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Multilayered Edible Coatings to Enhance Some Quality Attributes of Ready-to-Eat Cherimoya (*Annona cherimola*)

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Abstract: Multilayer coating can be applied on fresh fruit to protect and enhance its shelf life. This study evaluated the application of a multilayer protein and chitosan coating on fresh cherimoya. To determinate the effect of the multilayer coating on the shelf life on the fruit, total phenolic content, pH, °Brix, weight loss, and hardness values were tested. The ripening process is associated with an increase of soluble solids, and results showed that the presence of the multilayered coating maintains the total phenolic content, pH, and °Brix values over time while reducing the water loss. This effect is probably due to the presence of the coating that creates a barrier on the food surface that reduces the respiration rate and affects the ripening process, demonstrating the method's feasibility to be used to enhance the shelf life of fresh-cut cherimoya.

Keywords: edible coating; cherimoya; multicoating

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1. Introduction

Cherimoya (*Annona cherimola* Mill.) is a native fruit of South America that has gained relevance in recent years worldwide. It is characterized as a pulpy and very aromatic fruit, which makes it highly appreciated in international markets. The *Annonaceae* family features more than 100 genera and more than 2000 species [1–3], and it has been cultivated since 3400 B.C.

During recent years, cherimoya production has increased all over the world, and it is mainly consumed fresh. In addition, it has been used in traditional medicine to treat dysentery, inflammation, and rheumatism and as anticonvulsant [4–7]; several authors have studied the properties of different species of Annona and its application in medicine and food and as an alternative crop [8–10].

Cherimoya needs temperate climatic conditions and it is cultivated mainly in Spain, the United States, Mexico, Guatemala, and Peru [10]. It is characterized by having high contents of ascorbic acid, potassium, and minerals; when the cherimoya fruits are maturating, the pulp loses its firmness due to the enzyme action, mainly cellulases and polygalacturonases [2,10–12]. After harvest, fruits and vegetables continue the respiration process and thus the maturing process [13].

The consumption of ready-to-eat foods has increased in the last years due to the current lifestyles. Ready-to-eat cherimoya can be found in supermarkets, but the fruits have a low shelf life. An alternative that has been developed to solve this problem is the application of edible coatings based on natural polymers [14–16]. Edible coatings are applied as a monolayer and can be made from proteins [17–19], carbohydrates [20,21], oils, or a mixture of them [22,23]. It can be applied by immersion, spraying, or brushing and is considered fit for use to extend fresh fruit shelf-life without causing anaerobiosis and reducing decay without affecting their quality. The edible coating must have special requirements such as gas and moisture barrier, color, mechanical properties, and being non-toxic, among other characteristics [13].

Edible coatings have demonstrated their ability to protect foods [19,24–26], but in recent years, the application of multilayered coatings have demonstrated the improvement of fresh fruit shelf life. In this technique, oppositely charged solutions are used to dip the fruit, creating a layer-by-layer coating that enhances fruit properties [27–30].

Multilayer coatings have demonstrated their ability to improve or extend the performance of a monolayer material [31] and can improve their mechanical properties [32]. Moreover, taking in consideration the physicochemical characteristics of each layer, the adherence can be improve and thus the product shelf life as well [33]. Unfortunately, there are few studies about the improvement of shelf life in cherimoya. For example, Liu et al. [30] reported the use of a chitosan coating, which preserved some properties such as fruit firmness and membrane integrity of the fruit for a longer time compared with the control sample. Similar results were reported by Arnon [34] by applying polysaccharide-based edible multicoating on citrus fruit, improving the product shelf life. In addition, Martiñon [35] applied a multilayer coating based on chitosan and/or pectin on fresh cantaloupe, achieving an improvement in the characteristics of the fruit and in its shelf life. Moreover, Treviño-Garza [36] developed a layer-by-layer edible coating based on mucilages and chitosan and demonstrated their ability to maintain the quality and preservation of fresh pineapple.

The objective of this study was to evaluate the shelf-life-enhancing ability of fresh-cut, ready-to-eat cherimoya using multilayer, edible coatings based on proteins and carbohydrates.

2. Materials and Methods

Reagents and cherimoya fruits:

Commercial whey protein isolate (WPI) was obtained by Nature's Best (Hauppauge, NY, USA). Chitosan reagent (≥90% deacetylation; medium molecular weight) was obtained from Chemsavers (Bluefield, VA, USA); sorbitol and all other reagents were purchased from Sigma-Aldrich (Steinheim, Germany). Fresh cherimoyas were purchased from a local market. Fruits were prepared as described by Rossi-Márquez et al. [14]. Briefly, cherimoya samples were washed with distilled water and peeled. After, they were cut into 2 cm squares pieces. All samples were washed using a chlorinated solution (0.01 g/100 mL) for 5 min and then left to dry out for 5 min. Control and coated samples were chosen randomly.

Film forming solutions preparation:

Two different coating solutions were prepared. Solution A was prepared by dissolving 5 g of whey protein isolate (WPI) and 7 g of sorbitol in 100 mL of distilled water, and after, the pH was adjusted to pH 7.0. The solution was heated to 90 °C for 25 min to favor the protein denaturation. Then, the solution was allowed to cool at room temperature until use. Solution B was prepared to obtain 0.8 % *w/v* chitosan solution (CH) using 0.1 N HCl.

Coating application:

Previous studies have demonstrated the ability of whey protein films and/or chitosan to enhance the shelf life of fresh-cut fruits or vegetables [37–39]. Rossi et al. [14] showed that the presence of a whey-protein-based coating reduced the water loss of different vegetables and apples, improving the shelf life of the product. Moreover, due to the different charges of the molecules, it was decided to apply the protein solution first and the chitosan solution in a second stage.

To evaluate the effect of coating application, the fruit pieces previously prepared were divided in four groups and subjected to the follow treatments. Single-layered coating (SC) was obtained by dipping the fruit pieces into solution A (SC-A) or solution B (SC-B) for 5 min, draining, for 10 min and then dipping again in the same solution for 5 min and finally draining again for 10 min. For multilayered coating samples (MC) fruit pieces were immersed into solution A for 5 min and then drained for 10 min. After, they were immersed into solution B for 5 min and then drained for 10 min. For uncoated samples (UC), samples were immersed in distilled water with the same procedure used for SC samples. Samples were stored at 5 °C for either two, seven, or ten days in sealed, low-density polyethylene bags (5 pieces/bag) before they were analyzed (1 bag by each treatment).

Quality parameters:

Hardness:

Hardness was evaluated as described by Rossi-Marquez [14]. The methodology includes the use of an Instron universal testing machine model no. 5543A (Instron Engineering Corp., Norwood, MA, USA) rigged with a 2 kN load cell for compression mode with a cylindrical probe (55 mm in diameter). Samples were compressed to 50% of deformation. Analyses were performed with at least five samples per treatment.

Weight loss and pH:

The pH was determined using the AOAC method 981.12 [40] using a digital pH-meter (LAQUA pH 1100, Horiba Scientific, Kyoto, Japan). For weight loss, samples were weighted using a digital balance, and then, calculation was performed using the following equation:

$$Weight\ loss\ (\%) = \frac{initial\ weight - final\ weight}{initial\ weight} \times 100 \quad (1)$$

Total phenolic content:

Uncoated and coated cherimoya samples were analyzed by their phenolic contents using a modification of the Folin–Ciocalteu method [41] described by Rossi-Márquez et al. [14]. First, 10 g of each sample was homogenized in 80 mL of water, then centrifuged at $200\times g$ and 4 °C for 10 min. After, 0.5 mL of supernatant was mixed with 2.5 mL of Folin–Ciocalteu's reagent (100 g/L), and subsequently, 2 mL of sodium bicarbonate solution (75 g/L) was added. Samples were incubated for 1 h at 30 °C and after again for an additional 1 h at 4 °C. Finally, absorbance was measured at 760 nm. Total phenolic content is expressed as g of gallic acid equivalents (GAE)/kg of each sample (fresh weight).

Total Soluble Solids (°Brix) and Protein Content:

Total soluble solids (TSS) expressed in °Brix was determined to 25 °C by using the RHB-32ATC refractometer (Yhequipment CO., LIMITED, Shenzhen City, China). Protein content was measured using Kjedahl's method ASTM E258-07.

Statistical Analysis:

Results were analyzed using the JMP 8.0 software (SAS Institute, Cary, NC, USA), applying the Tukey–Kramer test ($p < 0.05$) to determine the differences.

3. Results and Discussion

3.1. Hardness (N)

A hardness test can indicate that the senescence process has occurred, and it is related to the fruit freshness as perceived by the consumer [42]. As shown in Figure 1, for the uncoated samples, there was a reduction on the hardness value over time, and this can be attributed to an enzymatic degradation of cell walls [13]. The coatings slightly increase the hardness of the fruit due to the thin layer generated on the fruit surface and help retain its texture for up to 2 days of storage. After 7 days of storage, a linear decrease in hardness was observed even in the coated fruits, which, in any case, remained harder than the uncoated fruits. This result can be attributed to the low-O_2 atmosphere created on the surface because of the presence of a barrier that can inhibit the activity of the enzymes associated in the cell wall degradation mechanism and solubilization of pectins [43]. Nevertheless, at 7 and 10 days of storage, the multilayer coating achieved a hardness of the fruit comparable with fresh fruits. These results may be due to the increased deposition of the acidic chitosan solution on the negative surface of the protein coating that improved the barrier properties, creating a modified atmosphere with high CO_2 and low O_2, which reduced the respiration rate, as shown previously in mangoes [44] and papaya [45]. Moreover, the antibacterial properties of the chitosan coating could have a role in the improvement of shelf life [46], as our results indicated that the of the multilayer coating could promote the shelf life of fresh cut fruits using natural ingredients.

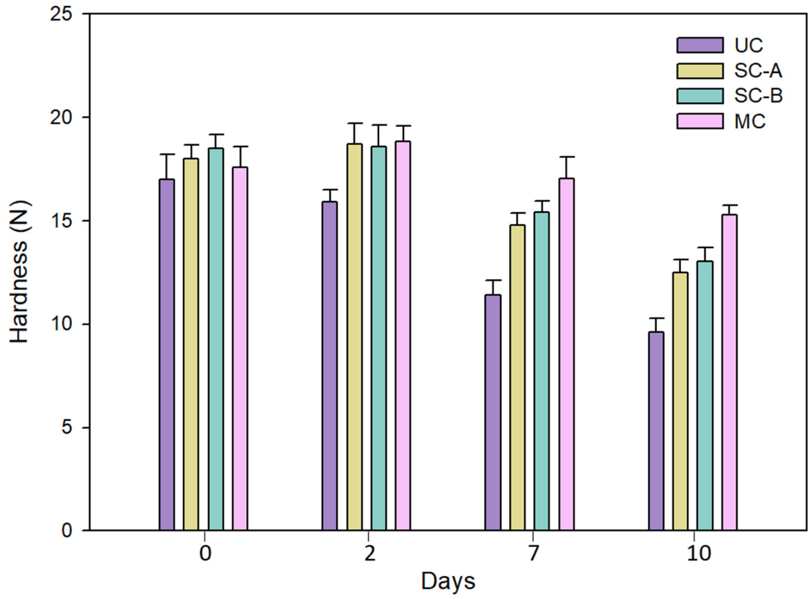

Figure 1. Hardness values for uncoated sample and for single-layer and multilayer coating treatment. N = 3; data shown are mean ± standard deviation.

3.2. Weight Loss and pH

As expected, there was a weight loss over time in all samples. Once the vegetable or fruit has been harvested, it loses weight due to the respiration process and begins a process of deterioration. As shown in Figure 2, the presence of a single coating reduced the weight loss of cherimoya samples, but the presence of multilayered coating significatively decreased the weight loss. This effect can be attributed to the creation of a wall on the cherimoya exterior that can reduce the respiration rate of the fruit. Yonemoto et al. [47] applied a wax coating on cherimoya fruit, reducing the weight loss, achieving a better fruit appearance, and reducing fruit spoilage.

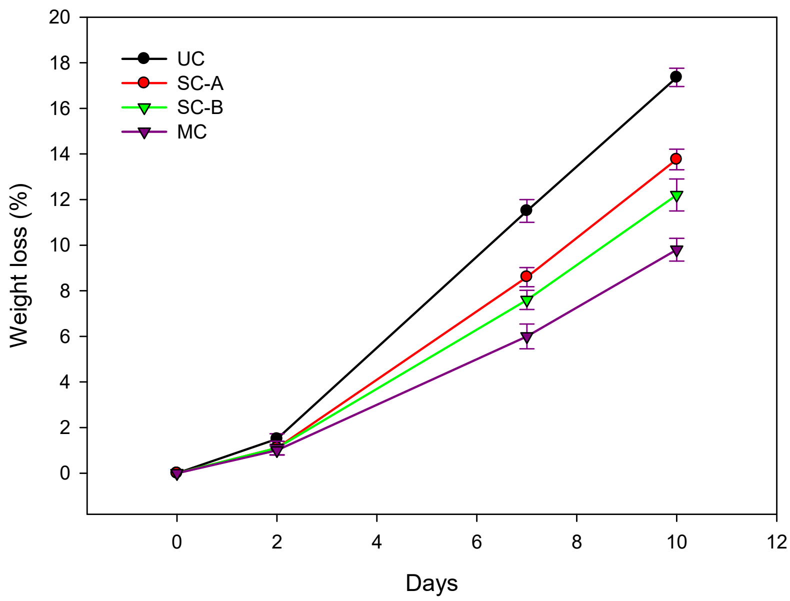

Figure 2. Weight loss of sliced cherimoya with different treatments: uncoated samples (UC), single coating A (SC-A), single coating B (SC-B), and multilayered coating (MC). N = 3; data shown are mean ± standard deviation.

The presence of a multilayered coating reduced the variability of the pH value and kept it constant over time (Figure 3). Several authors reported a pH decrease during cherimoya ripening [48,49] although, in this study, the pH did not undergo a significant change during the 10 days that the experiment lasted.

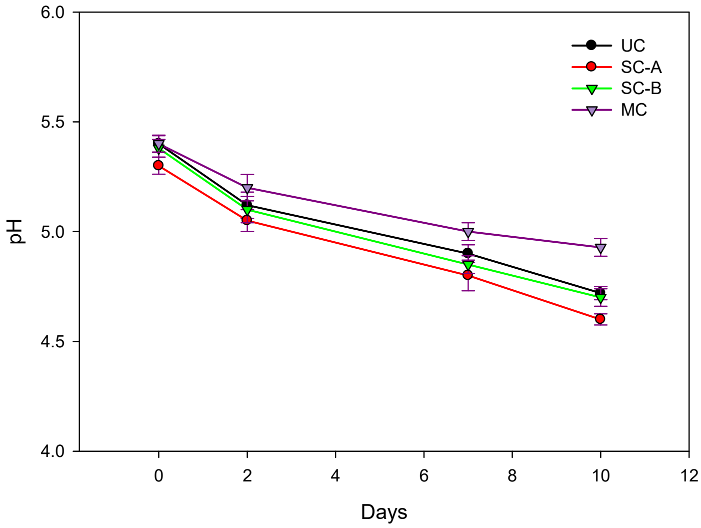

Figure 3. pH values of sliced cherimoya with different treatments: uncoated samples (UC), single coating A (SC-A), single coating B (SC-B), and multilayered coating (MC). N = 3; data shown are mean ± standard deviation.

3.3. Total Phenolic Content

Edible coatings can act as a barrier to prevent the gas transfer and protect the fruits against the oxidation process. Phenolics have a chemoprotective effect and can positively affect human health [50–52]. The presence of single-layer or multi-layer coating prevents phenolic loss.

Moreover, the multilayer coating on samples keeps the total phenolic values constant over time (Figure 4). These results are in agreement with different authors who demonstrated the ability of edible coatings to reduce the loss of phenolic content in fresh fruit [14,53,54].

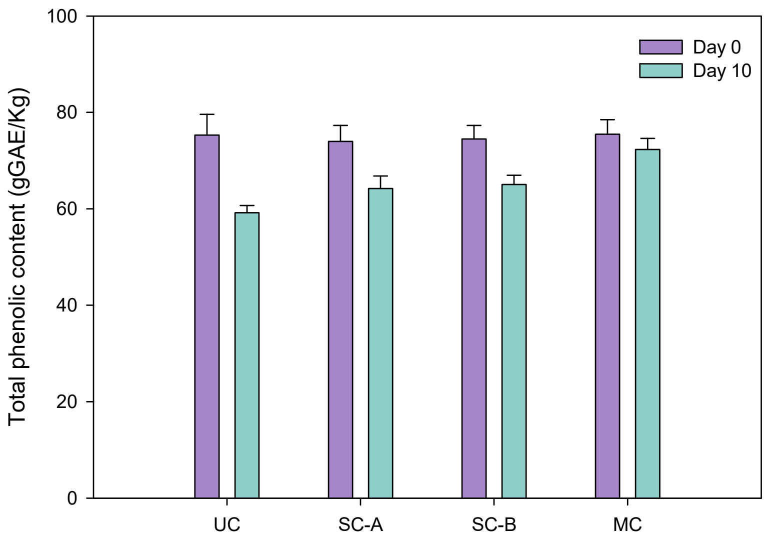

Figure 4. Coatings' effect on cherimoya phenolic content over time. Total phenolic content was detected in uncoated, single-layer, and multilayer coating on cherimoya at 0 and 10 days of their storage. N = 3; data shown are mean ± standard deviation.

3.4. Brix Percentage and Protein Content

The ripening process can be associated with an increase of soluble solids in cherimoya [55]. As was shown in Table 1, uncoated samples increased their °Brix over time, an effect that can be attributed to increases of glucose and fructose content, while the presence of the multilayer coating reduced the value and kept it at a proper level until the tenth day. These results are similar to those reported by Palma [56], who mentioned that ripened cherimoya fruits should have 18–24 °Brix values among varieties.

Table 1. Total soluble solid and protein content in uncoated and differently coated cherimoya samples during 10 days of preservation.

Samples	**Days**							
	0		**2**		**7**		**10**	
	Prot.	**Brix**	**Prot.**	**Brix**	**Prot.**	**Brix**	**Prot.**	**Brix**
UC	1.42 ± 0.21	23.2 ± 1.2	1.03 ± 0.13	25.3 ± 0.9	0.77 ± 0.1	28.5 ± 1.2	0.54 ± 0.06	30.9 ± 1.2
SC-A	1.44 ± 0.11	22.7 ± 1.4	1.35 ± 0.32	23.8 ± 1.2	0.85 ± 0.2	26.2± 1.0	0.73 ± 0.05	28.7 ± 0.9
SC-B	1.38 ± 0.11	23.6 ± 1.1	1.25 ± 0.12	24.5 ± 1.2	0.85 ± 0.2	25.2 ± 1.2	0.81 ± 0.2	27.4 ± 1.2
MC	1.42 ± 0.23	23.1 ± 1.5	1.32 ± 0.38	24.1 ± 1.3	1.16 ± 0.09	25.1 ± 1.5	0.94 ± 0.04	26.23± 1.2

Values are mean ± standard deviation (N = 5).

For protein contents, similar results were observed (Table 1), where the presence of the multilayer coating reduced the protein content loss over time. This may be due to a barrier effect of the coating, which can reduce the gas and liquid migrations, preventing the loss of the proteins. These results are comparable to those reported by Hernández-Fuentes et al. [10], who studied the bioactive components of cherimoya grown in Mexico.

In line with the results of this study, the use of multilayered coating made by using whey proteins and chitosan showed a positive effect during the storage days on the color retention and the visual appearance of MC-coated fruit pieces with respect to the other samples (Figure 5).

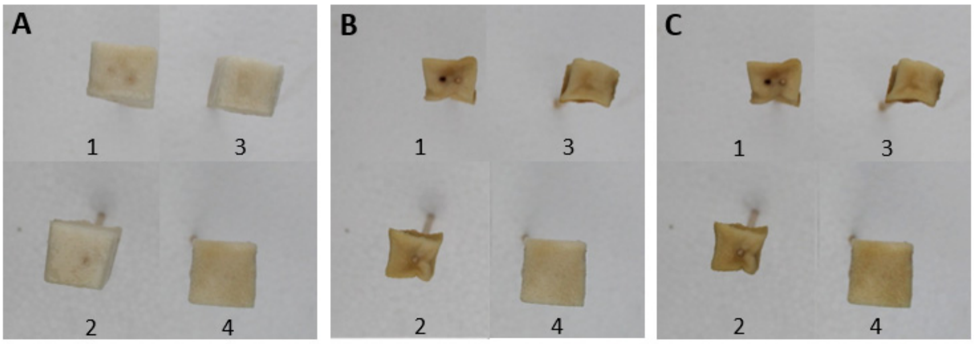

Figure 5. The visual appearance of cherimoya pieces after coating treatments during storage at 0 (panel **A**), 7 (panel **B**), and 10 days (panel **C**). 1, UC; 2, SC-A; 3, SC-B; 4, MC.

4. Conclusions

The progress of ripening is generally determined by several physicochemical changes. For cherimoya (*Annona cherimola* Mill.) fruit, ethylene production is a late event, where ripening is associated with a decrease in pulp firmness and a large increase in both soluble solids contents and titratable acidity [55]. Moreover, fresh-cut fruit continues to lose moisture due to transpiration after the skin is removed; therefore, the weight loss continues to increase during the storage, and the fruit turgidity decreases. Edible coatings are able to create a barrier on the fruit surface, creating a natural and transparent skin able to inhibit the respiration rate affecting the enzymatic degradation of the cell wall and to decrease the water loss, preserving the turgidity of fruit structure. These characteristics can be improved by the application of a multilayered coating that can be prepared using natural components such as proteins, carbohydrates, or a mix of them. The presence of the multilayer coating is able to counteract the increase of acidity and soluble solids and protein degradation, slowing down maturation process. In addition, preserving the total phenolic content of cherimoya fruit can be a useful tool to maintain their nutraceutical properties.

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