

Whitemouth croaker (*Micropogonias furnieri*) protein isolate and organoclay nanocomposite coatings on shelf life and quality of fresh-cut pear

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Abstract

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. The objective of this study was to apply edible coatings from protein isolate of Whitemouth croaker with organoclay Montmorillonite in fresh-cut pear, throughout the storage of 12 days at 4±1°C, and assess their properties and verify the effectiveness of this coating as a barrier against the weight loss of pear, aiming to increase its shelf life. The different coatings applied with and without montmorillonite in fresh-cut pear were effective during the 12 days of storage. The CPI and montmorillonite coating applied to Fresh-Cut pear showed lower weight loss (4.68%), lower microbial growth and a smaller decrease of firmness, lightness and pH, and therefore showed the best results in coating of fresh-cut pear.

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Introduction

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 and Lea, 2003; Chiumarelli *et al.*, 2011).

Vegetables processing may result in a dramatic loss of firmness in fruit tissues during storage due to the action of peptic 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 (Rojas-Grau *et al.*, 2009a). Besides using refrigerated cooling, many authors have tested biodegradable coatings to prolong life of the fruit and maintain quality (Park *et al.*, 2005; Tanada-Palmu and Grosso, 2005).

Edible coatings have been used in the fresh-cut industry as a strategy to reduce the deleterious effects that minimal processing on vegetable tissues. Furthermore 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.*, 2009a). In the meantime, consumption of fresh-cut fruits has increased due to demand for fresh, healthy, convenient, and additive-free prepared produce items (Rico *et al.*, 2007). Among many fruits, peeled ready to eat pear has drawn the attention of food industry and research as a novel minimally processed product (Li *et al.*, 2012)

Pear, a good source of antioxidant compounds such as phenolic, anthocyanin, and vitamin C, is a popular and commercially important cultivar which served as the main fresh-cut fruit item. Similar to other fresh-cut produces, processing operations could cause undesirable changes in fresh color, appearance, and nutrition throughout the storage. Enzymatic browning, degradation and oxidation of pigments, water loss, whitening, and surface dehydration are likely to occur as a consequence of wounding (Rojas-Grau *et al.*, 2009b). In this context, the objective of this study was to apply edible coatings from protein isolate of Whitemouth croaker with organoclays in Fresh-Cut pear, throughout the storage of 12 days at 4°C, and assess their properties and verify the effectiveness of this coating as a barrier against the weight loss of pear, aiming to increase its shelf life.

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Material and Methods

Material

Williams pears (*Pyrus communis* L.) was purchased in local market in the city of Rio Grande/RS – Brazil at commercially maturity stage, based on external color and firmness, physiological defect-free and visually detectable infections caused by microorganisms. Samples were transported in coolers to the laboratory where they were stored at 5 ± 1 °C until processing. The croaker protein isolate (CPI) was obtained from mechanically separated meat (MSM) of the industrialization of Whitemouth croaker (*Micropogonias furnieri*)” from the process of varying pH, using adapted methodology of Nolsoe and Underland (2009) and Freitas *et al.*, (2011). The organophilic clay utilized was Montmorillonite K10 (Sigma-Aldrich) with a particle size of 10 nm. The plasticizer used was glycerol (Vetec, Fine Chemicals).

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 croaker protein isolate (CPI) in distilled water in a beaker of 1000 ml. This aqueous dispersion was maintained with gentle and constant stirring for 20 minutes 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 1N NaOH (Merck) using pH meter bench (Marconi, PA 200) while maintaining constant stirring for 10 minutes. Then 5 g of Montmorillonite (MMT) were 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 homogenizer (Ultraturrax IKA, T25) for 5 minutes. For the preparation of pure CPI coating, the same procedure was carried out without addition of MMT (Cortez-Vega *et al.*, 2014). Once the film solutions were prepared, these were used for coating Fresh-Cut pear.

Preparation of fresh-cut pear

The minimally 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 pear was also cleaned with the same solution for 5 minutes. 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 slices (2.5 x 2.5 cm). Then, these slices 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 minutes.

Pear coatings

Dried and sanitized pear 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 minutes, they were then drained using sieves, and left to dry for 2-3 minutes. The samples for each treatment were packaged in unrecycled PET (Polyethylene Terephthalate) containers, with cover (SANPACK), whose external dimensions were 15.5 x 13.2 x 5.5 cm. The number of slices per package was standardized and stored in refrigerated conditions at 5 ± 1 °C for 12 days.

Physicochemical analysis of coated pear

The weight loss was obtained by taking the difference between the initial weight of the Fresh-Cut pear and that obtained one at the end of each storage time, according to the formula:

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

The results were expressed as percentage of weight loss.

The measures of the Fresh-Cut pear slices 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

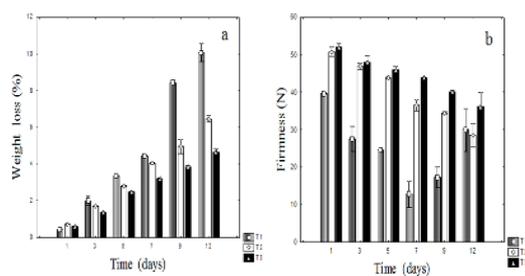


Figure 1. Effect of CPI coating with and without MMT on fresh-cut pear on the weight loss (a) and firmness (b), each value represents the mean of three replicates with standard deviation. T1 (control); T2 (fresh-cut pear coated with pure CPI); T3 (fresh-cut pear coated with CPI with the addition of MMT)

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 Abbé refractometer, with the correction temperature at 20 °C. The results were expressed in °Brix (AOAC, 2000).

Microbiological analysis of coated pear

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

Statistical analysis

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

Results and Discussion

Physicochemical analysis

The Fresh-Cut pear received CPI coating with and without addition of MMT, compared with a control sample, in order to assess their physical, chemical and microbiological characteristics. Figure 1 shows the values of weight loss and firmness versus a function of days of storage.

The control sample (T1), in Figure 1a, presented the greatest weight loss over time, reaching 10.06% in final of storage. This value was higher above that found for Fresh-Cut pear coated with pure CPI coating and CPI and MMT coating, which obtained an average of 6.48% and 4.68% respectively. From the 5th day of storage, the control treatment (T1) started to show a greater weight loss than the other

treatments, maintaining this trend until the end of storage. The weight loss values for the coated pears (T2 and T3) were lower than the levels reported by Xiao *et al.* (2011) who studied the effects of sodium chlorite with or without chitosan or carboxymethyl chitosan in quality maintenance during the storage of d'Anjou pears, and obtained weight loss values of 9.30% for the fruits coated with chitosan; 14.01% for those coated with carboxymethyl chitosan and 9.54% for those without any coating. The low values of weight loss observed in the present work for coated pears are due to the barrier action on water loss by the coatings, causing high relative humidity around the atmosphere of the fruit, thus reducing the gradient to the outside. These results demonstrate efficacy in weight loss and showed lower values to those obtained by Qi *et al.* (2011) who studied the effect of coatings based on chitosan in minimally processed apples concluding that they were not fully effective in weight loss, finding values weight loss of 19% for the control treatment and 15% for the coated apples.

The firmness of the samples of Fresh-Cut pear was influenced by storage time and coating application. It can be observed (Figure 1b) that the firmness of pears decreased over time, however, the treatment T1 showed greater decrease in firmness until the seventh day of storage (67.52%). After this period the firmness began to rise. This increase in firmness could be caused by partial dehydration of the surface leading to an abrasive surface (Gorny *et al.*, 2000), and also by maturation differences between the pieces of pears (Lesage and Destain, 1996).

The treatment T3 had the lowest decrease in firmness (30.55%), showing that the addition of croaker protein isolate (CPI) and montmorillonite (MMT) was efficient to maintain the firmness of these for longer than when using only pure CPI. Rojas-Grau, *et al.* (2008) evaluated the firmness of minimally processed apple samples coated with alginate and gellan. According to their results, calcium chloride promoted bonds between the polymer chains, thereby reducing the loss of firmness. Fontes *et al.* (2008) evaluated minimally processed apples coated with sodium alginate and cassava starch and observed hardening of the tissue. The authors attributed this to the addition of calcium salts in the coating formulation, because the calcium ions form complexes with the cell wall pectin, improving the structural integrity and providing higher firmness of tissues. In this work, calcium ions were not used to maintain firmness of minimally processed pears, showing that the use of nanoclays was efficient to maintain firmness of minimally processed pears for a longer period.

Table 1. Growth of mesophilic microorganisms (CFU g⁻¹) in fresh-cut pear stored at 4 ± 1 °C for 12 days

Days	Treatments		
	T1	T2	T3
1	1.52±0.59 ^{dA}	1.12±0.7 ^{dA}	1.10±0.5 ^{dA}
3	2.41±0.32 ^{dAB}	2.93±0.34 ^{cA}	2.01±0.23 ^{cB}
5	3.31±0.20 ^{cA}	3.24±0.30 ^{bcA}	3.21±0.25 ^{baA}
7	4.06±0.27 ^{bcA}	3.46±0.16 ^{bcB}	3.33±0.16 ^{bbB}
9	4.81±0.21 ^{abA}	3.69±0.27 ^{bbB}	3.45±0.23 ^{bbB}
12	5.44±0.18 ^{aaA}	4.56±0.32 ^{abB}	4.02±0.23 ^{acC}

Means followed by the same letter in the column and capital letter in the line did not differ by Tukey Test (p< 0.05). (T1) control. (T2) CPI; (T3) CPI + MMT.

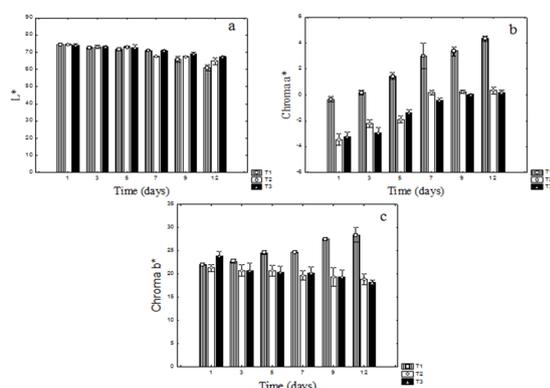


Figure 2. Effect of CPI coating with and without MMT of fresh-cut pear on the color, lightness (a), Chroma a* (b), Chroma b* (c). Each value represents the mean of three replicates with standard deviation. T1 (control); T2 (fresh-cut pear covered with pure CPI); T3 (fresh-cut pear covered with CPI with the addition of MMT)

Xiao *et al.* (2011) demonstrated that the use of chitosan and carboxymethyl chitosan helped to maintain firmness of the pieces of pears when stored for 10 days at 4 °C, and the use of these additives caused a steady increase of 0.25N (0 days) to 31.3N (10 days). These authors attribute this increase to the dehydration of the surface of the fruit, which led to a hardening of the pieces of the pear, thus the increase in resistance resulted in higher firmness measurements. The results obtained by these authors are not in agreement with the results of this study because the firmness remained however dehydration was not observed on the surface of the pear during the days of storage when using croaker protein isolate in Fresh-Cut pear.

Figure 2 shows the color values of Fresh-Cut pear as a function of days of storage. Lightness values decreased until the last day of storage for all

treatments. The treatment T1 showed the greatest browning (18.23%) compared to samples that were coated with CPI, T2 (12.57%) and T3 (9.19%). The lightness results in this study are in agreement with the results obtained by Perez Gago *et al.* (2006) who studied the effect of antioxidants and whey protein based films in color change in minimally processed apples, and concluded that the treatment incorporate protein-based films showed greater inhibition of enzymatic browning. The decrease in lightness values for minimally processed pears are in agreement with the study by Li *et al.* (2012) who also observed a decrease of the lightness values when using high and low concentrations of O₂ in packaging of minimally processed pears stored at 4 °C for 12 days.

Xiao *et al.* (2011) demonstrated in their work that the use of high concentrations of sodium chloride (1000 mg / L and 600 mg / L) were effective to slow the decrease in L* between 7 and 10 days, and after this time the L* values were lower than those found for the control sample. The work done by these authors is not in agreement with the present work, since, the use of CPI made the lightness values of the pieces of pear stay above the values of the control treatment (T1) within 12 days of storage.

Chroma a* values increased until the last day of storage for all treatments. The T1 showing the greatest oxidative browning, and T3 had the lowest browning in Fresh-Cut pear. These results agree with Xiao *et al.* (2011) who evaluated minimally processed pears treated with combined effects of sodium chloride and chitosan coating finding for all treatments an increase in Chroma a* over storage time. Olivas *et al.* (2003) also reported the positive effect of the incorporation of some additives (ascorbic acid, calcium chloride and sorbic acid) along with methylcellulose and

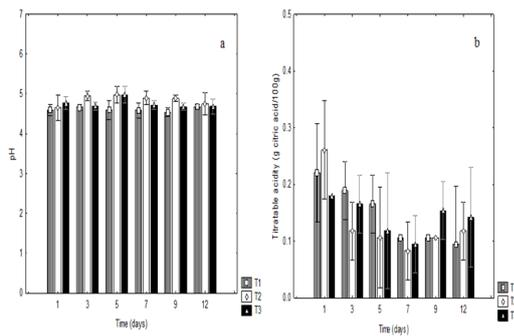


Figure 3. Effect of CPI coating with and without MMT on fresh-cut pear on the pH (a) and titratable acidity (b), each value represents the mean of three replicates with standard deviation. T1 (control); T2 (fresh-cut pear coated with pure CPI); T3 (fresh-cut pear coated with CPI with the addition of MMT)

methylcellulose-stearic coatings in the control of browning of freshly-picked d'Anjou pears.

The values of Chroma b^* decreased until the last day of storage for treatments T2 and T3. It was observed that T1 showed the largest increase of Chroma b^* (23.05%) this increase was observed until the last day of storage. This increase indicated a trend towards a more yellow color and therefore greater oxidative browning. The increase in Chroma b^* values in T1 agrees with the results obtained by Fontes *et al.* (2008) who also obtained higher values of Chroma b^* in the control treatment, whereas when these same authors used the application of preservative solution combined with different coatings, they found values ranging from 33.1 to 30.5. Figure 3 shows the pH and titratable acidity of Fresh-Cut pear according to the days of storage.

For the three treatments, the pH of the fruits showed similar behavior, according to the results, it can be seen that there was minimal reduction in pH during storage. The titratable acidity also had the same behavior for all three treatments, with a decrease in sample. Studying pear conservation of the Williams Cultivar, Brotel *et al.* (2010) reported pH values between 3.91 and 4.06 on the sixth day of storage of pears coated with starch added with calcium lactate and L-casein, but the results of these authors differ from those obtained in the present work, where higher pH values were obtained over the same period. According to Pinheiro *et al.* (2005), an indication that the fruit is in the process of senescence is the increase in the values of acidity and decrease in pH, resulting from the metabolism of organic acids, but in this study values inverse to those found by these authors were obtained.

Figure 4 shows the values of soluble solids, ($^{\circ}$ Brix) as a function of days of storage. With the

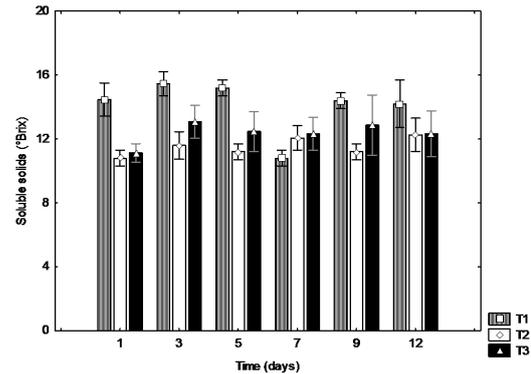


Figure 4. Effect of CPI coating with and without MMT on fresh-cut pear on the soluble solids, each value represents the mean of three replicates with standard deviation. T1 (control); T2 (fresh-cut pear coated with pure CPI); T3 (fresh-cut pear coated with CPI with the addition of MMT)

exception of the 7th day of storage, treatment 1 showed values of total soluble solids higher than the other treatments. The coated pears (treatments 1 and 2) presented higher total soluble solids values at the end of storage when compared to initial values. Treatment 3 showed an increase in $^{\circ}$ Brix values from day 1 to day 3 of storage and remained likewise until the end of storage, whereas treatments 1 and 2 had an increase from day 1 to day 3, however, these values had oscillations during the remainder of the period, with decreases and increases in the amounts of soluble solids. Martins (2010) showed that this behavior is a result of biochemical reactions resulting from the ripening. The increase in the content of total soluble solids observed in control sample (T1) and T2 can be the result of sugars accumulation which is concentrated by loss of moisture, a process that occurs during fruit ripening even though in small scale. This ripening was restricted in the treatment with CPI + MMT (T3). The values found in this study for soluble solids are in agreement with those of Brotel *et al.* (2010) who reported values of soluble solids, in average of 13.7 $^{\circ}$ Brix, being similar to those quoted by Silva *et al.*, (2002), who found values ranging between (11.7 and 15.1) $^{\circ}$ Brix in pear (*Pyrus communis* L.).

Microbiological analysis of Fresh-Cut pear

In Brazil, there is no specific legislation for minimally processed fruits and vegetables with the limits of tolerated counts. However, there is legislation for fresh fruits, in natura, prepared (peeled or selected or fractioned) sanitized, chilled or frozen, which stipulates maximum values of thermotolerant coliforms of 5×10^2 CFU g⁻¹ and absence of Salmonella in 25 g sample (BRASIL, 2001). The presence of *Escherichia coli* (<10² CFU g⁻¹) and

Table 3. Growth of yeast and mold microorganisms (CFU g⁻¹) in fresh-cut pear stored at 4 ± 1 °C for 12 days

Days	Treatments		
	T1	T2	T3
1	1.0±0.21 ^{dA}	0.82±0.32 ^{eA}	0.52±0.21 ^{fA}
3	1.97±0.36 ^{cdA}	1.62±0.21 ^{dA}	1.45±0.12 ^{eA}
5	2.95±0.12 ^{bcA}	2.42±0.16 ^{cb}	2.38±0.2 ^{dB}
7	3.44±0.21 ^{ba}	3.05±0.10 ^{cb}	2.97±0.10 ^{cb}
9	3.93±0.27 ^{ba}	3.69±0.2 ^{ba}	3.56±0.21 ^{ba}
12	5.03±0.7 ^{aA}	4.65±0.35 ^{aA}	4.05±0.12 ^{ab}

Means followed by the same letter in the column and capital letter in the line did not differ by Tukey Test (p< 0.05). (T1) control. (T2) CPI; (T3) CPI + MMT.

Salmonella in samples of Fresh-Cut pear was not detected, confirming the efficiency of the cleaning and the action of organic chlorine to disinfect the samples.

Table 1 shows the growth of mesophilic microorganisms in Fresh-Cut pear stored at 4 ± 1°C for 12 days. It can be observed in Table 1 that both treatments had similar behavior over the days of storage, and only after the fifth day did the treatments show significant differences between them. T2 and T3 had lower growth of mesophilic microorganisms in 12 days of storage in Fresh-Cut pear. These results are in agreement with Gomes *et al.* (2010) who also observed an increase in the growth of mesophilic microorganisms within each passing storage day of minimally processed pears treated with different pHs when stored at 4.5°C for 13 days. The results of this study are also in agreement with Perez-Cabrera *et al.* (2011) who demonstrated inhibition of mesophilic microorganisms when coatings with the ability to avoid browning (calcium and ascorbic acid) in Fresh-Cut pear were used. Table 2 shows the results for the growth of psychrotrophic microorganisms in Fresh-Cut pear stored at 4°C for 12 days.

The increase in storage days led to the increase of psychrotrophic microorganisms in Fresh-Cut pear, and T3 (CPI + MMT) differed significantly from T1 (control) but did not differ from T2 (CPI), showing that the use of CPI along with MMT was efficient to prolong the shelf life of pears. These results are in agreement with Oms-Oliu *et al.* (2008) who worked with modified atmospheres and these were effective for maintaining the shelf life of whole pears for 28 days, a value higher than when the same author compared with samples stored without the use of modified atmospheres.

Olivas *et al.* (2007) evaluated minimally processed apples coated with alginate stored at 4°C and found low levels of psychrotrophs (1 x 10¹ CFU g⁻¹) during the entire period of storage (15 days), a value lower than that found in this work for psychrotrophs (approximately 10⁶ CFU g⁻¹) in T1 and T2. Botrel *et al.* (2010) evaluated the use of calcium lactate applied in minimally processed pears and noted that there was an inhibition of the growth of enterobacteria in relation to the control sample. The same authors reported a lower count psychrotrophs also when calcium lactate was used as a coating. These results are in accordance with the present work which also showed that the use of coatings was effective to maintain and reduce microbial growth in Fresh-Cut pear relative to control sample.

Table 3 shows microbial growth compared to yeast and mold microorganisms for Fresh-Cut pear stored at 4°C for 12 days. Through the results, it can be observed that the samples of Fresh-Cut pear using coatings of croaker protein isolate with and without MMT showed similar behavior to the growth of yeasts and molds. However, the growth of these microorganisms in the T1 treatment was superior to treatments with coating. The treatment with CPI and MMT coating (T3) was the one that presented the lowest growth of yeasts and molds. The deterioration of minimally processed fruits and vegetables is usually detected by consumers when the count of yeasts and molds reaches levels above 5 log (CFU g⁻¹) (Jacxsens *et al.*, 1999). In this work T2 and T3 showed growth below 5 log (CFU g⁻¹) after 12 days of storage. These results are in agreement with the results found by Oms-Oliu *et al.* (2008) which found counts below 5 log (CFU g⁻¹) after 28 days of storage for whole pears when they used modified

atmospheres (CO₂ and O₂).

Conclusion

Edible coatings with Whitemouth Croaker Protein Isolate and organoclays-based reduced weight loss, microbial growth, loss of firmness and lightness of fresh-cut pear during 12 days of storage as compared with the control (uncoated sample). The use coats of croaker protein isolate coating and montmorillonite in Fresh-Cut pear showed lower weight loss (4.68%), than that of protein isolate coats without montmorillonite. The results showed that the use of fish protein isolate together organoclay nanocomposite is economically viable for the process of fresh-cut pear.

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