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ORIGINAL PAPER

Effect of an Edible Nanomultilayer Coating by Electrostatic Self-Assembly on the Shelf Life of Fresh-Cut Mangoes

Marthyna P. Souza · Antônio F. M. Vaz · Miguel A. Cerqueira · José A. Texeira · António A. Vicente · Maria G. Carneiro-da-Cunha

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Abstract This work aims at evaluating the effect of an alginate-chitosan nanomultilayer coating, obtained by electrostatic layer-by-layer self-assembling, in the quality and shelf life of fresh-cut mangoes. Coated and uncoated fresh-cut mangoes were stored under refrigeration (8 °C) for 14 days. The changes in mass loss, titratable acidity, pH, ascorbic acid content, total soluble solids, malondialdehyde content, browning rate, and microbial count were evaluated during storage. At the end of the storage period, lower values of mass loss, pH, malondialdehyde content, browning rate, soluble solids, microorganisms' proliferation, and higher titratable acidity were observed in the coated mangoes. The nanomultilayer coating did not improve the retention of vitamin C during storage of fresh-cut mangoes. Results suggest that chitosan-alginate nanomultilayer edible coating extends the shelf life of fresh-cut mangoes up to 8 days.

Keywords Edible coating · Nanolayers · Chitosan · Alginate · Fresh-cut mangoes

Introduction

Mango (*Mangifera indica* L. *Anacardiaceae*) is a popular subtropical fruit cultivated in the subtropical regions because of its high economical value (Sellamuthu et al. 2013). Recognized for its attractive color, delicious taste, rich aroma, exotic flavor, and high nutritional value, mango is a rich source of carotenoids and provides high contents of ascorbic acid and phenolic compounds (Shahnawaz et al. 2012; Liu et al. 2013).

Minimally processed fruits are one of the major growing sectors in food retail market (Robles-Sánchez et al. 2013), although the freshly cut mango still has a very limited offer in the world market. However, the popularity of tropical fruits in North American and European markets presents an excellent opportunity for the introduction of fresh-cut mango (Siddiq et al. 2013). Yet, fresh-cut processing induces chemical and biochemical changes besides increasing product respiration rate leading to a reduction of storage time (Azarakhsh et al. 2014). Edible coatings can contribute to extend the shelf life of these products. Coatings can control the internal atmosphere of fruits and retard their senescence, helping in their preservation once they provide a partial barrier to moisture, O_2, and CO_2 while also avoiding volatiles loss (Medeiros et al. 2012a). Polysaccharide-based coatings have been used to extend the shelf life of fresh-cut mangoes, using alginate, cassava starch, and/or chitosan as main components (Robles-Sánchez et al. 2013; Chien et al. 2007; Chiumarelli et al. 2011). Literature suggests that these materials can present improved functionality when used at a nanoscale (Carneiro-da-Cunha et al. 2010; Medeiros et al. 2012b).

In this context, Carneiro-da-Cunha et al. (2010) developed a chitosan and alginate-based nanolayered coating with enhanced mass transfer and mechanical properties. The nanolayers were produced using two polysaccharides with opposite charges, chitosan and sodium alginate, deposited by electrostatic layer-by-layer (LbL) self-assembly on aminolyzed/charged PET; however, this coating was not applied on foods. The objective of this work was therefore to evaluate the effect of alginate-chitosan nanomultilayer coating

M. P. Souza · M. G. Carneiro-da-Cunha (✉)
Biochemistry Department,
Universidade Federal de Pernambuco-UFPE,
Av. Prof. Moraes Rego s/n, CEP 50.670-420, Recife, PE, Brazil
e-mail: mgcc@ufpe.br

A. F. M. Vaz
Unidade Acadêmica de Medicina Veterinária, Universidade Federal de Campina Grande, CEP 58.7000-970, Patos, PB, Brazil

M. A. Cerqueira · J. A. Texeira · A. A. Vicente
CEB-Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

constructed by electrostatic self-assembly in the quality and shelf life extension of fresh-cut mangoes.

Material and Methods

Material

Sodium alginate was obtained from Kelco International Ltd (Portugal) and chitosan (90 % deacetylation) from Aqua Premier Co. Ltd. (Thailand). Lactic acid (90 %), ammonium molybdate, and hydrochloric acid were obtained from Merck (Germany); ethanol (99.8 %) and sodium hydroxide from Riedel-de Haën (Germany); and malondialdehyde (MDA) and oxalic acid-EDTA from Sigma (USA). Semi-ripe (titratable acidity=0.33±0.05 g citric acid/100 g mango; total soluble solids=11.8±1.5 °Brix) 'Tommy Atkins' mangoes were collected directly from the orchard of the Saint Francisco Valley in the city of Petrolina, Pernambuco (Brazil), at similar maturation state.

Preparation of Polyelectrolyte Solutions

The 0.2 % (*w/v*) sodium alginate solution was prepared by dissolving sodium alginate in distilled water and the 0.2 % (*w/v*) chitosan solution was prepared by dissolving chitosan in a 1.0 % (*v/v*) lactic acid solution. Both solutions were stirred using a magnetic stirrer (Fisaton, Brazil) at 200 rpm for 2 h at room temperature (25 °C) to achieve full dissolution. The pH of sodium alginate was adjusted to pH 7.0 with 1 mol L^{-1} sodium hydroxide while the pH of chitosan solutions was adjusted to 3.0 with 1 mol L^{-1} lactic acid and the solutions were termed Alg and Ch, respectively, according to Carneiro-da-Cunha et al. (2010). The opposite charges of these two solutions were confirmed by dynamic light scattering, presenting zeta potential values of −63.02±3.90 and +50.73± 3.61 mV for alginate and chitosan solution, respectively, showing that they can interact by electrostatic forces.

Mangoes Coating

Fifty 'Tommy Atkins' mangoes at a semi-ripe stage were selected and standardized, discarding those with injuries and at an inappropriate stage of maturation. Once selected, mangoes were immersed in sodium hypochlorite solution at 0.5 % (*v/v*) for 3 min, rinsed twice in tap water, and allowed to dry (Souza et al. 2010). After drying the mangoes in a sterile environment (laminar flow hood), they were individually peeled and sliced in eight pieces (3×3×1 cm) and termed fresh-cut mangoes. The samples were randomly divided into two groups (control and test), four pieces of each mango by group, totaling 200 pieces per group. Coating solutions (Alg and Ch) were applied on the test group by the layer-by-layer technique as described by Carneiro-da-Cunha et al. (2010); no coating was applied to the control group. Layer-by-layer technique consisted in the deposition of multiple nanolayers of Alg and Ch in fresh-cut mangoes surface. Briefly, fresh-cut mangoes were immersed into a 0.2 % (*w/v*) Alg solution pH 7.0 for 15 min and subsequently rinsed with deionized water with the same pH (7.0). The samples were dried under a flow of nitrogen (at 25 °C for 15 min) in order to speed up the process and the procedure was repeated using Ch as the polyelectrolyte and rising with deionized water at pH 3.0 (same pH of 0.2 % *w/v* Ch solution). This process was repeated with the alternate deposition of a total of five nanolayers (Alg-Ch-Alg-Ch-Alg). Then 16 samples of each group were placed in zipped bags (15×15 cm, Royal Pack, Brazil), which were closed tightly and stored at 8 °C and 93 % RH (in a room at controlled temperature and relative humidity) for further characterization.

Physical-Chemical Analyses of Mangoes

Fresh-cut mangoes were evaluated after 0, 2, 4, 8, and 14 days of storage. During storage time, mass loss, pH (potentiometry), total titratable acidity, total soluble solids, browning, ascorbic acid, and levels of malondialdehyde (MDA) were evaluated.

Mass Loss

Fresh-cut mangoes (test and control groups) were individually weighed on a semi-analytical balance (B-TEC-500, Brazil) and the results expressed in grams. The mass loss during the experiment was expressed in percentage, using the following equation:

$$W(\%) = \frac{m_i - m_t}{m_i} \times 100$$

Where m_i is the initial mass and m_t is the mass at time t. All determinations were performed with five replicates and results are given as the mean±standard deviation. No leakage of juice from the fruit pieces was observed during the experiments.

Total Soluble Solids

Fresh-cut mangoes (test and control groups) were pressed and the refractive index of the juice obtained was measured on a refractometer (AtagoAutomatc—Master T), with the content of total soluble solids expressed as degrees Brix. All determinations were performed with five replicates and results are given as the mean±standard deviation.

Determination of pH and Titratable Acidity

The pH of the juice was measured by potentiometry (Analyser, Brazil). Titratable acidity was determined by titrating 10 g of liquefied fresh-cut mangoes (Arno, Brazil) with 100 mL of distilled water with a 0.1 mol L^{-1} NaOH solution until pH 8.2 (Instituto Adolfo Lutz 1985). The results were expressed as percent (*w/w*) in grams of citric acid/100 g mango. All determinations were performed with five replicates and results are given as the mean±standard deviation.

Determination of Vitamin C

Vitamin C content was determined by the method of Bajaj and Kauer (1981). Initially, 5 g of fresh-cut mangoes (test and control groups) was homogenized in a domestic blender (Arno, Brazil) with 50 mL of 0.05 mol L^{-1} oxalic acid-EDTA and the extract was centrifuged for 5 min at 4,000×*g*. A solution of metaphosphoric acid-acetic acid (0.25 mL; 15 g of metaphosphoric acid in 40 mL of acetic acid and 100 mL of water) was added to a 2.5-mL aliquot of the supernatant, together with 0.5 mL of 5 % (*v/v*) sulfuric acid and 1 mL of 5 % (*w/v*) ammonium molybdate. After 15 min in the dark at room temperature (25 °C), the absorbance of the sample was measured at 760 nm. All determinations were performed with five replicates and results are given as the mean±standard deviation.

Determination of Malondialdehyde Content (MDA)

One gram of fresh-cut mangoes (test and control groups) was homogenized in a manual homogenizer of tissue cells with 4 mL of 0.1 % (*w/v*) trichloroacetic acid (TCA). The homogenate was centrifuged (4,000×*g*) and 0.5 mL of the supernatant obtained was mixed with 1 mL of thiobarbituric acid (0.5 % *w/v* in TCA 20 % *w/v*) and then subjected to heating at 100 °C for 30 min. The absorbance of the sample was measured at 532 nm and the results were expressed in micromole per gram mango. The amount of MDA-TBA complex (red pigment) was calculated from the extinction coefficient 155 mmol L^{-1} cm^{-1} (Djioua et al. 2009). All determinations were performed with five replicates and results are given as the mean±standard deviation.

Browning

The browning of fresh-cut mangoes (test and control groups) was measured by spectrophotometry according to Li et al. (2009). Two grams of fresh-cut mangoes was homogenized in a manual homogenizer of tissue cells with 5 mL of ethanol (95 %), centrifuged for 20 min at 4,000×*g* and the absorbance of the supernatant was measured at 420 nm. All determinations were performed with five replicates and results are given as the mean±standard deviation.

Microbiological Analyses

Microbiological analyses were carried out by counting the total mesophilic and psychrotropic microorganisms according to Medeiros et al. (2014). Samples of fresh-cut mangoes (1 g) were collected in sterile bottles containing 9 mL of maximum recovery diluent (0.1 % *w/v* peptone in 0.9 % NaCl solution) and then homogenized for 5 min. In a sterile environment, 10^{-1} to 10^{-14} dilutions using the maximum recovery diluent were done. One milliliter of each dilution was added to sterile Petri dishes, followed by the addition of approximately 15 mL of Plate Count Agar, at 40 °C. The samples were mixed immediately after pouring by rotating the Petri dish sufficiently to obtain evenly dispersed colonies after incubation. After complete solidification, the plates were inverted and incubated at 30 °C for 3 days to evaluate total mesophilic microorganisms and kept at 8 °C for 7 days to evaluate total psychrotropic microorganisms. All analyses were performed with five replicates. The results were expressed in log colony forming units per gram (log CFU/g mango).

Statistical Analyses

Statistical analyses were carried out using analysis of variance; comparisons between samples were performed using Student's *t* test. Differences were considered to be significant at $p<0.05$ (GraphPad® Prism, version 6, 2012, USA).

Results and Discussion

Mass Loss

Fresh-cut fruits are highly susceptible to mass loss, being therefore mass loss evaluation a very important parameter for fresh-cut fruit during storage (Azarakhsh et al. 2014). An excessive mass loss makes the product unsuitable for consumption and marketing. Figure 1 shows the mass loss of uncoated and coated fresh-cut mango during the 14 days of storage. In both groups, a mass loss was observed, probably resulting from respiration and transpiration processes. During respiration, there is a release of water and carbon dioxide by oxidation of carbohydrates, resulting in the mass loss of mangoes (Zhan et al. 2012). This mass loss occurs mainly due to water loss since the other components that can be lost (e.g., aromas, flavors and gaseous products of respiration) are practically undetectable in terms of mass loss (Souza et al. 2010).

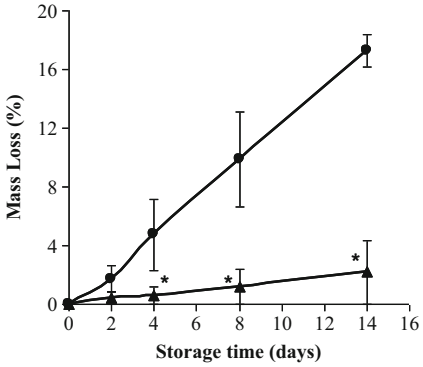

Fig 1 Mass loss of coated (*triangle*) and uncoated (*circle*) fresh-cut mangoes during 14 days of storage at 8 °C. Each *data point* is the average of five determinations and the *error bars* show the standard deviation. Significant difference between means (*$p<0.05$) compared to uncoated fresh-cut mangoes

It can be observed that at the fourth day of storage onwards there is a significant difference ($p<0.05$) between the coated and uncoated fresh-cut mangoes, being this difference higher at the day 14, being the values of 7.16±2.18 % for fresh-cut coated mangoes and 17.25±1.10 % for the uncoated mangoes. Results showed that the nanomultilayer coating acts as a barrier to water vapor avoiding the water loss (mass loss). Results are in agreement with the barrier capacity to water vapor of alginate-chitosan nanomultilayer presented by Carneiro-da-Cunha et al. (2010), who obtained values of WVP of 8.8×10^{-14} g m^{-1} s^{-1} Pa^{-1}. This improved barrier property of alginate-chitosan nanolayer as compared to the conventional coating of alginate (WVP of 24.6×10^{-11} g m^{-1} s^{-1} Pa^{-1}) (Hambleton et al. 2009) and chitosan (WVP of 3.8×10^{-11} g m^{-1} s^{-1} Pa^{-1}) (Souza et al. 2009) can be explained this effect by the electrostatic interactions between adjacent layers of alginate and chitosan, which lead to an increase of tortuosity of the matrix thus decreasing the diffusion of molecules through the matrix materials.

Medeiros et al. (2012a) showed that coated fresh-cut pears with κ-carrageenan-lysozyme-based nanomultilayer has low mass loss (3.5±0.6 %) after 7 days of storage when compared with uncoated fresh-cut pears (13.3±1.3 %). The WVP values of nanomultilayer coatings are lower than the values obtained for "conventional" coatings such as alginate and chitosan, 24.6 and 3.8×10^{-11} g m^{-1} s^{-1} Pa^{-1}, respectively.

Titratable Acidity and pH

The citric and the malic acids are the major organic acids present in mangoes and their content decreases during the ripening (Souza et al. 2010). This behavior is a natural tendency of the fruit, since the organic acids tend to decrease during maturation, as they are used as substrate for respiration (Calegaro et al. 2002). To avoid an excessive reduction of the electron transport chain and the subsequent production of free radicals, the mangoes when suffer mechanical injury or under refrigeration induce the synthesis of alternative oxidase (AOX) (Tovar et al. 2001).

Figure 2a shows that regardless the treatment, mangoes showed a reduction in the content of organic acids during storage time, being more pronounced in the uncoated mangoes ($p<0.05$), showing that alginate-chitosan nanomultilayer coating is effective in reducing the loss of acidity in mangoes, producing larger deviations in the metabolism of organic acids. This can be related with modification of the internal atmosphere of the mangoes promoted by the nanomultilayered coatings due to their gas mass transfer resistance and thereby minimize the amount of available oxygen. This will decrease the respiration rate and consequently the velocity of the electron transport chain, leading to a greater use of AOX. Pectin-chitosan-based nanomultilayered coating was effective in maintaining higher levels of titratable acidity in coated whole mangoes when compared to uncoated (Medeiros et al. 2012b).

Usually, during storage, an increase of pH occurs as a consequence of the fruits' ripening. In general, decomposition processes caused by hydrolysis, oxidation, or fermentation modifies the concentration of hydrogen ions in the food (Souza et al. 2010). Maintaining a more acidic pH in the coated fruit is beneficial because an acidic pH can guarantee a more controlled microbial growth (Tovar et al. 2001). In the present work, and in agreement with the previously described observations, an increase in pH was also registered for uncoated fresh-cut mangoes, whereas in the coated fresh-cut mangoes, no variation in pH was observed throughout storage time (Fig. 2b). This contributed to the maintenance of a favorable lower pH value in the coated fruits, being at same time a consequence and a reason for a higher stability of the fruits throughout storage under these circumstances. Equally, a chitosan- (β-cyclodextrin + trans-cinnamaldehyde complex)-pectin-based multilayer coating did not affect the pH of fresh-cut papaya and the values remained constant during 15-day storage (Brasil et al. 2012).

Total Soluble Solids

Total soluble solids (TSS) are used as an indicator of total sugar content in fruits providing information about the fruit maturation state. TSS are formed by water-soluble compounds of substances like sugars, acids, vitamin C, and some pectins (Souza et al. 2010). Therefore, an increase in TSS is a normal trend during fruit ripening and results from gradual degradation of starch and cell wall materials accumulated during preharvest period (Kienzle et al. 2011).

TSS values for coated and uncoated fresh-cut mangoes ranged from 11.3 to 12.3 °Brix at day 0; these results are ideal for marketing and consumption of mangoes 'Tommy Atkins'

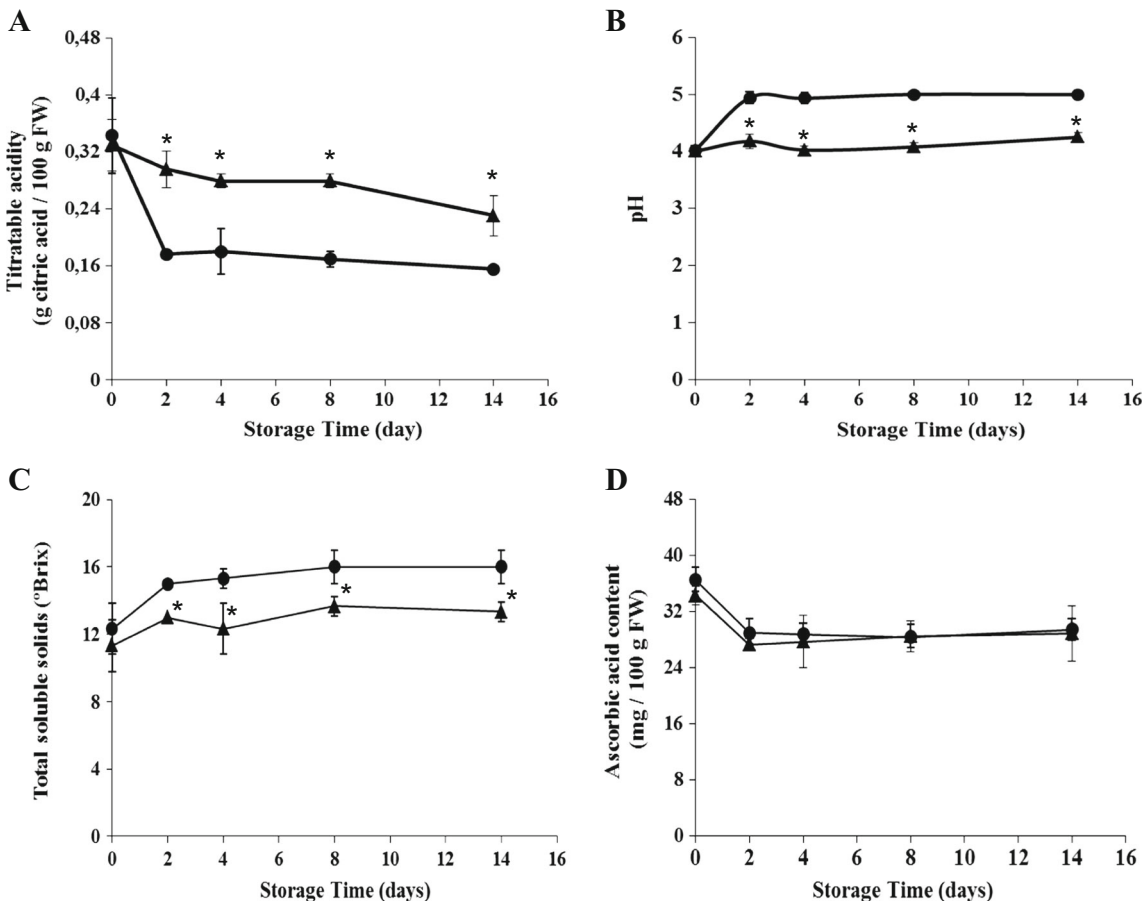

Fig 2 **a** Titratable acidity; **b** pH; **c** total soluble solids; and **d** vitamin C content of coated (*triangle*) and uncoated (*circle*) fresh-cut mangoes during 14 days of storage at 8 °C. Each *data point* is the average of five determinations and the *error bars* show the standard deviation. Significant difference between means (*$p<0.05$) compared to uncoated fresh-cut mangoes. *FW* fresh weight

(Hojo et al. 2009) and are in line with the values reported by Siddiq et al. (2013).

During storage, the increase of TSS both in coated and uncoated mangoes (Fig. 2c) was observed, leading the application of the multilayer coating to a small increase ($p<0.05$) when compared to the values obtained to uncoated mangoes. The lower values of TSS for the coated fruits are a direct result of water vapor barrier achieved by the application of the coating. Also, the decreased O_2 and CO_2 transfer rate due to the presence of the coating imposes a reduction in the metabolic activity of the fruits, leading to a further slowdown of polysaccharide degradation reactions and, therefore, to a lower TSS value (Medeiros et al. 2012b).

Vitamin C

Fruits are a natural source of ascorbic acid (vitamin C) being known that its level decrease during processing and ripening. Moreover, due to faster oxidative processes occurring in fresh-cut products, higher losses of vitamin C are expected (Djioua et al. 2009). This vitamin is one of the most sensitive components of foods and is often used as an indicator of the severity of post-harvest fruit damage (Özkan et al. 2004).

Figure 2d shows the concentrations of vitamin C for coated and uncoated fresh-cut mangoes. It can be notice that there are no significant differences ($p>0.05$) between vitamin C content of coated and uncoated fresh-cut mangoes during the storage time period under analysis. A reduction in the levels of vitamin C can be observed on the second day of storage for both coated and uncoated fresh-cut mangoes. Similar results were found for fresh-cut cantaloupe where the vitamin C content also decreased significantly during the time of evaluation and a chitosan- (β-cyclodextrin + trans-cinnamaldehyde complex)-pectin-based multilayer edible coating did not help retaining vitamin C (Martiñon et al. 2014); this same coating system provided different results with papaya where the multilayer fruits had higher vitamin C content than uncoated papaya during storage (Brasil et al. 2012). Equally, the use of alginate-based edible coatings reduced vitamin C loss of fresh-cut mangoes (Robles-Sánchez et al. 2013). The values obtained are higher than those reported by Sellamuthu et al. (2013) in 'Tommy Atkins' mangoes (17.01 mg/100 g) and by Liu et al. (2013) in different mango cultivars (Tainong No.1,

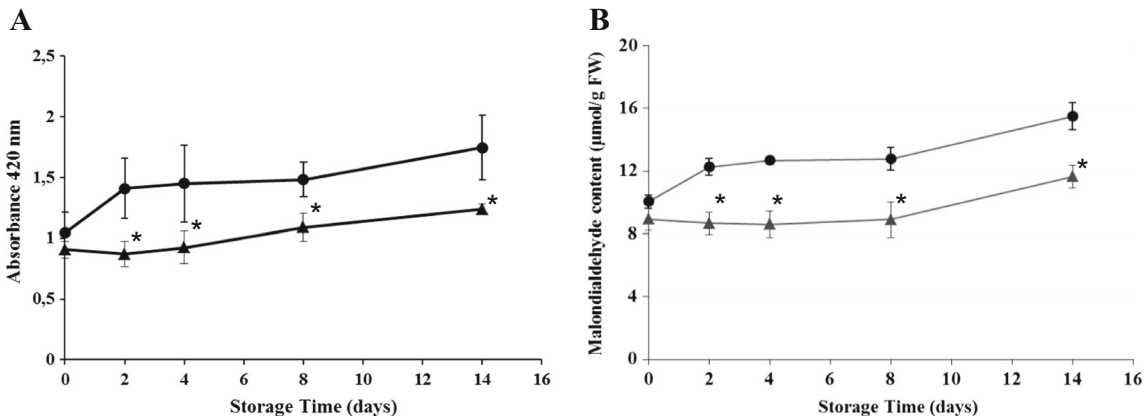

Fig 3 **a** Browning rate and **b** malondialdehyde content of coated (*triangle*) and uncoated (*circle*) fresh-cut mangoes during 14 days of storage at 8 °C. Each *data point* is the average of five determinations and the *error bars* show the standard deviation. Significant difference between means (*$p<0.05$) compared to uncoated fresh-cut mangoes. *FW* fresh weight

Irwin, JinHwang, and Keitt) with vitamin C contents ranging between 3.20 and 23.80 mg/100 g.

Browning

One of the main problems of fresh-cut mango during storage is tissue browning that can limit the shelf life of fresh-cut pieces (Robles-Sánchez et al. 2013). Tissue browning is easily detected and rejected by consumers. Cutting causes physiological and metabolic changes due to the activity of oxidation enzymes such as polyphenol oxidase and peroxidase, resulting in tissue browning (Oms-Oliu et al. 2010). The tissue browning of fresh-cut mangoes during storage time was also observed by Robles-Sánchez et al. (2013).

The browning increased with storage time for both coated and uncoated fresh-cut mangoes; however, for coated fresh-cut mangoes, it was lower than that of uncoated fresh-cut mangoes during the whole storage period (Fig. 3a). The chitosan-alginate nanomultilayer coating helps reduce the browning of the pulp probably by preventing oxidative or enzymatic browning due to limited O_2 transfer from the surrounding atmosphere.

Li et al. (2009) evaluated the effect of a packaging material obtained from a mixture of nano-Ag, nano-TiO_2, kaolin, and polyethylene on the shelf life of Chinese jujubes and a similar effect was observed in 12 day, the browning of jujube with this nano-packaging material was lower compared with the control. Application of the chitosan- (β-cyclodextrin + trans-cinnamaldehyde complex)-pectin-based multilayer edible coating helped in preserving the color attributes of fresh-cut papaya (Brasil et al. 2012).

Malondialdehyde Content (MDA)

As the final product of lipid peroxidation, MDA is often used as an index of cell oxidative damage under environmental stress (Xu et al. 2009). MDA content (Fig. 3b) was higher in uncoated fresh-cut mangoes when compared to the coated fresh-cut mangoes. While a significant increase in the MDA content of uncoated fresh-cut mangoes is observed already in

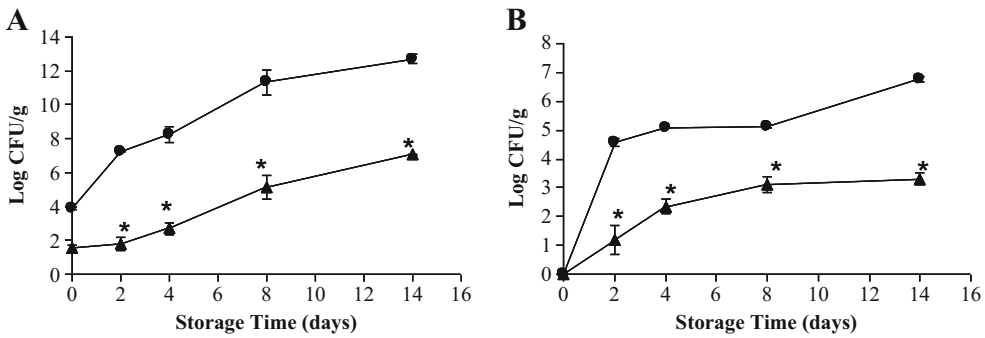

Fig 4 Microbiological counting of mesophilic (**a**) and psychotropic (**b**) microorganisms on coated (*triangle*) and uncoated (*circle*) fresh-cut mangoes during 14 days of storage at 8 °C. Each *data point* is the average of five determinations and the *error bars* show the standard deviation. Significant difference between means (*$p<0.05$) compared to uncoated fresh-cut mangoes. *FW* fresh weight

the second day of storage, for coated fresh-cut mangoes such an increase was only observed from the eighth day of storage onwards, suggesting that the use of this coating inhibits senescence processes involving lipid peroxidation. Similar results were found by Djioua et al. (2009), who analyzed the effect of hot water treatments of fresh-cut mangoes of the 'Keitt' variety. This behavior can be explained by the reduction in respiratory rate, since during the respiration process formation of high potential pro-oxidant molecules can occur (Ferrari 1998).

Microbiological Analyses

Fresh-cut fruits are a friendly substrate for microbial growth due to the high amount of moisture and sugars present on their surface, being expected changes in the microbial population of packaged fresh-cut produce (Wu et al. 2012). Figure 4a, b presents results for the growth of mesophilic and psychrophilic microorganisms (expressed as log CFU/g mango) on coated and uncoated fresh-cut mangoes during 14 days of storage. In the samples where a nanomultilayered coating was applied, lower counts of mesophilic microorganisms were consistently found when compared to uncoated samples (Fig. 4a); this antimicrobial/bacteriostatic effect persists until the last day of storage. Similar results were obtained by Chien et al. (2007) who evaluated the effect of chitosan coatings on mangoes microbial growth, attributing the reduced microbial growth to the antimicrobial activity of chitosan. The most widespread hypothesis for the antimicrobial activity of chitosan is the change in cell permeability due to the interactions between the positively charged chitosan molecules at acidic pH and the surface of bacterial cells that have a residual negative charge. This interaction leads to leakage of electrolytes and intracellular protein constituents (Mohamed et al. 2013; Pereda et al. 2011; Devlieghere et al. 2004).

Toxic substances may be produced when microbiological counts exceed 6.0 log CFU/g (Wu et al. 2012). In this present work after 8 days of storage, mesophiles did not exceed 6.0 log CFU/g for coated fresh-cut mangoes, while for uncoating fresh-cut mangoes this value has been exceeded after 2 days of storage (7.2 CFU/g).

The growth of psychrophilic microorganisms on fresh-cut mangoes is shown in Fig. 4b. A lag phase preceding the growth of psychrophilic microorganisms was not observed. The growth rate for psychrophilic microorganisms was higher in uncoated fresh-cut mangoes than in coated fresh-cut mangoes, as perceived by the slopes of the respective growth curves. These results suggest that application of the multilayered coating is an effective means to inhibit the growth of spoilage microorganisms (both mesophilic and psychroplilic) in fresh-cut mangoes. Brasil et al. (2012) and Martiñon et al. (2014) reported that chitosan- (β-cyclodextrin + trans-cinnamaldehyde complex)-pectin-based multilayered coating was effective in inhibiting aerobic bacteria, psychrophilic bacteria, yeast, and mold growth in fresh-cut papaya and cantaloupe.

Conclusion

The alginate-chitosan-based nanomultilayer coating improved the microbiological and physicochemical quality of fresh-cut mangoes during storage time. Microbiological analyses allowed concluding that the shelf life of fresh-cut mangoes could be extended up to 8 days at 8 °C when compared with uncoated fresh-cut mangoes (<2 days). The maintenance of quality and the extension of the shelf life of coated fresh-cut mangoes show that nanomultilayer coatings can be considered as a safe and effective treatment for fresh-cut mangoes.

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References

Azarakhsh, N., Osman, A., Ghazali, H. M., Tan, C. P., & Adzahan, N. M. (2014). Lemongrass essential oil incorporated into alginate-based edible coating for shelf-life extension and quality retention of fresh-cut pineapple. *Postharvest Biology and Technology, 88*, 1–7.

Bajaj, K. L., & Kauer, G. (1981). Spectrophotometric determination of L-ascorbic acid in vegetables and fruits. *Analyst, 106*, 117–120.

Brasil, I. M., Gomes, C., Puerta-Gomez, A., Castell-Perez, M. E., & Moreira, R. G. (2012). Polysaccharide-based multilayered antimicrobial edible coating enhances quality of fresh-cut papaya. *LWT—Food Science and Technology, 47*, 39–45.

Calegaro, J. M., Pezzi, E., & Bender, R. J. (2002). Utilização de atmosfera modificada na conservação de morangos em pós-colheita. *Pesquisa Agropecuária Brasileira, 37*, 1049–1055.

Carneiro-da-Cunha, M. G., Cerqueira, M. A., Souza, B. W. S., Carvalho, S., Quintas, M. A. C., Teixeira, J. A., & Vicente, A. A. (2010). Physical and thermal properties of a chitosan/alginate nanolayered PET film. *Carbohydrate Polymers, 82*, 153–159.

Chien, P., Sheu, F., & Yang, F. (2007). Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. *Journal of Food Engineering, 78*, 225–229.

Chiumarelli, M., Ferrari, C. C., Sarantópoulos, C. I. G. L., & Hubinger, M. D. (2011). Fresh cut 'Tommy Atkins' mango pre-treated with citric acid and coated with cassava (ManihotesculentaCrantz) starch or sodium alginate. *Innovative Food Science and Emerging Technologies, 12*, 381–387.

Devlieghere, F., Vermeulen, A., & Debevere, J. (2004). Chitosan: antimicrobial activity, interactions with food components and

applicability as a coating on fruit and vegetables. *Food Microbiology, 21*, 703–714.

Djioua, T., Charles, F., Lopez-Lauri, F., Filgueiras, H., Coudret, A., FreireJr, M., Ducamp-Collin, M. N., & Sallanon, H. (2009). Improving the storage of minimally processed mangoes (*Mangifera indica* L.) by hot water treatments. *Postharvest Biology and Technology, 52*, 221–226.

Ferrari, C. K. B. (1998). Oxidação lipídica em alimentos e sistemas biológicos: mecanismos gerais e implicações nutricionais e patológicas. *Revista de Nutrição, 11*, 3–14.

Hambleton, A., Frédéric Debeaufort, F., Bonnotte, A., & Voilley, A. (2009). Influence of alginate emulsion-based films structure on its barrier properties and on the protection of microencapsulated aroma compound. *Food Hydrocolloids, 23*(8), 2116–2124.

Hojo, R. H., São-José, A. R., Hojo, E. T. D., Alves, J. F. T., Rebouças, T. N. H., & Dias, N. O. (2009). Qualidade de manga 'Tommy Atkins' pós-colheita com uso de cloreto de cálcio na pré-colheita. *Revista Brasileira Fruticultura. Jaboticabal, 31*, 62–70.

Instituto Adolfo Lutz. (1985). Normas Analíticas: Métodos químicos e físicos para análises de alimentos, vol. 1, third ed. São Paulo, Brazil, 533pp

Kienzle, S., Sruamsiri, P., Carle, R., Sirisakulwat, S., Spreer, W., & Neidhart, S. (2011). Harvest maturity specification for mango fruit (*Mangifera indica* L. 'Chok Anan') in regard to long supply chains. *Postharvest Biology and Technology, 61*, 41–55.

Li, H., Li, F., Wang, L., Sheng, J., Xin, Z., Zhao, L., Xiao, H., Zheng, Y., & Hu, Q. (2009). Effect of nano-packing on preservation quality of Chinese jujube (*Ziziphus jujuba* Mill. var. inermis (Bunge) Rehd). *Food Chemistry, 114*, 547–552.

Liu, F. X., Fu, S. F., Bi, X. F., Chen, F., Liao, X. J., Hu, X. S., & Wu, J. H. (2013). Physico-chemical and antioxidant properties of four mango (*Mangifera indica* L.) cultivars in China. *Food Chemistry, 138*, 396–405.

Martiñon, M. E., Moreira, R. G., Castell-Perez, M. E., & Gomes, C. (2014). Development of a multilayered antimicrobial edible coating for shelf life extension of fresh-cut cantaloupe (*Cucumis melo* L.) stored at 4 °C. *LWT - Food Science and Technology, 55*, 341–350.

Medeiros, B. G. S., Pinheiro, A. C., Teixeira, J. A., Vicente, A. A., & Carneiro-da-Cunha, M. G. (2012a). Polysaccharide/protein nanomultilayer coatings: construction, characterization and evaluation of their effect on 'Rocha' pear (*Pyrus communis* L.) shelf-life. *Food Bioprocess Technology, 5*, 2435–2445.

Medeiros, B. G. S., Pinheiro, A. C., Carneiro-da-Cunha, M. G., & Vicente, A. A. (2012b). Development and characterization of a nanomultilayer coating of pectin and chitosan—evaluation of its gas barrier properties and application on 'Tommy Atkins' mangoes. *Journal of Food Engineering, 110*, 457–464.

Medeiros, B. G. S., Souza, M. P., Pinheiro, A. C., Bourbon, A. I., Cerqueira, M. A., Vicente, A. A., & Carneiro-da-Cunha, M. G. (2014). Physical characterisation of an alginate/lysozyme nanolaminate coating and its evaluation on 'Coalho' cheese shelf life. *Food Bioprocess Technology, 7*, 1088–1098.

Mohamed, C., Clementine, K. A., Didier, M., Gérard, L., & Noëlle, D. C. M. (2013). Antimicrobial and physical properties of edible chitosan films enhanced by lactoperoxidase system. *Food Hydrocolloids, 30*, 576–580.

Oms-Oliu, G., Rojas-Graü, M. A., González, L. A., Varela, P., Soliva-Fortuny, R., Hernando, I. H. M., et al. (2010). Recent approaches using chemical treatments to preserve quality of fresh-cut fruit: a review. *Postharvest Biology and Technology, 57*, 139–148.

Özkan, M., Kirca, A., & Cemeroglu, B. (2004). Effects of hydrogen peroxide on the stability of ascorbic acid during storage in various fruit juices. *Food Chemistry, 88*, 591–597.

Pereda, M., Ponce, A. G., Marcovich, N. E., Ruseckaite, R. A., & Martucci, J. F. (2011). Chitosan-gelatin composites and bi-layer films with potential antimicrobial activity. *Food Hydrocolloids, 25*, 1372–1381.

Robles-Sánchez, R. M., Rojas-Graü, M. A., Odriozola-Serrano, I., González-Aguilar, G., & Martin-Belloso, O. (2013). Influence of alginate-based edible coating as carrier of antibrowning agents on bioactive compounds and antioxidant activity in fresh-cut Kent mangoes. *LWT - Food Science and Technology, 50*, 240–246.

Sellamuthu, P. S., Denoya, G. I., Sivakumar, D., Polenta, G. A., & Soundy, P. (2013). Comparison of the contents of bioactive compounds and quality parameters in selected mango cultivars. *Journal of Food Quality, 36*, 394–402.

Shahnawaz, M., Sheikh, S. A., Panwr, A. A., Khaskheli, S. G., & Awan, F. A. (2012). Effect of hot water treatment on the chemical, sensorial properties and ripening quality of Chaunsa mango (*Mangifera indica* L.). *Journal of Basic Applied Sciences, 8*, 328–333.

Siddiq, M., Sogi, D. S., & Dolan, K. D. (2013). Antioxidant properties, total phenolics, and quality of fresh-cut 'Tommy Atkins' mangoes as affected by different pre-treatments. *LWT - Food Science and Technology, 53*, 156–162.

Souza, B. W. S., Cerqueira, M. A., Casariego, A., Lima, A. M. P., Teixeira, J. A., & Vicente, A. A. (2009). Effect of moderate electric fields in the permeation properties of chitosan coatings. *Food Hydrocolloids, 23*, 2110–2115.

Souza, M. P., Cerqueira, M. A., Souza, B. W. S., Teixeira, J. A., Porto, A. L. F., Vicente, A. A., & Carneiro-da-Cunha, M. G. (2010). Polysaccharide from *Anacardium occidentale* L. tree gum (Policaju) as a coating for Tommy Atkins mangoes. *Chemical Papers, 64*, 475–481.

Tovar, B., Garcıa, H. S., & Mata, M. (2001). Physiology of pre-cut mango II. Evolution of organic acids. *Food Research International, 34*, 705–714.

Wu, Z. S., Zhang, M., & Wang, S. (2012). Effects of high pressure argon treatments on the quality of fresh-cut apples at cold storage. *Food Control, 23*, 120–127.

Xu, W. T., Peng, X., Luo, Y. B., Wang, J., Guo, X., & Huang, K. L. (2009). Physiological and biochemical responses of grapefruit seed extract dip on 'Redglobe' grape. *LWT - Food Science and Technology, 42*, 471–476.

Zhan, L., Li, Y., Hu, J., Pang, L., & Fan, H. (2012). Browning inhibition and quality preservation of fresh-cut romaine lettuce exposed to high intensity light. *Innovative Food Science and Emerging Technologies, 14*, 70–76.