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# Coatings Based on Gelatin and Chitosan in the Conservation of Papaya (*Carica papaya* L.) Minimally Processed

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**Abstract:** Minimally processed fruits undergo changes that require careful monitoring. This study aimed to assess the effectiveness of edible coatings based on chitosan and gelatin using various application techniques for minimally processed papayas. The treatments included: control (distilled water), 1% chitosan, 4% gelatin, 1% chitosan + 4% gelatin (blend), and 1% chitosan with a 4% gelatin undercoat (layer-by-layer). The coatings' infrared spectroscopies (FTIR) were analyzed, and the fruits' fresh mass, firmness, pulp color (L*, a*, b*, and hue angle), pH, titratable acidity, soluble solids, ascorbic acid, phenolic compounds, lycopene, β-carotene, total sugars, and catalase activity were measured. The results revealed that gelatin and the layer-by-layer treatment positively influenced the preservation of minimally processed papaya. These coatings effectively reduced fresh mass loss while maintaining firmness and the characteristic orange color of the mesocarp. Furthermore, the treated samples consistently exhibited low soluble solids content during the storage period, with minimal variations in acidity, thereby influencing the maturation process. Gelatin coatings demonstrated low polyphenol content, while the layer-by-layer treatment showed no significant changes in vitamin C levels. Lycopene and β-carotene levels remained stable throughout the storage period, with a slight increase observed in total sugars. Consequently, the application of gelatin polymers and the undercoat treatment (LBL) represents a viable alternative for extending the shelf life of minimally processed papayas for at least eight days.

**Keywords:** blend; layer-by-layer; edible toppings; minimally processed; postharvest



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## 1. Introduction

Papaya (*Carica papaya* L.), a fruit belonging to the Caricaceae family, originates from tropical regions and is highly valued worldwide [1]. Brazil, being the second largest papaya producer globally [2], plays a crucial role in its cultivation. The major papaya producing states in Brazil are Espírito Santo, Bahia, and Ceará [3].

Papaya's nutritional composition, rich in vitamins A, C, and antioxidants, coupled with its low levels of sodium, fat, and calories [4,5], make it a highly desirable fruit for consumption. Moreover, its sensory appeal further contributes to its popularity among consumers.

In Brazil, papaya is commonly consumed fresh, as well as in the form of candies, juices, vitamins, and minimally processed products, which have gained significant acceptance [6]. The demand for minimally processed vegetables has risen in recent years due to their

convenience. Minimally processed vegetable sales began in Brazil in the 1990s, and the sector has since grown and established a strong presence in the domestic market [7]. The appeal lies in their ease of preparation, reduced storage space, smaller portion sizes, and minimized waste [8].

Minimally processed vegetables undergo a series of stages, including selection, classification, and washing, peeling, cutting, slicing, sanitizing, rinsing, centrifuging, and packaging, in order to maintain the freshness of the product [9] and add value. However, these processing steps subject the vegetables to mechanical stress, which can lead to water loss, increased respiration rate, enzymatic browning, and changes in flavor and aroma [10]. Although minimally processed papaya is well received by consumers, its shelf life is limited to approximately two working days [6], necessitating the use of technologies to maintain its quality.

In this regard, edible coatings offer potential solutions for preserving minimally processed fruits. Edible coatings employ biodegradable materials derived from polysaccharides, proteins, and lipids [11]. They can be described as thin materials directly applied to the surface of fruit and vegetables [12].

Among the materials suitable for edible and/or biodegradable coatings, gelatin and chitosan are frequently utilized. Gelatin, derived from collagen through partial hydrolysis, possesses excellent film-forming properties and acts as an effective external barrier, effectively preventing changes in the surrounding environment [13]. Chitosan, on the other hand, is derived from chitin through deacetylation and demonstrates great potential in food coating production [14]. It can also serve as a controlled-release carrier for drugs and additives, with various applications such as microspheres, flakes, nanoparticles, fibers, and films [15]. Moreover, chitosan is non-toxic, biodegradable, cost-effective, and exhibits antimicrobial activity [16].

The application of coatings through techniques that allow adjustment of coating properties according to specific process requirements has gained significant research attention [17]. Notably, the layer-by-layer technique and the utilization of blends have shown promising results, as they enable the use of multiple biopolymers. The layer-by-layer technique involves the deposition of alternating layers of materials with opposite charges, facilitating adhesion of the coating to the fruit surface [18]. This technique can be applied using various methods such as immersion, spray, electrodeposition, magnetic assembly, electrocoupling, filtration, microfluids, fluidized beds, centrifugation, and immobilization [19].

Blends, incorporating a combination of two or more polymers [20], offer the advantage of obtaining materials that possess unique properties not found in individual components [21]. These materials are tailored to enhance tensile strength, impact resistance, and solubility reduction [22], resulting in coatings with superior adhesion characteristics to the fruit surface.

Thus, the present study aims to evaluate the effectiveness of gelatin and chitosan-based coatings on the preservation of minimally processed papaya using various techniques.

## 2. Materials and Methods

### *2.1. Plant Material*

The papaya fruits were obtained from a rural property located in the municipality of Mossoró-RN, located in the mesoregion of the west of Potiguar, at 5°11′17″ south latitude and longitude 37°20′39″ west. Papayas of the 'Papaya' variety were harvested at the point of commercial maturation. The fruits were transported in plastic boxes to the Postharvest Physiology Laboratory of the Center for Human, Social, and Agrarian Sciences (CCHSA) of the Federal University of Paraíba (UFPB), Bananeiras campus.

Fruits were initially selected by size, color, and absence of mechanical damage and were washed with running water to remove possible dirt. Minimal processing was carried out at a temperature of approximately 10 °C. The utensils used were (trays, cutting blades, stainless steel table, and benches). The papayas were cut longitudinally and the seeds

removed with the aid of a spoon, and were immersed in an active chlorine solution at 200 ppm for 10 min, and then allowed to dry naturally, as shown in the steps of minimum papaya processing.

A chitosan solution (Polymar, Reference: 9012-76-4) with a degree of deacetylation of 82.89% was prepared at a concentration of 1.0% (*w*/*v*), in distilled water and in an acetic acid solution 1, 0% (*w*/*v*), with the aid of a heating plate (Tecnal, model TE 0181) at a temperature of 45 °C for 2 h until complete dissolution, as described by Yoshida [22]. The final pH of the solution was 3.92.

A colorless and flavorless gelatin solution (Dr. Oetker) was prepared by dissolving in distilled water at a concentration of 4% (*w*/*v*). The solution was homogenized on a hot plate (Tecnal, model TE 0181) at a temperature of 60 °C for 30 min. The final pH of the solution was 5.57. After dissolution, both solutions were added to glycerol at a concentration of 1% (*w*/*v*).

The papayas were immersed in five types of coatings (Figure 1): (1) control (distilled water) for about 30 s and left to dry naturally, (2) chitosan (1%, *p*/*v*) for about 30 s and left to dry naturally, (3) gelatin (4%, *p*/*v*) for about 30 s and left to dry naturally, (4) blend (chitosan 1%, *w*/*v*+ gelatin 4%, *w*/*v*) for about 30 s and left to dry naturally, and (5) layer-by-layer (chitosan 1%, *w*/*v* under gelatin 4%, *w*/*v*) the fruits were immersed for 30 s, in the first layer composed of 1% chitosan, and after natural drying, they were immersed in a 4% gelatin solution for 30 s and left to dry naturally. Then, the minimally processed papayas were packaged in polystyrene trays and wrapped with polyvinyl chloride (PVC, 12 µm), and stored at a temperature of 5.0 ± 1°C and RH of 80 ± 5% for 10 days.

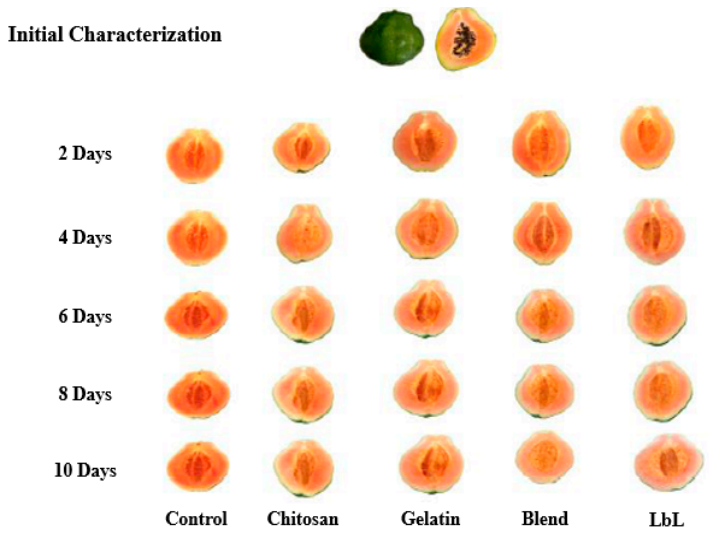


**Figure 1.** Effects of chitosan and gelatin coatings on freshly processed papayas in control treatments and coated with chitosan, gelatin, blend, and layer-by-layer storage for 10 days at 5 °C.

The experimental design used was completely randomized, in a 5 × 6 factorial scheme (treatments: control; 1.0% chitosan; 4% gelatin; 1.0% chitosan + 4% gelatin and 1.0% chitosan under gelatin 4%) and six storage periods (0, 2, 4, 6, 8, and 10 days). The experimental unit consisted of one fruit and each treatment had 4 repetitions, totaling 104 papayas. The results were submitted to analysis of variance using AgroEstat software, version 1.1 [23]. When significant differences were found, a regression analysis was performed. Means were compared by Tukey's test ($p \leq 0.05$).

### *2.2. Physical Analyses*

Physical analyses were carried out, such as FTIR, fresh mass, firmness, and color, and are described below. Fourier transform infrared spectroscopy (FTIR) of the coatings was recorded between 400 and 4000 $cm^{-1}$ with 8 average scans and 8 $cm^{-1}$ resolution (Cary 600 series FTIT Spectrometer). The coatings on the surface of the fruits were evaluated by

image recording with the aid of an epifluorescence microscope (Axion Imager A2) using ZEN software. Fresh mass loss: mass loss was obtained by the difference between the initial mass and the final mass at each storage time, with the aid of an electronic scale of 50–6000 g ± 2 g (UX8200S, SHIMADZU / precision Marte scale) according to Equation (1). The results were expressed in percentage of mass loss.

$$\text{Loss of mass } (\%) = \frac{[\text{initial mass} - \text{final mass}]}{\text{initial mass}} * nit \quad (1)$$

The firmness of the epicarp was measured using a digital penetrometer (PTR-300, São Paulo, Brazil), with a 5 mm tip (5–200 N ± 1 N) evaluated in the equatorial region of the papayas. The results are expressed in Newtons.

The color of the mesocarp of the fruits was measured using a portable colorimeter (Delta Vista color d.0, São Leopoldo, RS, Brazil) with standard illuminant D65, observation angle of 0°, and calibrated with color standard (white), using the CIELab System. The brightness (L*: 0 = black, 100 = white), a* coordinate: ranging from +a red to −a green, b* coordinate: ranging from +b yellow to −b blue, and hue angle (h°).

### *2.3. Physical-Chemical Analyses*

Physical-chemical analyses were carried out such as: soluble solids, titratable acidity, pH, ascorbic acid, lycopene, β-carotene, phenolic compounds, total sugars, proteins, enzymes, and minerals. Soluble solids were determined with the aid of a digital manual refractometer (MA871, Milwaukee, WI, USA). The results were expressed as a percentage [24]. Titratable acidity (TA) was determined by maceration of 5 g of papaya pulp in a mortar in 50 mL of distilled water and titrated with 0.1 N NaOH until light pink in color. The results were expressed as percentage of citric acid [25]. The pH was determined in a digital potentiometer (PA200, Marconi—Piracicaba, São Paulo, Brazil), according to the techniques of [25].

Ascorbic acid (AA) was determined by UV-visible spectrophotometry (Bel photonics—UV W51), according Pearson [24]. Approximately 5.0 g of the sample was weighed in a beaker. To this was added 10 mL of 0.4% oxalic acid and the solution was stirred for 5 min. The sample was transferred to a 10 mL volumetric flask and made up to volume with oxalic acid. The solution was filtered. The spectrophotometer was zeroed with distilled water at a wavelength of 520 nm. In a test tube, 1 mL of 0.4% oxalic acid was transferred, 9 mL of DFI dye solution was added and the L1 reading was performed. Then, some crystals of ascorbic acid were added to the test tube to decolor the solution, and the L1A reading was taken. One milliliter of the filtrate was transferred to two test tubes. In one of them, 9 mL of distilled water was added. The apparatus was again zeroed with this solution. In the other tube, 9 mL of DFI was added and the L2 reading was performed. A few crystals of ascorbic acid were added to this test tube and the L2A reading was taken.

Lycopene and β-carotene: β-carotene and lycopene were determined using the method of [26]. Furthermore, 300 mg of the pulp was used, shaking vigorously with 10 mL of an acetone–hexane mixture (4:6), and then filtered. Concentrations were determined by observing measurements at absorbances of 453, 505, 645, and 663 nm. Results were expressed in mg of lycopene/g, and mg of β-carotene/g. For determination, Equations (2) and (3) were used.

$$\beta - \text{carotene} = (0.216 \times A_{663}) - (1.22 \times A_{645}) - (0.304 \times A_{505}) + (0.452 \times A_{453}) \quad (2)$$

$$\text{Lycopene} = (0.0458 \times A_{663}) - (0.204 \times A_{645}) - (0.372 \times A_{505}) + (0.0806 \times A_{453}) \quad (3)$$

Total phenolic compounds (FC) were determined according to the methodology described by Waterhouse [27]. For the extraction of phenolic compounds, 1 g of the sample was weighed and ground in a mortar. Then, the samples were transferred to 50 mL flasks, where they were left to rest for 30 min. Subsequently, they were filtered on filter paper,

and 600 µL aliquots were transferred to test tubes and 1525 µL of water was added, 125 µL of Folin–Ciocalteusolution, which were stirred and left to rest for 5 min. After resting, 250 µL of 20% sodium carbonate was added, followed by further stirring, where they were placed in a water bath at 30°C for 30 min. The samples were read in a UV-visible spectrophotometer (Bel photonics—UV W51) at a wavelength of 765 nm. Results were expressed in mg of gallic acid 100 $g^{-1}$ FW.

Total sugars were determined by the anthrone method, described by Yemm and Willis [28]. Furthermore, 0.5 g of the samples were weighed, ground in a mortar, and diluted in 50 mL of distilled water. Afterwards, they were left to rest and a filtration was carried out. The reagents were placed in the test tubes, following the same order as the standard curve. Then, the sample, water, and 0.2% anthrone were added. The tubes were placed in a water bath at 100 °C for 3 min, followed by a bath in ice water and subsequent readings. The standard curve was prepared with glucose and readings performed in a spectrophotometer at an absorbance of 620 nm.

Total proteins were determined using the method by Boaretto [29], using 100 mM potassium phosphate buffer (pH 7.5), 1 mM EDTA, and 3 mM dithiothreitol (DTT). To perform the extraction, 1 g of plant material was used and 3 mL of extraction buffer was added, later centrifuge data rotation of 10,000 rpm for 30 min. After centrifugation, the supernatant was collected for the determination of total proteins by the method of [30]; moreover, part of the supernatant was kept in a freezer at −80 °C for the quantification of catalase. Enzyme catalase activity was determined in a spectrophotometer by monitoring the degradation of $H_2O_2$ at 240 nm during the 1min period [31]. The reaction consisted of plant extract, 100 mM potassium phosphate buffer (pH 7.5), and $H_2O_2$. Results were expressed in µmol $min^{-1}$ mg protein.

## 3. Results

### *3.1. Physical Analyses*

The FTIR spectra of gelatin, chitosan, gelatin + chitosan (blend), and chitosan-gelatin (layer-by-layer) films were evaluated, and the results are presented in Figure 2. Typical bands characteristic of chitosan and gelatin films were observed.

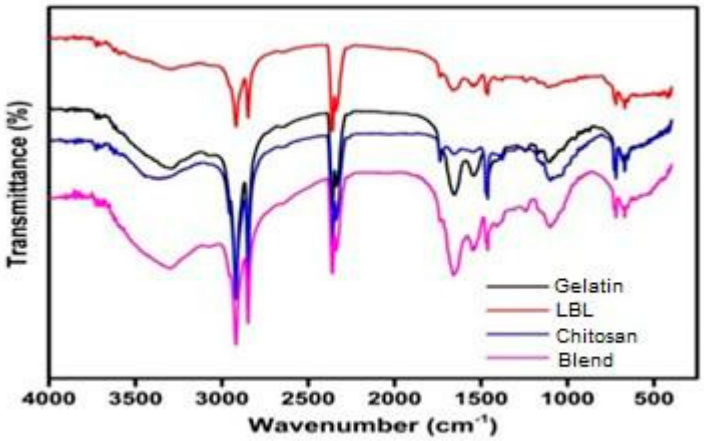


**Figure 2.** Fourier transform infrared spectroscopy (FTIR).

Regardless of the treatment, there was a progressive loss of fresh mass over the storage days, and a significant difference ($p < 0.01$) in fruit storage time was observed (Figure 3A). Gelatin-coated fruits exhibited the lowest mass loss during the storage period, followed by layer-by-layer and blend treatments. The control and chitosan-coated fruits showed the highest weight loss.

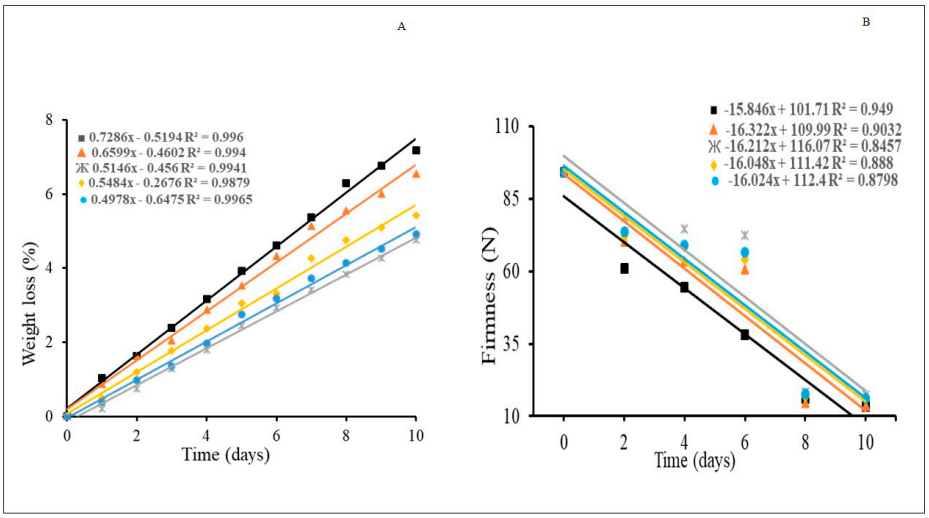


**Figure 3.** Effects of chitosan and gelatin-based coatings, (**A**) weight loss and (**B**) firmness in freshly processed papayas from the control (■) and chitosan (▲), gelatin (ж), blend (♦), and layer-by-layer treatments (●) stored for 10 days at 5 °C.

Fruit firmness decreased as the storage time increased, irrespective of the type of coating (Figure 3B). There was a significant difference ($p < 0.01$) in both time and the interaction between types of coatings and storage time. Gelatin-coated fruits had the highest firmness during the storage period, measuring 17.44 N. Layer-by-layer and blend treatments resulted in firmness values of 16.26 and 15.84 N, respectively. The control and chitosan-coated fruits had the lowest firmness, measuring 13.35 and 13.83 N, respectively.

Luminosity (L*) showed a significant difference ($p < 0.01$) as a function of time and the interaction between coating and storage time. Luminosity values ranged between 27.15 and 32.85 for the minimally processed papayas in this study (Figure 4A). The coated fruits consistently displayed higher luminosity values throughout the storage period. Gelatin-coated fruits exhibited the highest L* values, initially increasing until the fourth day and then decreasing until the last day, with an average of 31.06 (Figure 4A). Blend-treated fruits showed an increase in L* values until the sixth day, followed by a reduction until the last day, with an average of 30.19 (Figure 4A). Layer-by-layer-treated and chitosan-coated fruits showed a continuous increase in L* values until the last day of storage, with averages of 29.52 and 29.34, respectively. The control fruits had the lowest L* values, which decreased until the sixth day, averaging 28.59.

The chroma a* values, representing the degree of variation from red to green, decreased for all treatments starting from the second day, and there was a significant difference ($p < 0.01$) in storage time. The a* color coordinate of papayas ranged from 10.92 to 16.75 (Figure 4B), indicating a reddish color (positive a* values). Gelatin-coated fruits had the lowest a* values from the second day of evaluation, averaging 12.42. Layer-by-layer, chitosan, and blend treatments had averages of 12.47, 12.85, and 13.07, respectively. The control fruits showed an initial increase and the highest values of a* until the sixth day, followed by a decrease until the last day, with an average of 13.40.

Variations and reductions in color coordinate b* (Figure 4C) were observed for all treatments over the storage days, and there was a significant difference ($p < 0.01$) as a function of time and the interaction between coating and storage time. Fruits coated with gelatin had the lowest b* values, averaging 21.61. Layer-by-layer, blend, and chitosan-coated fruits had averages of 22.30, 22.57, and 23.20, respectively. The control treatment exhibited the highest values and variations of b* (Figure 4C).

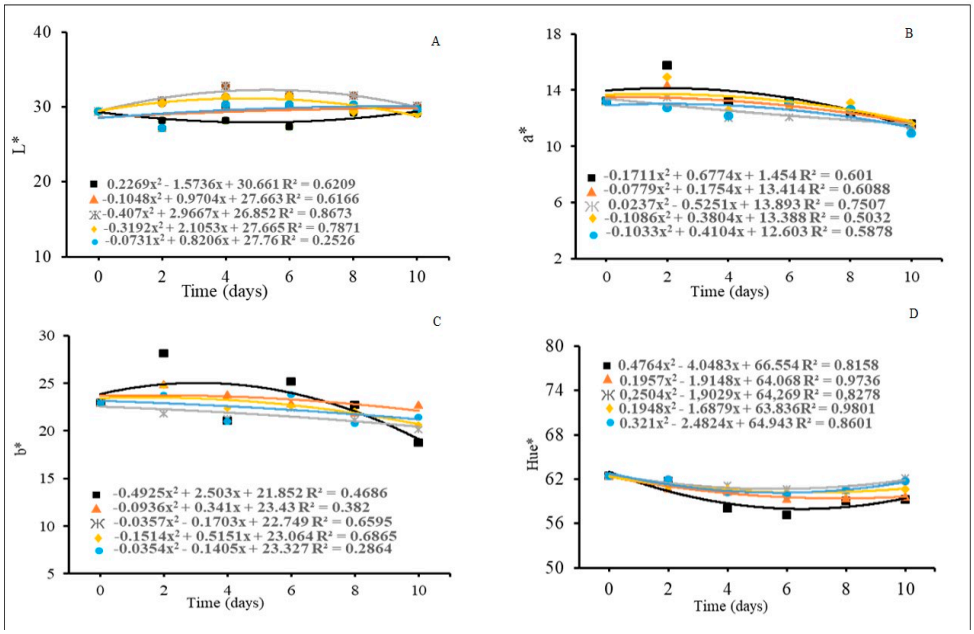


**Figure 4.** Effects of chitosan and gelatin-based coatings (**A**) luminosity, (**B**) a*, (**C**) b*, and (**D**) hue, of minimally processed papayas in the control (■), chitosan (▲), gelatin (⋇), blend (♦), and layer-by-layer treatments (●) stored for 10 days at 5 °C.

The values of the hue angle (h°) showed a significant difference ($p < 0.01$) in fruit storage time. Hue angle values ranged from 58.05 to 62.44 (Figure 4D). According to the CIELAB system, a smaller angle indicates a redder fruit, while a larger angle indicates a more yellow fruit. Gelatin-coated and layer-by-layer treated fruits exhibited the highest h° values throughout the storage period, with slight reductions until the eighth day and an increase on the last day, averaging 61.40 and 61.12, respectively (Figure 4D). Blend-treated fruits had a reduction in h° values until the sixth day, followed by an increase until the last day of evaluation, with a mean of 60.88. Chitosan-coated fruits showed reductions in h° values until the eighth day, with a slight increase on the last day, averaging 60.33. The lowest h° values during the storage period were observed in the control treatment, with more pronounced reductions over the days, averaging 59.61.

### *3.2. Physicochemical Characteristics*

Regarding pH, it was observed that there was a significant difference ($p < 0.01$) in time and in the interaction between coatings and storage time. The pH of papayas ranged from 5.50 to 6.49 (Figure 5A). The fruits of the gelatin and blend treatments showed the highest pH values until the sixth day of evaluation, with a subsequent reduction until the last day, with averages of 6.18 and 6.14, respectively. The fruits of the layer-by-layer and chitosan treatments showed constant pH values over the days, with averages of 5.98 and 6.02, respectively. The greatest variations in pH values during the storage period were from the control treatment fruits, with increase and decrease over the days; the pH of these fruits averaged 6.11.

In relation to the soluble solids (SS) contents, small variations were observed over the days (Figure 5B) and that there was a significant difference ($p < 0.01$) in the storage time. Fruits coated with blend, gelatin, and layer-by-layer treatments showed the lowest SS contents, with small increases until the last day of evaluation, with averages of 10.1, 10.4, and 10.5%, respectively. The highest SS contents were from the fruits of the control treatment and those coated with chitosan, with variations, showing an increase and a reduction until the last day. The means of SS in these fruits were 10.7 and 10.6%, respectively.

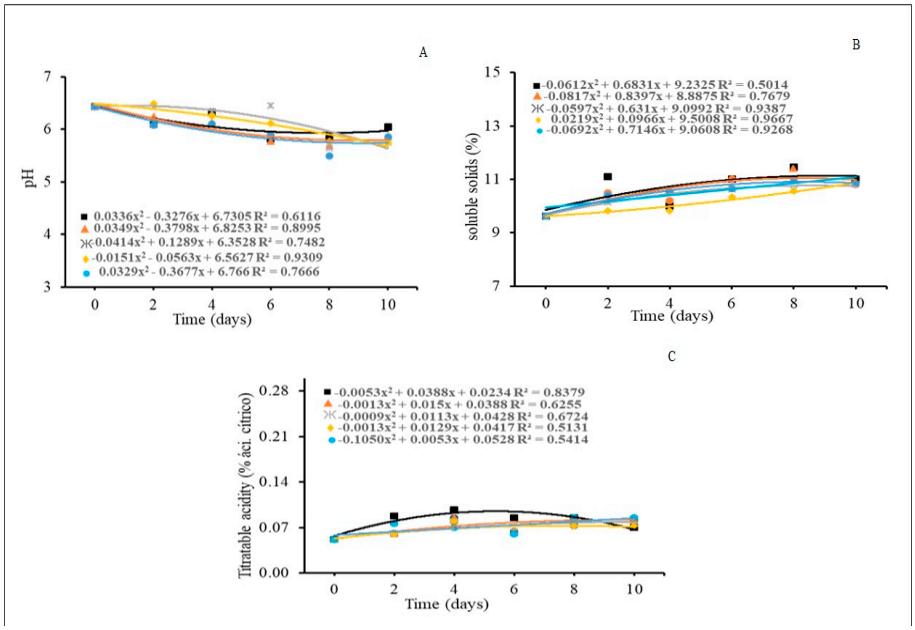


**Figure 5.** Effects of chitosan and gelatin coatings (**A**) pH, (**B**) soluble solids, and (**C**) titratable acidity of minimally processed papayas in control (■), chitosan (▲), gelatin (ж), blend (♦), and layer-by-layer treatments (●) stored for 10 days at 5 °C.

The titratable acidity (TA) contents are shown in Figure 5C, where there was a significant difference between the treatments, storage period, and in the interaction between the coatings and storage period ($p < 0.01$). The coated fruits presented the lowest levels of AT until the eighth day of evaluation, with a constant increase in these values, with 0.07% average for citric acid. The fruits of the control treatment showed an increase and the highest TA values occurred until the eighth day of evaluation, with an average of 0.08%.

For ascorbic acid (AA) there was a significant difference ($p < 0.01$) between treatments and storage time. Ascorbic acid levels in papayas ranged from 10.70 to 17.80 mg 100 $g^{-1}$ (Figure 6A). The gelatin-coated fruits showed an increase in AA levels and the highest values until the sixth day of evaluation, with an average of 14.65 mg 100 $g^{-1}$. The fruits coated layer-by-layer showed an increase until the second day, with a small reduction on the fourth day, followed by an increase until the last day of storage, with an average of 13.05 mg 100 $g^{-1}$. The fruits coated with the blend treatment increased their AA levels until the second day, with a subsequent reduction until the last day, with an average of 12.41 mg 100 $g^{-1}$.

It was observed for the phenolic compounds (PC) that there was a significant difference ($p < 0.01$) in the time and in the interaction between coating and storage period. The PC content ranged in the minimally processed papayas from 85.35 to 105.09 mg AG 100 $g^{-1}$ FW (Figure 6B). Fruits coated with gelatin had the lowest levels of PC until the eighth day, with an average of 87.27 mg AG 100 $g^{-1}$. The fruits covered with the layer-by-layer treatment showed the highest PC levels until the fourth day, where it later showed a reduction until the last day, with an average of 94.99 mg AG 100 $g^{-1}$. The fruits from treatments with chitosan and blend had a linear increase in PC levels over the days, with averages of 99.97 and 98.38 mg AG 100 $g^{-1}$, respectively. The fruits of the control treatment showed a linear increase in PC, with higher values in the last days of evaluation, with an average of 101.01 mg AG 100 $g^{-1}$ FW.

For the lycopene contents, it was observed that there was a significant difference ($p < 0.01$) in the time and in the interaction between coatings and storage time. Lycopene levels in papayas ranged from 0.02 to 0.08 mg 100 $g^{-1}$ (Figure 6C). The fruits of the layer-by-layer treatment showed the lowest lycopene contents with small reductions throughout the storage period, with an average of 0.03 mg 100 $g^{-1}$. The fruits of the gelatin and chitosan treatments showed small increases in lycopene levels along the days, with a reduction

on the last day of evaluation, with an average of 0.05 mg 100 $g^{-1}$. The highest levels of lycopene were found in the fruits of the control and those coated with the blend until the sixth day, followed by a reduction in these values until the last day.

Regarding the β-carotene levels, it was observed that there was a significant difference ($p < 0.01$) in the time and in the interaction between coating and storage time. The levels of β-carotene in minimally processed papaya ranged from 0.03 to 0.08 mg 100 $g^{-1}$ (Figure 6D). The fruits of the layer-by-layer treatment showed the lowest levels of β-carotene throughout the conservation period, with an average of 0.04 mg 100 $g^{-1}$. The fruits coated with gelatin showed a reduction in β-carotene levels until the sixth day, followed by an increase until the eighth day, and a small reduction on the last day, with an average of 0.04 mg 100 $g^{-1}$. The fruits of the control treatment showed variations in β-carotene levels over the days, where it showed the highest levels of this pigment until the eighth day, with an average of 0.07 mg 100 $g^{-1}$.

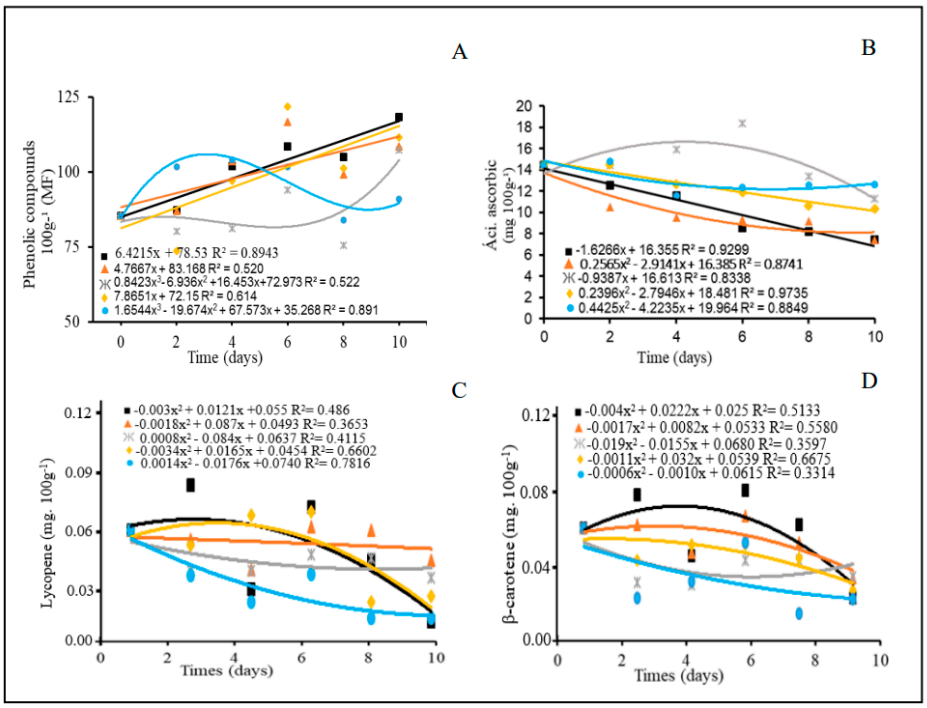


**Figure 6.** Effects of chitosan and gelatin-based coatings (**A**) ascorbic acid, (**B**) phenolic compounds, (**C**) lycopene, and (**D**) β-carotene contents of minimally processed papayas in the control treatments (■), coated with chitosan (▲), gelatin (ж), blend (♦), and layer-by-layer (●) stored for 10 days at 5 °C.

For total sugars, it was observed that there was a significant difference ($p < 0.01$) in storage time and in the interaction between coating and storage time. Total sugar levels in minimally processed papayas ranged from 1.22 to 3.58 g 100 $g^{-1}$ (Figure 7). Fruits coated with blend and layer-by-layer showed a tendency to increase in total sugar contents throughout the storage period, with averages of 2.73 and 2.52 g 100 $g^{-1}$, respectively. The fruits coated with chitosan and gelatin showed an increase and the highest levels of sugars until the sixth day, with a subsequent reduction, with averages of 2.47 and 2.61 g 100 $g^{-1}$, respectively, followed by the fruits of the control treatment, with an average of 2.40 g 100 $g^{-1}$.

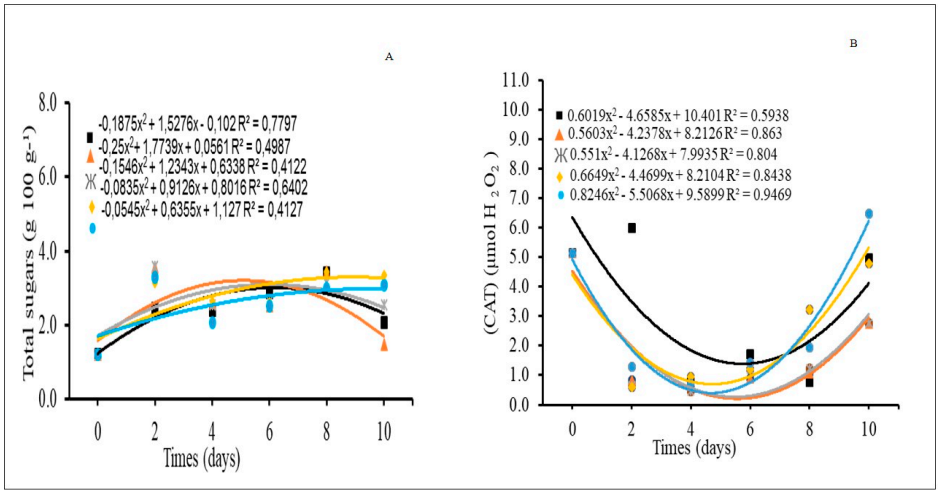


**Figure 7.** Effects of chitosan and gelatin-based coatings (**A**) total sugar, (**B**) catalase activity (µmol $H_2O_2$ fresh mass$^{-1}$) contents of minimally processed papayas in control (■), chitosan-coated (▲), gelatin (ж), blend (♦), and layer-by-layer treatments (●) stored for 10 days at 5 °C.

For the catalase enzyme, it was found that there was a significant effect ($p < 0.01$) on storage time and on the interaction between treatment and storage time (Figure 7B). A reduction in the activity of this enzyme was observed until the sixth day for all treatments, with a subsequent increase until the last day. The fruits of the blend and layer-by-layer treatments had an increase in catalase activity from the sixth day, where they showed the highest activity on the last day. The fruits coated with chitosan and gelatin had the lowest activity of this enzyme during the storage period. The fruits of the control treatment showed the highest activity of this enzyme until the sixth day of evaluation.

## 4. Discussion

### *4.1. Physical Analyses*

Typical broad bands in chitosan films appeared in the spectra from 3000 to 3600 cm$^{-1}$ and were attributed to the amide A band (elongation), and other bands were also identified at 2879 cm$^{-1}$ (C–H elongation of alkyl groups) and 1470 cm$^{-1}$ $CH_2$ (angular strain). Typical broad bands in gelatin films appeared in the spectra of 1632 cm$^{-1}$ (attributed to the C=O elongation) and1239 cm$^{-1}$ (attributed to the C–N and N–H elongation in the amide).

According to Abugoch [32] and Lima [33], chitosan films demonstrated characteristic bands at 1637 and 1570 cm$^{-1}$ (attributed to an amide bond), 3400–3500 cm$^{-1}$ (attributed to O–H and N–H stretching), and 900 and 1150 cm$^{-1}$ (assigned to pyranose rings and amino groups).Characteristic gelatin bands appeared at 1632 cm$^{-1}$ (attributed to C=O elongation), 1549 cm$^{-1}$ (attributed to N–H elongation in amide bonds), and 1239 cm$^{-1}$ (attributed to C–N and N–H elongation in amide) as per Gennadios [34] and Pranoto et al. [35].

According to Poverenov et al. [36], gelatin and chitosan components in blend and layer-by-layer exhibited the characteristic bands in superposition once the components were combined in equimolar amounts. According to Lima et al. [37], the films that the coatings form on the surface of the fruits reduce water loss and dehydration, thus preventing weight loss and wilting. This shows that fruits coated with gelatin, layer-by-layer, and blend were efficient in maintaining the mass loss of minimally processed papayas.

The fruits coated with chitosan lost more mass when compared to the other coatings, this may have occurred because the chitosan film did not form the protective layer well, or the coating caused stress and acted in the opposite way. The fruits without coatings were the ones that lost more fresh mass over the days, which can be justified because they have no protection, thus causing greater transpiration in these fruits. According to Kumar [38], the greatest mass losses in fruits occur due to the migration of water present in the fruit to

the environment, being attributed to transpiration of the stomata and direct evaporation through the epidermal cells.

Poverenov et al. [36], when studying edible gelatin and chitosan coatings using the layer-by-layer technique and by blend in minimally processed melons, observed that the fruits coated with the blend, gelatin, and chitosan treatments had lower mass losses over the days when compared to the blend, gelatin, and chitosan treatments.

The maintenance of firmness in vegetables essentially depends on factors such as tissue turgor and cell wall degradation [39], which is directly related to the loss of vegetable mass. The greater firmness of fruits coated with gelatin, layer-by-layer, and blend over the days are associated with the film that these coatings formed on the surface of minimally processed papayas, acting in the reduction of the metabolic rate of the fruits, resulting in low internal concentration of available $O_2$, which inhibited the degradative processes of the cell wall and the solubilization of pectins, as can be seen in Brackmann et al. [40] and Castañeda et al. [41].

The fruits coated with chitosan and the control fruits (without coating) showed the lowest firmness over the days, which may be related to water loss and loss of cell wall integrity, which occurs due to several mechanisms, such as, the solubilization of protopectins that occurs during the natural ripening process and by the action of hydrolytic enzymes [42].

When studying edible coatings of gelatin and chitosan using the layer-by-layer technique and by blend in minimally processed melons [36], it was observed that the coated fruits showed the highest firmness until the last day of storage, with emphasis on the layer-by-layer technique, being similar to this research, as the treatment presented the second-best firmness in papaya.

The fruits covered with gelatin and the blend treatment showed greater luminosity over the days, characterizing lighter fruits, which can be attributed to the solutions ap-plied to the surface of the minimally processed papayas, preventing the ripening of the fruits over the days (Figure 4A).

The luminosity of the fruits coated with layer-by-layer and chitosan treatments had a constant increase in L* values over the days, showing maintenance of this characteristic during the conservation period. The lowest values of L* and reduction were found in uncoated fruits, showing darker fruits, which is related to the degree of maturation of this pulp, or its degradation.

When studying multilayer antimicrobial edible coatings based on polysaccharide in the conservation of minimally processed papaya, Brasil et al. [43] observed that the uncoated fruits (control) had lower luminosity when compared to the coated fruits. The coated fruits were lighter when compared to the control, these fruits had lower a* values, which is related to the film that the coatings formed on the surface of the papayas, preventing the fruits from becoming dark over the days, characteristic of mature fruits (Figure 4B).The control fruits showed darker pulp over the days, which may be related to the oxidation of carotenoids, or indicates oxidative darkening of the pulp. Brasil et al. [43] also observed in minimally processed papaya fruits with multilayer antimicrobial edible coating based on polysaccharide, that a* values decreased for all treatments over the days.

The b* coordinate indicates the color variation from yellow to blue. The coated fruits showed values of this coordinate and few variations over the days, which is justified by the maintenance of the orange–yellow color of the pulps of the minimally processed pa-payas. Uncoated fruits showed an increase in b* values and variations over the days, which may be related to the darkening of the pulp and the presentation of carotenoids.

The coated fruits showed higher h° values over the days, which indicates that these pulps were more yellow, which may be because the coatings prevented these pulps from becoming darker (Figure 4D). The uncoated fruits, on the other hand, showed lower values and sharp reductions over the days in h°, showing that these fruits had darker pulps over the days, as shown in Figure 1.

### *4.2. Physicochemical Characteristics*

The pH of fruits coated with gelatin and blend showed a reduction from the sixth day onwards (Figure 5A), which can be attributed to the production of organic acids through biochemical reactions [44]. In contrast, the layer-by-layer and chitosan treatments did not exhibit significant variations in pH values over the storage period. These coatings acted as barriers, preventing an increase in respiratory rate and maintaining a low pH, which is desirable for controlling microbial growth in fresh fruits [45]. This observation aligns with previous studies on papaya coated with edible coatings, where a decrease in pH was observed for all treatments during the storage period [6].

Fruits coated with blend, gelatin, and layer-by-layer treatments exhibited lower soluble solids (SS) values (Figure 5B), indicating the creation of a modified atmosphere by the coatings. According to Lima et al. [37], this modified atmosphere acted as a barrier, delaying metabolic reactions and resulting in fewer variations and lower SS values compared to control fruits and those coated with chitosan. Conversely, control fruits and those coated with chitosan showed higher SS values, indicating an increased degree of maturation. This finding is consistent with a study by Brasil et al. [43], where control fruits had the highest SS levels until the ninth day of evaluation.

The coated fruits displayed lower titratable acidity (TA) values (Figure 5C), which can be attributed to the barrier effect of the coatings on the pulp's surface, inhibiting ripening. In contrast, control fruits exhibited an increase in TA values, as reported by Trigo et al. [46], indicating that TA levels rise during ripening and senescence due to organic acid release. Similar behavior was observed in the study by Brasil et al. [43], where coated fruits showed minimized effects of respiration and ripening compared to the control.

Vitamin C (ascorbic acid, AA) contents varied among different coatings, with fluctuations observed during the storage period. The reduction in AA content may be attributed to the damage caused by minimal processing, which can stimulate degradation reactions that consume ascorbic acid [47]. Among the coatings, the layer-by-layer treatment exhibited the lowest AA loss compared to gelatin and blend treatments.

Control fruits and those coated with chitosan showed the lowest AA contents, with a linear reduction observed in control fruits and a reduction from the fourth day onwards in fruits coated with chitosan (Figure 6B). The observed AA losses in these fruits may indicate pulp oxidation, where ascorbic acid content tends to decrease during maturation due to enzymatic action (ascorbate oxidase) or oxidative enzymes such as peroxidase. A similar trend was observed in a study by Martiñon et al. [48], where the ascorbic acid content decreased over time in chitosan-coated melon.

The lowest levels of lycopene were found in fruits treated with the layer-by-layer coating throughout the storage period, indicating that this coating minimized the effect of maturation (Figure 6C). Gelatin- and chitosan-coated fruits exhibited slight increases in lycopene levels over time, followed by a reduction on the last day of evaluation. The control and blend treatments showed an initial increase in lycopene contents, followed by a reduction from the sixth day onwards, which may be associated with degradation during respiratory processes and intensified during ripening and senescence. This reduction in lycopene content in papaya is related to its oxidation [49]. A study by the same authors reported an increase in lycopene content in chitosan-coated papaya during cold storage [50].

The layer-by-layer coating applied to minimally processed papaya pulp showed the lowest β-carotene levels throughout the storage period, possibly due to its low oxygen permeability (Figure 6D). The other treatments, including gelatin, blend, chitosan, and control, showed variations in β-carotene levels, which could be attributed to oxidation of this pigment during fruit maturation. An increase in β-carotene content was reported in papaya coated with 1% chitosan, with the control treatment exhibiting the highest levels of this pigment [51].

A gradual increase in total sugars (Figure 7A) was observed in fruits coated with blend and layer-by-layer coatings, without significant variations over time. In contrast, chitosan, gelatin, and control treatments exhibited variations in total sugar content, with an increase

possibly related to starch conversion and a subsequent decrease associated with energy consumption for metabolic processes.

The increase in catalase activity observed in fruits coated with blend and layer-by-layer treatments indicates enhanced cellular protection against stress factors (Figure 7B). On the other hand, fruits coated with chitosan and gelatin showed the lowest catalase activity during the storage period, while control fruits exhibited the highest activity until the sixth day of evaluation. Catalase is an antioxidant enzyme that degrades hydrogen peroxide ($H_2O_2$) without requiring an electron donor. It acts as a defense mechanism against stress factors [51,52].

## 5. Conclusions

The application of gelatin and chitosan-gelatin (layer-by-layer) coatings demonstrated a significant positive effect on the preservation of minimally processed papaya. These coatings successfully delayed fruit maturation, extending the shelf life by at least eight days. Additionally, they effectively minimized fresh mass loss while preserving fruit firmness and the vibrant orange color of the pulp. Throughout the storage period, these treatments consistently maintained low soluble solids contents, resulting in minimal acidity variations and influencing the ripening process. Gelatin coatings exhibited low polyphenol content, while the layer-by-layer treatment consistently maintained vitamin C levels. Moreover, there were no notable variations in lycopene and β-carotene levels over time, indicating the stability of these important pigments. Although there was a slight tendency for total sugars to increase, no significant variations were observed. Notably, the layer-by-layer treatment displayed an increase in catalase activity, suggesting enhanced cellular protection. Consequently, the utilization of gelatin and chitosan polymers in a layer-by-layer configuration presents a viable and effective alternative for preserving minimally processed papayas.

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