_2O_2), forming a blue-stable radical cation, ABTS+, that absorb at 600 nm and an antioxidant molecule present in the fluid will suppress this color absorption in a

concentration-dependent manner [68]. However, we evaluated the capacity of the coatings to decrease the antioxidant capacity loss by two radical scavenging assays which not necessary correlate, since the fruits have different antioxidant molecules such as enzymatic and non-enzymatic antioxidant activity in such a way that several systems (radical scavenger and lipid oxidation inhibitors) should be addressed. Even though both methodologies are fast, easy to reproduce, and are highly used to assess the antioxidant activity of different fruits. The results indicate that the highest antioxidant capacity obtained by the ABTS method as Trolox equivalent (TEAC μmol Trolox/g extract) was 7.07 μmol Trolox/g extract relative to the treatment with 0.5% of RGEO (T3). ABTS and DPPH methods could be confirmed as an excellent antioxidant activity. ABTS results demonstrated an antioxidant activity of 7.07 μmol Trolox/g extract and from the DPPH method an EC_{50} of 223 g/L. Previous studies have reported an antioxidant capacity for these methods of 717.49 μmol Trolox/g for DPPH and 3.63 μmol Trolox/g in ABTS [69].

Additionally, it was determined that T2 and T5 antioxidant capacity from day one to day twelve only decreases from 65% to 46% and 55% to 44%, respectively, while the uncoated fruits (T1) the antioxidant capacity reduced from 65% to 18%. The above demonstrates a positive effect on the antioxidant activity preservation by the CS + RGEO treatments. Statistical data show that there is a significant difference ($p < 0.05$) in the procedures.

4. Conclusions

The incorporation of *Ruta graveolens* L. essential oil in the chitosan edible coatings allowed to obtain stable emulsions of low viscosity, small particle size, and secure adhesion on the gooseberry surfaces. The application of the treatments in the fruits improved the physical-chemical stability, preventing the consumption of organic acids as reflected by the results in the titratable acidity, pH, maturity index, and weight loss studies.

Microbiological analysis showed a decrease in the population of microorganisms, due to the antimicrobial and antifungal characteristics of the components. CS + RGEO 1.5% (T5) presented the highest reduction in the colony-forming units concerning the control.

Despite the low acceptance in the sensory analysis of T4 and T5, we recommend T3 as a suitable treatment for cape gooseberry fruits from the physical-chemical preservation results as well as the reasonable microbial spoilage control.

The CS + RGEO coating applications also preserve the antioxidant property of case gooseberries after twelve days of treatment under storage according to the DPPH and ABTS results. Several studies are currently conducted to encapsulate the RGEO for edible coating applications to decrease the aroma and flavor on the coatings and improve consumer acceptance. All the results in the present study demonstrated the positive effect of the implementation of CS + RGEO edible coatings on the physical-chemical, antioxidant, and microbial decay of *Physalis peruviana*. They could be promising candidate treatment for the food industry.

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