



Using edible coatings from Whitemouth croaker (*Micropogonias furnieri*) protein isolate and organo-clay nanocomposite to improve the conservation properties of fresh-cut 'Formosa' papaya



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ABSTRACT

Edible coatings have been used in the fresh-cut industry as a strategy to reduce the deleterious effects of minimal processing on plant tissues. The objective of this study was to apply edible coatings from protein isolate of Whitemouth croaker with organo-clay montmorillonite in minimally processed papaya slices, throughout the storage of 12 days at 5 °C, and assess their properties and verify the effectiveness of this coating as a barrier against the weight loss of papaya, aiming to increase its shelf-life. The different coatings applied with and without montmorillonite in minimally processed papaya were effective during the 12 days of storage. The croaker protein isolate (CPI) and montmorillonite (MMT) coating applied to minimally processed papaya showed lower mass loss (5.26%), lower microbial growth and a smaller decrease of firmness, lightness and pH, and therefore showed the best results in coating of minimally processed papaya.

Industrial relevance: Papaya is very much appreciated in its minimally processed, but this type of processing causes injuries so that its shelf-life is reduced. This perishability of papaya needs a good preservation strategy. The present results demonstrated the feasibility of using edible coatings on the basis of fish protein isolate and organo-clay nanocomposite, with low cost and high shelf-life compared to the control sample. The study further confirms that the viability of fish protein of low value for the industries becomes a great potential to increase the shelf-life of minimally processed papaya, without changing the characteristics in relation to odor and appearance of minimally processed fruit.

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1. Introduction

Fish proteins have properties that are advantageous in the preparation of biofilms, such as the ability to form networks, plasticity and elasticity, showing good oxygen barrier, but its water vapor barrier is low due to their hydrophilic nature. These properties may be improved by applying nanotechnology, including materials such as nanoclays. The croaker has lower market prices in relation to other species. The edible covers of fish protein with clay can extend the shelf life and improve the quality of fruits by providing barriers to mass transfer, improving integrity or handling and/or the functional loads such as antimicrobial agents and antioxidants.

Edible plasticized films and coatings are thin, flexible materials made from biopolymers and capable of forming a continuous matrix by adding food grade plasticizers. They are usually manufactured by the wet method, which is based on the drying of a film-forming solution or dispersion by casting on a convenient support. Furthermore, the wet

process is generally preferred in order to form edible preformed films or to applied coatings directly onto food products (Alfaro, Fonseca, & Prentice, 2012; Araujo-Farro, Podadera, Sobral, & Menegalli, 2010; Colla, Sobral, & Menegalli, 2006; Guilbert, 2000; Sobral, Menegalli, Hubinger, & Roques, 2001).

Fresh-cut fruit and vegetables generally consist in washed, cut, treated with sanitizing agents and packaged products and stored under refrigerated conditions (Del Nobile, Conte, Scrocco, & Brescia, 2009; McKellar et al., 2004). The cutting or slicing operations modify the metabolic process of vegetal tissue and increase its susceptibility to spoilage, inducing a reduction of the shelf life (Del Nobile et al., 2009). Quality and shelf life of fresh-cut fruits are reduced by water loss, senescence processes, microbial growth, colour and texture changes, due to the tissue injuries caused by peeling, slicing and cutting. Thus, in spite of their convenience, fresh-cut mangoes may show browning and undesirable texture changes during storage (Beaulieu & Lea, 2003; Chiumarelli, Ferrari, Sarantópoulos, & Hubinger, 2011).

Several treatments have been studied in order to maintain quality and to prolong the shelf-life of the fruit products (Beaulieu & Lea, 2003; Chiumarelli et al., 2011; Fontes, Sarmento, Spoto, & Dias, 2008; Qi, Hu, Jiang, & Tian, 2011; Rodrigues, Pereira, Ferrari, Sarantópoulos, & Hubinger, 2008; Rojas-Grau, Tapia, & Martin-Belloso, 2008).

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Processing operations may result in a dramatic loss of firmness in fruit tissues due to the action of pectic enzymes and the most common way of softening control in fresh-cut fruits is the use of treatments with calcium salts texture enhancers, which may also be added to edible coatings to minimize softening during the storage of fresh-cut fruits (Rojas-Grau, Soliva-Fortuny, & Martín-Belloso, 2009). Besides using refrigerated cooling, many authors have tested biodegradable coatings to prolong life of the fruit and maintain quality (Park, Stan, Daeschel, & Zhao, 2005; Santos, Martins, Salas-Mellado, & Prentice, 2011; Tanada-Palmu & Grosso, 2005).

Edible coatings have been used in the fresh-cut industry as a strategy to reduce the deleterious effects of minimal processing on plant tissues. Edible coatings may contribute to extend the shelf life of fresh-cut fruits by reducing moisture and solute migration, gas exchange, respiration, and oxidative reaction rates, as well as by reducing or even suppressing physiological disorders (Rojas-Grau et al., 2009).

The papaya is an example of a product whose consumption is often limited by the size and inconvenience of peeling, making the processed form expand its marketing; it allows the consumer in many different occasions and its use in different food service (Souza & Durigan, 2007). In this context, the objective of this study was to apply edible coatings from protein isolate of Whitemouth croaker with organoclays in minimally processed papaya slices, throughout the storage of 12 days at 5 °C, and assess their properties and verify the effectiveness of this coating as a barrier against the weight loss of papaya, aiming to increase its shelf-life.

2. Materials and methods

2.1. Material

The Formosa variety of Papaya (*Carica papaya* L.) was purchased in local shops in the city of Rio Grande/RS. The fruits were selected according to size, color and elongated shape, as indicated by Lima, Ramos, Marcellini, Batista, and Faraoni (2005) and had an average weight of 2.0 kg, 50 to 75% yellow skin, physiological defect and free of visually detectable infections caused by microorganisms. Samples were transported in coolers to the laboratory where they were stored at 4 ± 1 °C until processing. The croaker protein isolate (CPI) was obtained according to the adapted methodology of Nolsoe and Undeland (2009) and Freitas, Gauterio, Rios, and Prentice (2011) to solubilize and isolate protein by the process of varying pH, as mechanically separated meat (MSM) from the industrialization of Whitemouth croaker (*Micropogonias furnieri*). The organophilic clay utilized was Montmorillonite K10 (MMT, Sigma–Aldrich) with a particle size of 100 nm. The plasticizer used was glycerol (Vetec, Química Fina).

2.2. Methods

2.2.1. Preparation of film solution

The film solution was prepared by the casting technique. The polymer coating was developed initially in the preparation of a dispersion of 35 g of Whitemouth croaker protein isolate (CPI) in distilled water in a beaker of 1000 mL (Martins, Costa, Damodaran, & Prentice, 2011). This aqueous dispersion was maintained with gentle and constant stirring for 20 min with a stirring propeller shaft (Fisatom, 713D) at 30 °C in thermostatic ultrasonic bath (QUIMIS, 214 D2), for hydration of the CPI. After the hydration, the dispersion pH was adjusted to 11.2 with the addition of 1 N NaOH (Merck) using pH meter bench (Marconi, PA 200) while maintaining constant stirring for 10 min. Then 5 g of MMT was added and the temperature was elevated to 80 °C. After complete dissolution of the CPI and MMT 10.5 g of glycerol previously dissolved in distilled water at the temperature of the film solution (80 °C) was added maintaining the pH at 11.2. Subsequently, the film solution was placed in a homogenizer (Ultra-turrax IKA, T25) for 5 min. For the preparation of pure CPI coating, the same procedure

was carried out without addition of MMT. Once the film solutions were prepared, these were used for coating minimally processed papaya.

2.2.2. Preparation of minimally processed papaya

The minimal processing was performed at a temperature of about 10 °C with the previously sanitized utensils in a solution of organic chlorine (dichlorocyanurate) at the concentration of 2 g L^{-1} . The selected papaya was also cleaned with a solution of organic chlorine at the concentration of 2 g L^{-1} for 15 min. The operators were properly protected with gloves, aprons, hats and masks, in order to protect the product, as much as possible, from contamination. The raw material was subjected to manual removal of the peel and seeds and afterwards it was manually cut into cubes of approximately 2.5 cm^3 . Then, these pieces were rinsed with chlorinated water (0.2 g L^{-1}) to eliminate cellular spilled juice. Water was drained using sieves for a period of 2–3 min (Pizato, Cortez-Vega, Souza, Prentice-Hernández, & Borges, 2013).

2.2.3. Papaya coatings

Dried and sanitized Papaya was divided into three lots: Treatment 1 (T1, control), Treatment 2 (T2, pure CPI coating) and Treatment 3 (T3, CPI coating with MMT). The T2 and T3 were immersed in a film solution for 5 min, they were then drained using sieves, and left to dry for 2–3 min.

The samples for each treatment were packaged in unrecycled PET (Polyethylene Terephthalate) containers, with cover (SANPACK), whose external dimensions were $15.5 \times 13.2 \times 5.5$ cm. The number of pieces per package was standardized and stored in refrigerated conditions at 4 ± 1 °C.

2.2.4. Physicochemical analysis of coated papaya

The weight loss was obtained by taking the difference between the initial weight of the minimally processed papaya and that obtained one at the end of each storage time, according to the formula:

$$(\%) \text{Weight loss} = [(initial\ mass - final\ mass) / (initial\ mass)] \times 100.$$

The results were expressed as percentage of weight loss.

The measures of the papaya-cubes firmness were determined by using a texture analyzer (Stable Micro Systems, TA.XT.plus). A cylindrical probe in the pre-test speed of 4 mm s^{-1} , post-test of 8 mm s^{-1} , test of 2 mm s^{-1} and penetration depth of 5 mm was used. The results were expressed in Newton (N).

Color analysis was evaluated by using a Minolta colorimeter, model Chroma Meter CR400. The parameters of luminosity L^* [0 (black) to 100 (white)], Chroma a^* [green chromaticity (–60) to red (+60)] and Chroma b^* [blue chromaticity (–60) to yellow (+60)] were verified.

The pH was determined by using the method described by AOAC (2000). The pH was measured using a digital pH meter (Marconi, PA 200). It was prepared a suspension of 20 g of sample in 100 mL of distilled water, thus measuring the pH with the assistance of a pH meter.

Total titratable acidity was determined and calculated as the volume in mL of NaOH 0.1 mol L^{-1} , required to titrate 10 mL of the diluted sample and homogenized in 100 mL of water. The results were expressed as percentage of citric acid (AOAC, 2000).

Content of total soluble solids was determined in a bench-type refractometer Abbé, with the correction temperature at 20 °C. The results were expressed in °Brix (AOAC, 2000).

2.2.5. Microbiological analysis of coated papaya

Microbiological tests performed were psychotrophic, total and thermotolerant coliforms, *E. coli*, moulds and yeasts, and *Salmonella* sp., following the methods described in APHA (2001).

2.3. Statistical analysis

The results were analyzed statistically by the analysis of variance (ANOVA) using the software Statistica® 7.0 (StatSoft, Inc., Tulsa, USA). Mean separation was determined using the Tukey test at $P \leq 0.05$.

3. Results and discussion

3.1. Selection of the formulations of cover

The minimally processed papaya received CPI coating with and without addition of MMT, compared with a control sample, in order to assess their physical, chemical and microbiological characteristics.

Fig. 1. shows the values of mass loss, soluble solids, ($^{\circ}$ Brix) and firmness as a function of days of storage.

Note in Fig. 1a, that the control sample (T1) had the greatest weight loss over time, reaching the final with a mass loss of 10.83%, a value well above that found for papaya coated with pure CPI coating and CPI coating with the addition of MMT, which obtained an average of 5.90% and 5.26% respectively. After 12 days of storage, when comparing the values of mass loss of papaya CPI coated with and without montmorillonite compared with those of the control group, a significant difference among the three treatments is visible. Teixeira, Durigan, Mattuiz, and Rossi Júnior (2001) evaluated the mass loss of samples of minimally processed papaya, stored at different temperatures for 7 days. The samples lost an average of 7.92%, 7.29% and 8.15% of the initial content when stored at 3 °C, 6 °C and 9 °C, respectively. Using an average temperature of 4 ± 1 °C, this work was compared with the data obtained in this present work finding the best weight loss results after 12 days of storage.

The values of $^{\circ}$ Brix (Fig. 1b) increased until the last day of storage for all treatments. The T2 and T3 did not differ significantly until the fifth day of storage. The treatment T1 was significantly different from

the first day with treatments of croaker protein isolate coating with and without MMT. The values of $^{\circ}$ Brix on papaya without coating increased as ripening increased due to increased degradation or biosynthesis of polysaccharides and increase due to moisture loss because of the accumulation of sugars in the tissues. According to Carmo (2004), the metabolic processes related to the advancement of ripening, probably due to dissociation of some molecules and enzymes structural soluble compounds, have direct influence on soluble solids, in which the fruit in advanced stages of maturation exhibits the highest levels of soluble solids, in agreement with the results found in this work. The T2 and T3 showed minimal increase in the values of $^{\circ}$ Brix, demonstrating that the CPI coatings were satisfactory as an oxygen barrier.

The firmness (Fig. 1c) of the samples of minimally processed Formosa papaya was influenced by storage time and the application of coating. It can be seen that the firmness of the papaya decreased with time, however, T1 showed lower firmness. Treatment T3 had lower decrease in firmness (29.08 N). The T2 and T3 did not differ significantly until day 7 of storage. Treatment T1 showed 69.76% loss of firmness to the last day of storage. The treatment T3 had 17.64% loss of firmness. This work was compared to Tapia et al. (2008) who evaluated the firmness of minimally processed papaya samples coated with gellan gum and alginate. Agreeing with the results obtained in this study, the coated samples showed higher firmness than the control sample. The decrease in strength can be directly linked to increased mass loss of papaya cubes. Peixoto, Silva, Martins, Torres, and Guimarães (2008) evaluated the firmness of papaya minimally processed samples, submitted to treatment with calcium chloride and ascorbic acid. According to their results, the addition of calcium chloride maintained the firmness values close to 30 N within 10 days of refrigerated storage, calcium crosslinks with pectin and thus influences firmness. However, as in this study, there was a steady reduction in the control sample (15 N) and the sample treated with ascorbic acid (17.5 N).

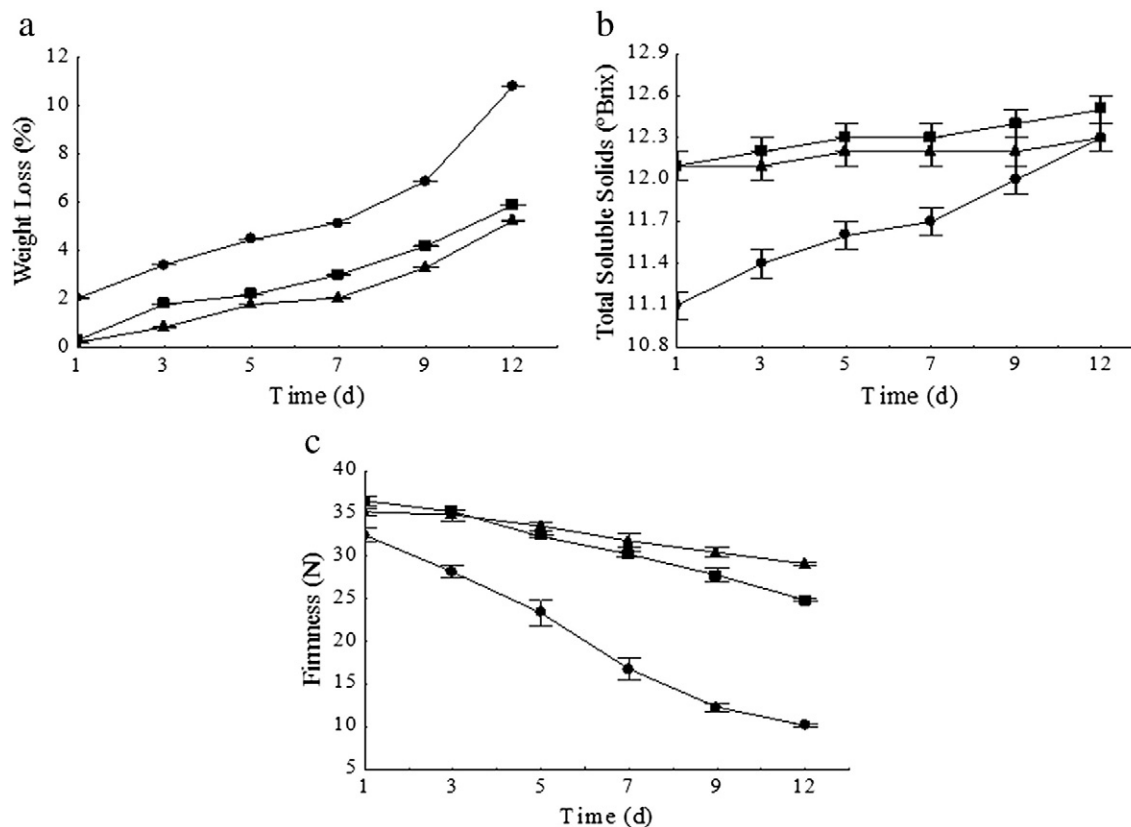


Fig. 1. Effect of CPI coating with and without MMT in minimally processed papaya, on the mass loss, $^{\circ}$ Brix and firmness, each value represents the mean of three replicates with standard deviation. Where: ● T1 (control), ■ T2 (papaya covered with pure CPI), ▲ T3 (papaya covered with CPI with the addition of MMT).

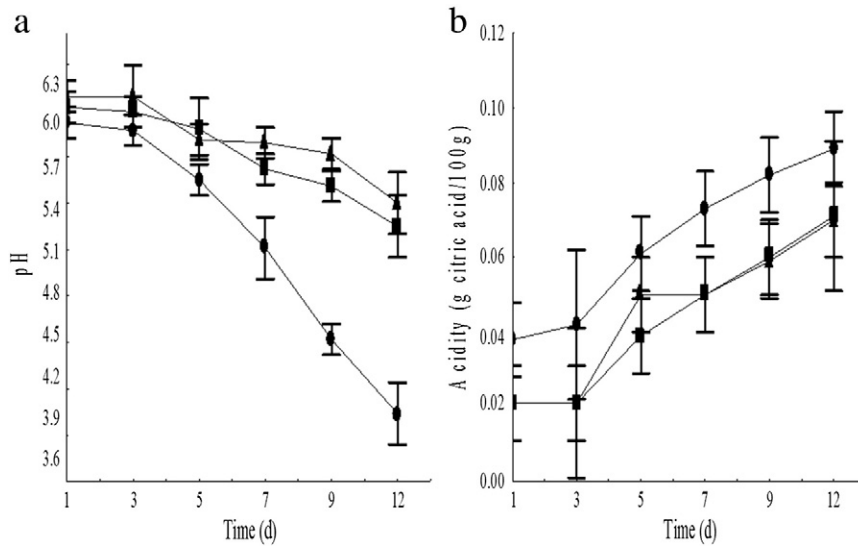


Fig. 2. Effect of CPI coating with and without the MMT on minimally processed papaya on the pH and titratable acidity, each value represents the mean of three replicates with standard deviation. ● T1 (control), ■ T2 (papaya coated with pure CPI), ▲ T3 ((papaya coated with CPI with the addition of MMT).

Fig. 2. shows the pH and titratable acidity of minimally processed papaya according to the days of storage.

It can be seen from Fig. 2a that the pH of papaya decreased with time, however, T1 showed a lower pH (4.04) than other treatments. After 12 days, by comparing pH values of papaya coated with CPI with and without MMT compared to the control group, significant difference among the three treatments was showed. According to the results, it can be observed that there was a decrease in pH during storage.

The decrease of these values may be associated with production of organic acids such as malic and citric acids during storage due to biochemical reactions (Lima et al., 2005). This agrees with Godoy et al. (2003) where the pH of minimally processed papaya samples stored under refrigeration for 9 days in different containers is reduced during storage, ranging from 5.57 to 4.83. Oliveira Júnior, Cordeiro, Carlos, Coelho, and Araújo (2000) while assessing the pH of minimally processed papaya samples stored at different temperatures for 8 days

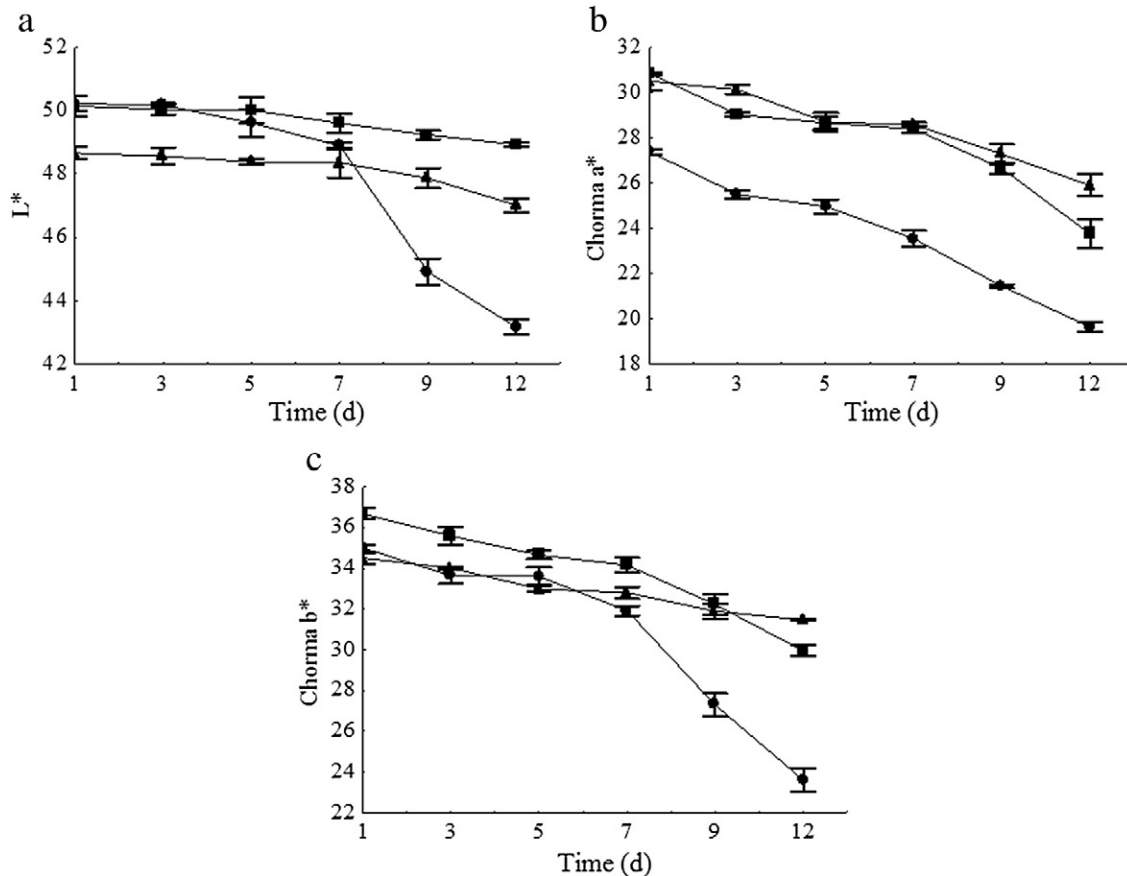


Fig. 3. Effect of CPI coating with and without the in MMT of minimally processed papaya on the color, each value represents the mean of three replicates with standard deviation. ● T1 (control), ■ T2 (papaya covered with pure CPI), ▲ T3 (papaya covered with CPI with the addition of MMT).

discovered a reduction in pH during the storage period, ranging from 5.65 to 5.30. This agrees with the results obtained in this study.

It can be seen in Fig. 2b that the acidity values of the minimally processed papaya samples, both T1 and the treatments of croaker protein isolate coatings with and without addition of montmorillonite behaved inversely to the pH, because the acidity increased along storage time. Treatment T1 was significantly different from treatments using croaker protein isolate film coatings with and without addition of MMT, agreeing with Sarzi, Durigan, Donadon, Teixeira, and Lima (2002a) who also found an increase in the levels of titratable acidity of papaya cut into pieces, attributing the increased acidity to the increase of galacturonic acid as a result of enzymatic activity. Godoy et al. (2003) found an increase of 0.05% to 0.10% for total titratable acidity of Formosa papaya stored in trays at the end of nine days of storage; the values were similar to those obtained in this work.

Fig. 3. shows the color values of minimally processed papaya as a function of days of storage.

The brightness values decreased until the last day of storage for all treatments. It was observed in treatment T1, which showed the highest darkening (14.06%) in relation to the samples that were coated with CPI. Treatment T2 presented the lowest darkening during the storage time (2.43%). The results show no significant difference between T1 and T2 until the fifth day of storage, after this period a significant difference between the two treatments is shown. Treatment with CPI coating and MMT (T3) showed lower brightness when compared with other treatments since the beginning of the analysis. These results agree with the work of Wiley (1994) who observed in the coloring of papaya pulp subjected to different types of cuts for 10 days of storage, an increase in brightness in the halves ($L^* = 58.71$ to $L^* = 61.63$) and stability in slices ($L^* = 56.27$ to $L^* = 56.20$), no significant biochemical reactions resulting from the contact with enzymes and substrates or differences attributable to temperature were observed.

The Chroma a^* values decreased until the last day of storage for all treatments. It was observed that the treatment T1 showed the greatest loss of value of the Chroma a^* (28.27%) compared to samples which were coated with the CPI. Treatment T3 showed the smallest decrease of Chroma a^* (15.08%). The results show that there was an increase in the intensity of green up to the last day of storage.

The values of Chroma b^* decreased until the last day of storage for all treatments. It was observed that the treatment T1 showed the greatest loss of Chroma b^* (32.52%) compared to samples which were coated with CPI with and without addition of MMT. Treatment T3 showed the smallest decrease of Chroma b^* (8.81%) until the last day. The results show that there was an increase in the intensity of blue until the last day of storage. Just as for the Chroma a^* , the decrease in the values of Chroma b^* may indicate an oxidative darkening, agreeing with Fontes et al. (2008) who observed in the pulps of apples treated with alginate MP had the lowest mean L^* (darker coloring) among treatments. However, these values did not differ statistically from the control treatment from the 9th day and from dextrin to day 13.

Table 1

Growth curve of psychrotrophic microorganisms ($\log \text{CFU g}^{-1}$) of minimally processed papaya samples using different coatings stored at 4 ± 1 °C.

Time (days)	Treatments		
	T1	T2	T3
0	2.30 ± 0.08 ^{fA}	2.30 ± 0.05 ^{fA}	2.30 ± 0.05 ^{eA}
1	3.43 ± 0.11 ^{eA}	2.82 ± 0.08 ^{eB}	2.32 ± 0.07 ^{eC}
3	4.42 ± 0.09 ^{dA}	3.43 ± 0.11 ^{dB}	3.18 ± 0.11 ^{dB}
5	6.65 ± 0.12 ^{cA}	5.09 ± 0.13 ^{cB}	4.83 ± 0.09 ^{cB}
7	7.64 ± 0.11 ^{bA}	6.01 ± 0.09 ^{bB}	5.47 ± 0.12 ^{bCC}
9	7.81 ± 0.05 ^{bA}	6.18 ± 0.10 ^{bB}	5.70 ± 0.11 ^{abC}
12	10.53 ± 0.15 ^{aA}	6.84 ± 0.08 ^{aB}	6.36 ± 0.08 ^{aC}

T1 (Control) uncoated papaya, T2 (papaya coated with CPI), T3 (papaya coated with CPI and MMT). Means followed by same small letter in the column and capital letter on the line do not differ by Tukey test ($P \leq 0.05$).

Table 2

Growth curve of yeasts and molds ($\log \text{CFU g}^{-1}$) of minimally processed papaya samples using different coatings stored at 4 ± 1 °C.

Time (days)	Treatments		
	T1	T2	T3
0	1.65 ± 0.12 ^{fA}	1.65 ± 0.09 ^{fA}	1.65 ± 0.11 ^{eA}
1	2.11 ± 0.09 ^{fA}	1.95 ± 0.07 ^{eA}	1.69 ± 0.10 ^{eB}
3	2.57 ± 0.13 ^{eA}	2.04 ± 0.11 ^{eB}	1.90 ± 0.07 ^{eB}
5	3.72 ± 0.15 ^{dA}	3.46 ± 0.11 ^{dAB}	3.25 ± 0.11 ^{dB}
7	4.89 ± 0.07 ^{cA}	4.66 ± 0.08 ^{cAB}	4.43 ± 0.13 ^{cB}
9	5.94 ± 0.10 ^{bA}	5.64 ± 0.12 ^{bB}	5.44 ± 0.11 ^{bB}
12	7.03 ± 0.03 ^{aA}	6.58 ± 0.14 ^{aB}	6.21 ± 0.10 ^{aC}

T1 (Control) uncoated papaya, T2 (papaya coated with CPI), T3 (papaya coated with CPI and MMT). Means followed by same small letter in the column and capital letter on the line do not differ by Tukey test ($P \leq 0.05$).

3.2. Microbiological analysis of papaya

Tables 1 and 2 show the growth curve of psychrotrophic microorganisms, yeast and mold samples of minimally processed papaya, using croaker protein isolate coatings with and without MMT over 12 days of storage at 4 ± 1 °C.

Through these results, it can be seen that the sample using minimally processed Formosa papaya using croaker protein isolate coatings with and without MMT showed similar behavior on the growth of psychrotrophic microorganisms. However, the growth of these microorganisms in the control treatment (T1) was superior to that of treatments with coating. Treatment CPI nanocomposite coating and MMT (T3) was the one with the lowest growth of microorganisms.

Among inorganic agents, nanoparticles (MMT) received great attention from the scientific world, due to the high biocidal effects on many species of microorganisms. The combination of coating with silver-montmorillonite (Ag-MMT) controlled microbial growth better than the sole coating treatment. In particular, fresh-cut carrots with active coating did not overlap the microbiological limit imposed for fresh-cut vegetables (Costa, Conte, Buonocore, Lavorgna, & Del Nobile, 2012). Andrade (2006) in evaluating the growth of psychrotrophic microorganisms in minimally processed papaya found that, regardless of the treatment (control sample, ascorbic acid and calcium chloride), there was an increase in the number of psychrotrophic microorganisms, reaching between the 6.3×10^3 and $8.0 \times 10^3 \log \text{CFU g}^{-1}$, these results are consistent with those obtained in this study with the variation of 2.03×10^3 to $10.53 \times 10^3 \log \text{CFU g}^{-1}$.

Through the results, it can be observed that samples of Formosa papaya minimally processed using croaker protein isolate coats with and without MMT showed similar behavior to the growth of molds and yeasts. However, the growth of these microorganisms in the T1 treatment was superior to the treatment using coating. Treatment with CPI coating and MMT (T3) presented the lowest growth of molds and yeasts. The results show a significant difference between T1 and the treatments with coatings (T2 and T3) until the last day of storage.

The presence of total coliforms and thermotolerant coliforms ($<10^2 \text{CFU g}^{-1}$) was not detected, as well as *Salmonella* (absence in 25 g) in all treatments of minimally processed papaya, confirming the effectiveness of hygienic and the action of organic chlorine in the disinfection of the samples. According to Brazilian legislation, prepared fresh fruit (peeled or selected or fractional), the presence of thermotolerant coliforms within $5 \times 10^2 \log \text{CFU g}^{-1}$ and the absence of *Salmonella* in 25 g of sample sanitized and refrigerated are permitted (Brasil, 2001). The present results agree with those obtained by Sarzi, Durigan, Donadon, Teixeira, and Lima (2002b). The authors found that the hygienic conditions adopted during the processing of minimally processed papaya were efficient, because the presence of total coliforms and thermotolerant coliforms was not detected during the storage for up to 14 days.

4. Conclusion

Whitemouth croaker protein isolate and organoclays-based edible coatings could reduce weight loss, lower microbial growth and loss of firmness, lightness, and pH of fresh-cut Formosa papaya during 12 days of storage as compared with the control (uncoated sample). The use of croaker protein isolate with montmorillonite coatings in minimally processed papaya showed lower mass loss (5.26%), than that of protein isolate coats without montmorillonite.

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