



Impact of edible coatings based on cassava starch and chitosan on the post-harvest shelf life of mango (*Mangifera indica*) ‘Tommy Atkins’ fruits

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Abstract

The mango has short postharvest shelf life which varies from 6 to 10 days at room temperature in its fresh form. The objective of this study was to evaluate the effect of usage of edible coatings based of cassava starch and chitosan on post-harvest shelf life of mango. Mangoes of Tommy Atkins variety were covered with nine different formulations of coatings in a factorial block experimental design. The fruits were stored at 25 °C and evaluated during storage for the weight loss (%), color parameters, sensory attributes evaluated by trained panel, the rate of CO₂ production and microbiological contamination on the peels. Results analysed by linear regressions and ANOVA, demonstrated that chitosan showed significant effect on weight loss (%) and on values of L*, a*, b*, chroma, °hue, peel color, texture, aroma and time which correlated well for suitability of fruit consumption. The formulation containing 0.25% of chitosan and 0.5% of cassava starch showed most favorable results as it presented a post-harvest shelf life of 3 days more than the control fruits and lower rates of CO₂ production, showing that this coating actually decreased the rate of the respiratory processes of mango, without compromising the proper ripening of the fruit.

Keywords: *Mangifera indica*; edible coatings; modified atmosphere; chitosan; cassava starch; physico-chemical characteristics.

Practical Application: This work on finding a suitable edible coating formulation for fresh mango ‘Tommy Atkins’ fruits has large practical application as it extends the postharvest shelf life of mango fruits by an extra three days at ambient conditions. The ripe mango fruit quality is achieved after 12 days of storage when an edible formulation containing 0.25% chitosan and 0.5% cassava starch was used as coating of the fruit. Thus it is obvious that this work presents enormous practical applications.

1 Introduction

The mango (*Mangifera indica* L.) is one of the most consumed fruits in the world, either in its fresh form or as derived products, such as pulp, juice, nectar, jam and jelly (Canuto et al., 2009). Brazil is a major producer of mango (Pinto et al., 2011; Souza et al., 2011) and it exported 156 thousand tons of mango in the year 2015, leading the country to occupy 7th place among the world's largest mango exporters (Singh et al., 2013). Moreover, due to the large European demand for tropical fruits, this market still presents large growth potential (Hojo et al., 2009). However, the fruits marketed especially in the international market, require high quality products (Gomes et al., 2010). Thus, in order for the country to participate more effectively in the international market, it is necessary to use technologies that extend and guarantee fruit quality after harvest along with an increased shelf life (Hojo et al., 2009).

At room temperature the mango has a short postharvest life, varying from 6 to 10 days, reaching 14 days at low temperatures (Yamashita et al., 2001). The high susceptibility of the mango to the cold injury (Miguel et al., 2011) and post-harvest diseases

(Singh et al., 2013) are among the main factors that limit the distribution and commercialization of the product, causing damages to agriculture and trade. In Brazil, the post-harvest losses of mangoes are considerable, due to several factors mainly due to the high perishability and susceptibility of the mango to diseases and physiological deterioration of the fruit due to ripening, resulting in consequent loss of water, wrinkling and wilting, which reduces commercial value of the fruits substantially (Assis et al., 2004; Fischer et al., 2009; Neves et al., 2007).

To increase shelf life of fruits, including mangoes, currently plastic films with selective permeability to CO₂ and O₂ are utilized. These materials produce a modified atmosphere around the fruits, decreasing the concentration of O₂ and increasing the CO₂ concentration. As a consequence, there is a reduction in respiratory rate and delay in fruit maturation (Singh et al., 2013). The commonly used films are polyethylene, polypropylene, and various polymers (Neves et al., 2002; Sanches et al., 2011). However, environmental reasons associated with new trends in the consumer market have led the food industry to engage

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in the development of nature friendly materials with a lower environmental impact (Lorevice et al., 2014; Souza et al., 2012; Mali et al., 2010; Farias et al., 2012).

Thus, in recent years, edible coverings and biodegradables have generated interest of the food industry because of its advantages that differentiate them from non-edible packaging. These materials usually improve the visual appearance of the fruits to which they are applied; they are non-toxic, can be ingested along with the product, they are of low cost and have low or no environmental impact (Botrel et al., 2007). If these are made with industrial waste, they contribute further to the preservation of the environment.

Edible films and coverings can be made from various types of materials. The commonly used are polysaccharides (cellulose, starches and derivatives, vegetable or microbial gums, etc.), proteins (gelatin, zein, gluten, etc.) and lipids (waxes and lipid derivatives) (Fakhouri et al., 2007). However, the commercial use of edible films formulated from the usual biodegradable matrices has been limited by problems related to the usually poor mechanical properties and poor moisture barrier compared to synthetic polymers (Souza et al., 2012).

One of the materials still unexplored in Brazil as a component of fruit biofilms is chitosan, although some national and international research work has shown that it can be very advantageous in the development of new materials for use in technological and biomedical applications (Laranjeira & Fávere, 2009; Assis, 2010). Chitosan is the second most abundant polysaccharide available in nature and can be obtained from the deacetylation reaction of chitin (Aider, 2010). Chitin has a crystalline or amorphous structure, insoluble in water, organic solvent and some dilute acids and bases (Antonino, 2007). While chitin, due to its high degree of acetylation, is a highly insoluble product, chitosan is soluble in acidic aqueous solutions, such as those prepared with acetic acid (Goy et al., 2004). The use of chitosan as a source of antifungal and bactericidal action is widely reported in the literature (No et al., 2002; Shen et al., 2010). Moreover chitosan also reduces the permeability of the material to water and oxygen vapors (Bangyekan et al., 2006; Maciel et al., 2012; Zamudio-Flores et al., 2010).

The use of chitosan as an edible cover allied to other materials such as cassava starch can bring in significant results. Starch alone functions in prolonging the shelf life of some fruits, although there are limitations with respect to its mechanical and barrier properties since it is highly susceptible to moisture, has poor protection of water vapor (Guilbert et al., 1996) and do not provide food protection to microorganisms (Aider, 2010; Bourtoom & Chinnan, 2008; Suzuki et al., 2005).

Currently, all commercially produced chitin is obtained from crabs and shrimp shells, which result from the processing industry of these crustaceans (Berger et al., 2011). As Brazil is a major shrimp producer, the use of these residues in the form of edible coatings, besides being of great importance for the preservation of the environment, can constitute an efficient and economic method of extending the post-harvest shelf life of tropical fruits, including mango, fruit of significant economic importance to the country.

Thus the objective of the present research was to develop edible coatings based on cassava starch and chitosan, and to verify their impact of usage on the physico-chemical characteristics and post-harvest shelf life of the mango of 'Tommy Atkins' variety.

2 Material and methods

2.1 Materials

Mango (*Mangifera indica*) fruits of the 'Tommy Atkins' variety were obtained from the orchard of Fazenda Frutal, located in the municipality of Neópolis-SE. The fruits were harvested at half-ripe maturation stage and were brought by land transportation to the Laboratory of Processing of Plant Origin Products pertaining to the Food Technology Department of the Federal University of Sergipe, São Cristóvão.

For the preparation of coatings, commercial chitosan obtained from the industry, Polymar Ciência e Nutrição S/A was used, and the cassava starch used was of the Dinha Bá brand, produced and purchased in the state of Sergipe.

2.2 Methods

Nine formulations of different edible coatings were developed by combining three levels of cassava starch (0%, 0.25% and 0.5%) and with three levels of chitosan (0%, 0.25% and 0.5%), by means of a complete factorial design (Hayashi et al., 2012), resulting in obtaining the 9 treatments as specified in Table 1.

2.3 Sanitization of fruits, preparation and application of coatings

The sanitization of the fruits was carried out in a solution of 200 mg/L of active chlorine solution for 10 minutes. Later the fruits were rinsed in 3 mg/L of active chlorine solution and placed in trays for drying at ambiente temperature (29 ± 2 °C).

The coatings were prepared following procedures described by Vásconez et al. (2009). Initially, the chitosan was dispersed in 1% (w/v) of aqueous acetic acid solution. In the sequence, under gentle stirring, glycerol was added until the concentration of this reagent in the final solution of chitosan was equal to 1.28% (w/v). The cassava starch solutions were prepared by dissolving the starch in aqueous solution containing 0.64% (w/v) glycerol followed by heating the suspension in a shaking water bath until complete gelatinization of the cassava starch was achieved. The coating formulations (Table 1) were prepared by

Table 1. Complete block design used for the development of nine formulations of edible coatings showing the actual values of the independent variables, namely, starch and chitosan concentrations.

Factor A	Factor B		
	Cassava Starch ¹		
Chitosan ¹	0%	0.25%	0.5%
0%	Treatment 1 (Control)	Treatment 2 (T2)	Treatment 3 (T3)
0.25%	Treatment 4 (T4)	Treatment 5 (T5)	Treatment 6 (T6)
0.50%	Treatment 7 (T7)	Treatment 8 (T8)	Treatment 9 (T9)

¹Concentration expressed as g/100g of final solution.

adding the chitosan solutions to the gelatinized starch solutions until the complete homogenization.

The sanitized fruits were immersed in different coating solutions, where the fruits were maintained for three minutes and were then spread on a nylon net until the surface was perfectly dried. The control treatment was done by immersing the fruits in aqueous solution without addition of any coating material.

2.4 Assessment of post-harvest life

The fruits of all previously described treatments were placed in open aluminum trays and stored in BOD chambers at 25 °C. Physico-chemical, sensorial and microbiological analyses were performed in each of the treatments listed in Table 1, at the beginning and during the days of storage.

Weight loss

The weight loss of the fruits was determined following the methodology of Scanavaca et al. (2007), using an electronic semi-analytical balance, with a sensitivity of 0.01g. A representative sample of each treatment, consisting of a tray containing 4 fruits, was weighed on the initial day and at 5, 7, 8, 9, 12 and 14 days of storage of the product. Weight loss during storage was expressed as a percentage (%) relative to the initial weight of the sample.

Color

The color of the mango peel in each treatment was evaluated in the Konica Minolta CR - 400 colorimeter. The fruits were divided and demarcated in eight regions and the readings were made in the central point of each demarcation, followed by calculation of mean value and standard error of the readings of each parameter

(L*, a*, B*, chroma and °hue). The analyses were performed in a representative sample of each treatment, containing 4 fruits on the initial day and at 5, 7, 8, 9, 12 and 14 days.

Sensory analysis

The development of fruit maturation along storage was evaluated by a trained sensory panel team, composed of 8 members. The training of the sensorial team was conducted by presenting to the members, mango fruit in three stages of maturation - green, mature ripe and over-ripe. Also the detailed description of the color, aroma and texture of the fruits in each maturation stages - green, mature ripe and over-ripe was presented to the panel members After the training period, a screening test was applied to the members as described by Biasoto et al. (2010), and individuals who demonstrated good discriminative ability ($pF_{sample} \leq 0.04$), good reproducibility in the trials ($pF_{frequency} \geq 0.05$) and consensus with the other team members for all evaluated attributes were selected to compose the trained sensory team which evaluated the post-harvest shelf life of the fruits of each treatment listed in Table 1.

The trained panel members evaluated the color of the peel, the texture and the aroma of the fruits expressing their perceptions in an unstructured scale of 9 cm, anchored at the far left, the middle and the right in green, mature ripe and over-ripe terms, respectively, and in the same way, the quality of the fruits was evaluated for their consumption, considering the three selected parameters, as shown in Figure 1. The samples were presented in a coded form with a three-digits number and the analysis performed by trained panel members. The sensorial analyses were also carried out in a representative sample of each treatment, containing 4 fruits on the initial day and at 5, 7, 8, 9, 12 and 14 days of fruit storage.

SENSORY EVALUATION OF THE MATURATION STAGE OF THE MANGO

Name: _____ Date: _____

Please initially evaluate the COLOR, TEXTURE and AROMA of each mango sample and then express in the appropriate scale the degree of maturation of the fruit

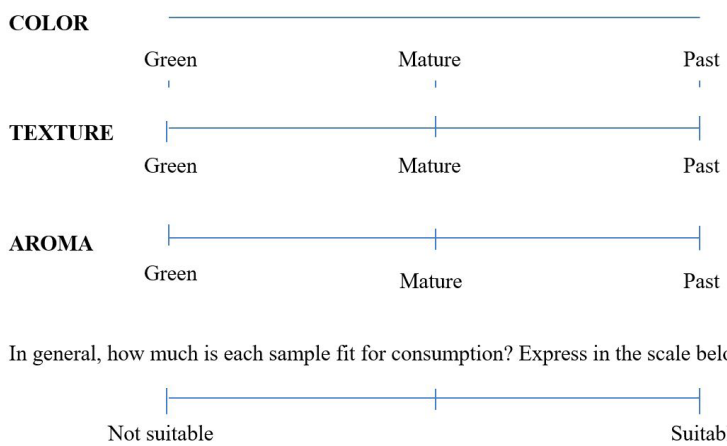


Figure 1. Sensory evaluation form used in the training and evaluation of the mango samples.

Respiration rate

The fruit respiration rate was determined in the control treatment and in the treatment that showed the best performance in the extension of post-harvest shelf life of mango, selected by analyzing all the treatments for weight loss, color change and sensorial quality of the fruit. To determine the fruit respiration rate in these two treatments, 3 fruits from each treatment were placed in glass bottles of 1.73 L each, which were hermetically sealed. After 30 minutes, 1 cm³ aliquot was injected with the aid of a chromatographic syringe in a 6100 GC (Young Lin) gas chromatograph, equipped with a thermal conductivity detector (TCD), using Rt-Q-Bond Plot column. The carrier gas was nitrogen, with a flow of 4 mL.min⁻¹; the electric current was maintained at 85 mA. The temperatures of the column, the injector and the detector were 30, 30 and 200 °C, respectively. The quantification was done by comparing the areas of the peaks produced by the samples, with areas of the peaks produced by the injection of standard aliquots of known concentrations. The respiration rate was calculated by means of the results expressed as a percentage of CO₂, taking into account the volume of the bottle, the mass of the mangoes and the time the bottle remained closed; these values were expressed in milligrams of CO₂ kg⁻¹.h⁻¹. The values were expressed in a chart by allocating the storage time in the abscissa and the mean respiration rate of each treatment in the ordinate, accompanied with their respective standard error of deviation. The analysis was performed in triplicate on days 0, 1, 4, 6, 8, 11 and 13 of fruit storage.

Microbiological analysis

Microbiological analysis was carried out throughout the storage both in the control treatment and in the treatment that demonstrated best performance in the extension of the postharvest shelf life of the mangoes. Thus, the fruits of these two treatments were immersed in sterile plastic bags containing 500 mL of peptone water. Later 25 mL of this solution was transferred to an erlenmeyer containing 225 mL of peptone water and a 10⁻¹ dilution was prepared, from which dilutions 10⁻² and 10⁻³ were obtained. The counting of aerobic mesophilic bacteria was carried out using the seeding technique in depth in Standard Medium for Counting (PCA), incubating at 35 °C for 48 hours and the results expressed in CFU.g⁻¹, according to methodology adapted from Maistro et al. (2012).

The total counts of molds and yeasts were carried out using a surface sowing method using Potato Dextrose Acidified (PDA) medium, incubated at 25 °C for 5 days according to the methodology adapted from Vantarakis et al. (2011) and the results were expressed in CFU.g⁻¹. For the counting of total and thermotolerant coliforms, the classical technique of the Most Probable Number was determined by using the lauryl sulfate tryptase, bright green and EC broths. The results were expressed in NMP/g (Oliveira et al., 2011). The analyses were performed in triplicate at the beginning and at the end of storage of the fruits.

2.5 Statistical analyses

Initially for the variables such as weight loss, color (L*, a*, b*, chroma and °hue) and sensory attributes (color, aroma, texture and consumption suitability), the effects of added chitosan and cassava starch were evaluated during all the storage period by Analysis of Variance (ANOVA) with three sources of variation, viz. chitosan, cassava starch and storage time. The interactions between the sources of variation were also included in the ANOVA. In case where the source of variation had a level of significance (p) equal to or lower than 5%, the effect was considered statistically significant.

In the sequence, linear regressions between the storage time of the mangoes and the results of the physico-chemical characteristics and sensorial attributes were calculated. The coefficient of angles of each regression was used to estimate the rate of change in the physico-chemical characteristics and sensorial attributes in the fruits during the storage of the same and to evaluate the impact of the treatments studied in the post-harvest shelf life of the mangoes. For each analysed variable (weight loss, L*, a*, b*, etc.), the lower the angular coefficient, the less altered the fruit would be and hence, the more efficient was the treatment in the extension of its post-harvest shelf life of the mango.

3 Results and discussion

3.1 Weight loss

Table 2 presents the results of ANOVA and it shows that in the present study, chitosan had a significant impact (p = 0.0002) on mango weight loss during storage of fruits for 14 days at 25°C, which did not occur with cassava starch (p = 0.6431). The fact

Table 2. Values and significance levels of F of the effect of chitosan and cassava starch on the physical and sensory parameters of mango.

Characteristics	Chitosan		Cassava starch		Interaction - Chitosan x Cassava starch	
	F	pF ¹	F	pF ¹	F	pF ¹
Weight loss	9.56	0.0002	0.44	0.6431	3.53	0.0095
L	59.8	< 0.0001	11.96	< 0.0001	4.21	0.0022
a*	8.63	0.0002	0.09	0.9136	25.01	< 0.0001
b*	35.8	< 0.0001	7.83	0.0004	0.90	0.4649
Chroma	88.8	< 0.0001	7.53	0.0006	32.77	< 0.0001
°hue	4.85	0.0079	0.4	0.6715	12.22	< 0.0001
Maturation rate (Color)	39.85	< 0.0001	7.93	0.0005	2.84	0.0252
Maturation rate (Texture)	7.31	0.0008	2.07	0.1281	5.23	0.0005
Maturation rate (Aroma)	14.2	< 0.0001	1.8	0.1682	3.31	0.0118
Consumption suitability	22.11	< 0.0001	0.99	0.3727	3.84	0.0049

¹ Level of significance of the F values for each variation source.

that the chitosan and cassava starch interaction was significant ($p = 0.0095$) indicates that the effect promoted by chitosan varied according to the level of cassava starch addition in the coatings.

Figure 2 shows the weight loss suffered by mango fruits in all treatments listed in Table 1 during storage at 25 °C for 14 days. The linear regressions shown in Figure 2, as well as their corresponding significance levels (p) and determination coefficients (R^2) are shown in Table 3. In each equation, the angular coefficient represents the rate of weight loss suffered during storage, and hence higher this coefficient, the faster is the weight loss.

From the data presented in Figure 2 and Table 3 it is possible to observe that, in general, coatings treated fruits with a higher level of chitosan (0.5%) presented lower weight loss, especially the T9 treatment, which was formulated with 0.5% cassava starch and 0.5% chitosan coating. On the 9th day of storage, the fruits

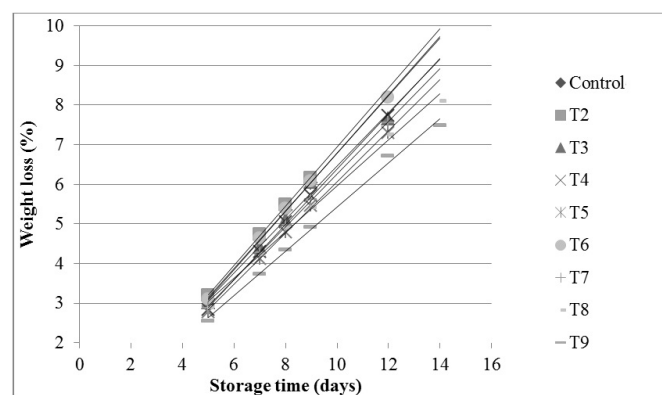


Figure 2. Linear regressions between weight loss and storage time of fruits of control and other different treatments. Chitosan (%) and cassava starch (%) concentrations were: Control (0, 0); T2 (0, 0.25), T3 (0, 0.5), T4 (0.25, 0), T5 (0.25, 0.25), T6 (0.25, 0.5), T7 (0.5, 0), T8 (0.5, 0.25), T9 (0.5, 0.5).

Table 3. Linear regressions between weight loss (%) of mango fruits subject to different treatments during 14 days of storage at 25 °C.

Treatments	Chitosan (%)	Cassava starch (%)	Equation
Control	0	0	$y = 0.7369x - 0.5782$ $R^2 = 0.9997$; $p = 0.0001$
T2	0	0.25	$y = 0.7469x - 0.5212$ $R^2 = 0.999$; $p = 0.0005$
T3	0	0.5	$y = 0.6728x - 0.2672$ $R^2 = 0.9915$; $p = 0.0003$
T4	0.25	0	$y = 0.6990x - 0.6083$ $R^2 = 0.9994$; $p < 0.0001$
T5	0.25	0.25	$y = 0.6467x - 0.4048$ $R^2 = 0.9992$; $p < 0.0001$
T6	0.25	0.5	$y = 0.7250x - 0.4687$ $R^2 = 0.9995$; $p < 0.0001$
T7	0.5	0	$y = 0.6659x - 0.3949$ $R^2 = 0.9992$; $p < 0.0001$
T8	0.5	0.25	$y = 0.582x + 0.137$ $R^2 = 0.9953$; $p < 0.0001$
T9	0.5	0.5	$y = 0.5585x - 0.1556$ $R^2 = 0.9961$; $p < 0.0001$

of the T9 treatment lost on an average approximately 4.9% of their initial weight, while the fruits of the control treatment had a weight loss of about 6.03% (Figure 1). In addition, treatments containing 0% and 0.25% of chitosan (T2, T3, T4 and T6) had similar weight loss to the control treatment, suggesting that the addition of chitosan at levels equal to or less than 0.25%, generally does not reduce mango weight loss.

Souza et al. (2011) when studying the effect of chitosan-based coatings on ‘Tommy Atkins’ mangoes, also observed a smaller loss of fresh mass throughout the storage of fruits treated with the coating; the mangoes treated with chitosan at 1% had a mass loss of 3.28%, while the fruits of the control treatment, which did not have any coating lost 3.8% of their initial weight.

3.2 Color

Table 2 shows that chitosan had a significant impact ($p \leq 0.05$) on parameters L^* , a^* , b^* , chroma and $^{\circ}$ hue, while cassava starch also had a significant impact ($p \leq 0.05$) on values of L^* and b^* . The effects of the coatings were more expressive on the parameters a^* , b^* and $^{\circ}$ hue, whose linear regressions as a function of storage time are presented in Table 4.

Observing the angular coefficients of the equations shown in Table 4, which represent the rates of change of the a^* parameter in the fruits peel, it is verified that the treatments that had 0.5% of chitosan in its formulations, such as the treatments T7, T8 and T9 (Table 1), showed alteration rates close to zero, indicating that their peels remained the same green color at the beginning of storage. The other treatments presented alteration rates of the parameter a^* similar to the control treatment. In general, these results suggest that at addition levels close to 0.5%, chitosan markedly reduces changes in the green tint present in the mango after harvesting. The data presented in Table 4 also concludes that mango fruits coated with 0.5% chitosan formulations also showed lower b^* and $^{\circ}$ hue alteration rates than the other treatments.

Similar results were also reported by Srinivasa et al. (2002), when they studied the application of chitosan biofilm and the use of low density polyethylene film in mangoes. The authors attributed this phenomenon to a retardation of the degradation of chlorophyll in the fruit peels due to the modified atmosphere created by the coatings. According to the authors, the increase in CO_2 levels and the O_2 consumption by the fruits promoted low O_2 levels around the stored fruits, which results in reducing the respiration rate of the mangoes. This maintained the green color of the peel, preventing the appearance of the yellow/orange color resulting from the carotenoids present in the mangoes.

3.3 Sensorial analysis

Table 2 shows that chitosan had a significant impact ($p \leq 0.05$) on the maturation rate of the mango and also on the speed at which the fruits became suitable for consumption when these parameters were evaluated for color, texture and aroma of the fruits. In turn, cassava starch had a significant impact ($p \leq 0.05$) only when the fruit maturation rate was judged through the peel. There were several significant chitosan starch interactions ($p \leq 0.05$) indicating that the effect promoted by chitosan varied depending on the level of starch addition in the coating.

Table 4. Linear regressions between the a*, b* and °hue values of mango fruits of the different treatments during 14 days of storage at 25 °C with respective coefficients of determination (R²) and levels of significance (p).

Treatments	Chitosan (%)	Cassava starch (%)	Equation parameter a*	Equation parameter b*	Equation parameter °hue
Control	0	0	$y = 0.8533x + 0.7881$, R ² = 0.9219; p = 0.0095	$y = 0.6518x + 24.4283$ R ² = 0.9170; p = 0.0104	$y = -0.9314x + 81.1371$ R ² = 0.8432; p = 0.0277
T2	0	0.25	$y = 1.2836x - 0.9719$ R ² = 0.9741; p = 0.0018	$y = 0.6493x + 26.9387$ R ² = 0.9039; p = 0.0130	$y = -1.6467x + 87.6214$ R ² = 0.9443; p = 0.0057
T3	0	0.5	$y = 1.5533x + 7.4317$ R ² = 0.9653; p = 0.0005	$y = 1.0549x + 21.8432$ R ² = 0.9331; p = 0.0017	$y = -1.2255x + 67.8458$ R ² = 0.9578; p = 0.0007
T4	0.25	0	$y = 1.0933x + 10.2656$ R ² = 0.9778; p = 0.0002	$y = 0.9737x + 20.2988$ R ² = 0.9248; p = 0.0022	$y = -0.5554x + 61.2231$ R ² = 0.9334; p = 0.0017
T5	0.25	0.25	$y = 1.1303x + 5.1643$ R ² = 0.8986; p = 0.0040	$y = 0.6895x + 23.3705$ R ² = 0.9567; p = 0.0007	$y = -0.4352x + 68.3166$ R ² = 0.5823; p = 0.0776
T6	0.25	0.5	$y = 0.7615x + 1.0308$ R ² = 0.8592; p = 0.0078	$y = 0.8316x + 20.7352$ R ² = 0.7807; p = 0.0196	$y = -0.6558x + 76.1199$ R ² = 0.9103; p = 0.0031
T7	0.5	0	$y = 0.1596x + 5.1514$ R ² = 0.8504; p = 0.0089	$y = 0.2074x + 21.7227$ R ² = 0.5190; p = 0.1063	$y = -0.1108x + 72.3689$ R ² = 0.1643; p = 0.4253
T8	0.5	0.25	$y = 0.0993x + 8.9628$ R ² = 0.6034; p = 0.0399	$y = 0.2927x + 23.2246$ R ² = 0.8011; p = 0.0065	$y = 0.0414x + 65.7613$ R ² = 0.0517; p = 0.6239
T9	0.5	0.5	$y = 0.0403x + 5.5122$ R ² = 0.7919; p = 0.0073	$y = 0.3537x + 22.3625$ R ² = 0.7677; p = 0.0097	$y = 0.1959x + 69.9648$ R ² = 0.7266; p = 0.0148

The maturation rates of the mangoes evaluated by the sensory panel through the color of the fruit peels are expressed through the linear regressions represented in Figure 3. In this figure, the 4.5 value of the maturation scale indicates that the mango was characterized with the ripe fruit color, according to the trained sensory panel. Values lower than 4.5 indicate that the mango was still green, whereas values higher than 4.5 indicate that the fruits were between the mature ripe and the late stage, an undesirable condition for both traders and consumers of the product. Thus, in Figure 3, the value 4.5 of the scale indicates the end of the post-harvest shelf life of each treatment.

It could be observed from Figure 3 that the fruits of the control treatment (without any coating), T2 (0.25% starch and 0% chitosan) and T3 (0.5% starch and 0% of chitosan) were already ripe on the 9th day of storage, while the fruits of the T6 treatment (0.5% starch and 0.25% chitosan) reached ripe maturity around the 12th day of storage of the fruits. This demonstrates that this treatment extended the shelf life of the mangoes by 3 days compared to the treatments without addition of chitosan. On the other hand, the fruits of the other treatments, especially the treatments containing 0.5% of chitosan (T7, T8 and T9) were not considered mature nor at the end of the storage, since these fruits had retained the green color in their peels. In fact, after the 12th day of storage, the panel members indicated the appearance of black spots in the peels of the fruits in the treatments containing 0.5% of chitosan (T7, T8, T9) which led to rotting, despite the color of the peels remaining green. These data are consistent with the observed results for the color parameter a* in relation to the T7, T8 and T9 treatments, whose fruits did not present a significant increase in the values of a*.

Figure 4 shows the linear regressions between the storage time of the mangoes of each treatment for 14 days at 25 °C and the degree of suitability for fruits consumption, according to the evaluation of the trained sensory panel members, who in

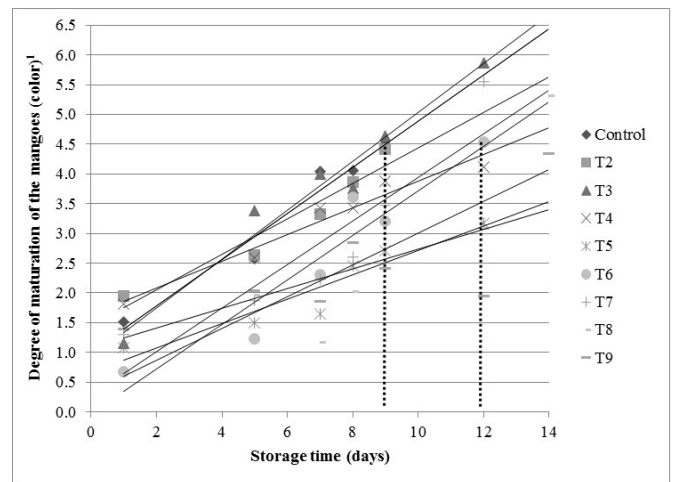


Figure 3. Linear regressions between the degree of maturity of the mangoes evaluated by the peel color and the storage time for control and different treatments. Chitosan (%) and cassava starch (%) concentrations for different treatments were: Control (0, 0); T2 (0, 0.25), T3 (0, 0.5), T4 (0.25, 0), T5 (0.25, 0.25), T6 (0.25, 0.5), T7 (0.5, 0), T8 (0.5, 0.25), T9 (0.5, 0.5). ¹ Unstructured 9-point scale (0 = green, 4.5 = mature, ripe, 9 = Over ripe stage - undesirable).

this analysis evaluated in an integrated manner all the sensory parameters previously analysed such as peel color, aroma and texture of the fruits. In Figure 4, the value 0 on the scale used by the panel members represents fruit not suitable for consumption (green or over-ripe fruits) while values close to 9 indicate fruits more suitable for consumption.

Figure 4 shows that at the beginning of storage, all treatments received from the panel members scores varying from 0.42 and 0.86, indicating that the mango fruits of all treatments were not suitable for consumption, since these fruits pertained to the green maturation stage. During the storage of fruits, an

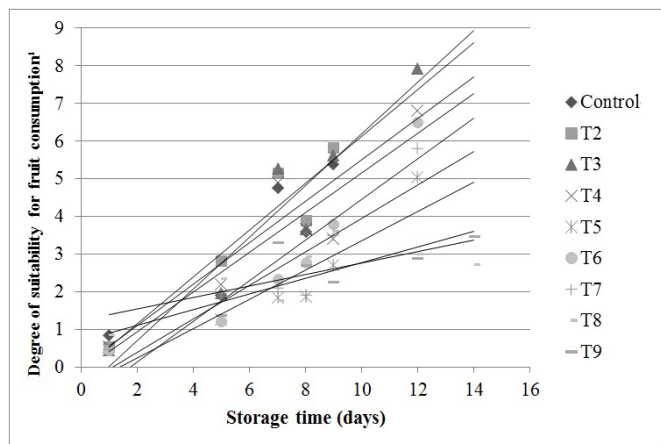


Figure 4. Linear regressions between the degree of suitability for fruit consumption and storage time for control and different treatments. Chitosan (%) and cassava starch (%) concentrations for different treatments were: Control (0, 0); T2 (0, 0.25), T3 (0, 0.5), T4 (0.25, 0), T5 (0.25, 0.25), T6 (0.25, 0.5), T7 (0.5, 0), T8 (0.5, 0.25), T9 (0.5, 0.5).¹ Unstructured 9 - point scale (0 - not adequate; 9 - adequate).

increase in the suitability values was observed for all treatments (Figure 4), with more significant increases for the fruits of the treatments control, T2, T3 and T4. This indicates that the mangoes of these treatments reached the ripe stage faster than those of the other treatments.

In Figure 4, it can be observed that the highest value of suitability reached by the control treatment was 5.38, which occurred on the 9th day of storage. After this period, the fruits of the control treatment began to show signs of rotting as black spots developed in the peel color, along with an excessively soft texture and an extremely sweet and fruity aroma, indicating mango fruits were at the last maturation stage.

Figure 4 also shows that the T5, T6 and T7 treatments had a more gradual increase in their suitability for consumption values during storage. For these treatments, the highest values of suitability in the scale used by the panel members were 5.01 (T5), 6.5 (T6) and 5.79 (T7), and it occurred on the 12th day of storage, with the exception of the T6 treatment, with suitability value expressively superior to that presented by the fruits of the control treatment on the 9th day of storage. This indicates that in addition to the T6 treatment which delayed the ripening speed of the mangoes, it apparently favored the biochemical processes of the fruits in such a way that they developed more favorable sensorial attributes compared to the fruits of the control treatment, which did not have any coatings.

On the other hand, the treatments T8 and T9 which had 0.5% of chitosan in their formulation, associated to 0.25% and 0.5% of starch, respectively, even at the end of 14 days of storage did not reach suitability values of 4.0 on the scale used, indicating that when the sensory panel evaluated the peel color, texture and aroma of mango fruits together, the individuals' perception was that these treatments did not develop sensorial characteristics that would qualify them as suitable for consumption even after 14 days of storage.

From the above, it can be concluded that the sensorial evaluations indicated the T6 treatment (0.5% cassava starch and 0.25% chitosan) as the best formulation for the mango coatings of the 'Tommy Atkins' variety, extending the post-harvest shelf life of the fruits by 3 days, as well as promoting mangoes with sensorial characteristics more favorable than those of the control treatment, which did not have any coating. Although treatments containing higher levels of chitosan, notably the T8 and T9 markedly delayed changes in fruit color parameters (a^* , b^* and $^{\circ}$ hue), this effect was undesirable from the point of view of sensorial quality control of the mangoes, since it did not acquire the desirable reddish-yellow colors, usually associated by consumers with ripe fruit and suitability for consumption.

3.4 Respiration rate

Figure 5 shows the respiration rate of control fruits (without any coating) and the treatment fruits with 0.5% starch and 0.25% chitosan (T6 treatment) during 13 days of storage, calculated from the production of CO_2 by the samples. The T6 treatment was selected for this study because it was the one that, according to the results discussed above, presented a better impact on the post-harvest shelf life of the mango, increasing its shelf life without compromising the sensorial quality attributes that the consumer uses most in selection of Tommy Atkins mango fruits, such as the color of the peel, texture and aroma of the fruits.

As can be seen in Figure 5, at the beginning of storage, the fruits of the control treatment and the treatment with the coating had similar respiration rates, which did not differ to each other at ($p \leq 0.05$), indicating that the fruits of the two treatments were in the same stage of maturation. However, already on the 1st day of storage, it was observed that the fruits having coating of 0.5% starch and 0.25% chitosan (T6), stood out from the fruits of the control treatment (without any coating), because these presented a significantly lower respiration rate ($p \leq 0.05$), a fact that was maintained on all other days of these mangoes storage.

Since respiration is the process by which the vegetable tissue consumes O_2 and produces CO_2 , the lower respiration rates observed in the fruits having coating of 0.5% starch and 0.25% chitosan indicate that the coating generated a barrier to O_2 , causing a decrease in the level of this gas around the mangoes which consequently reduced the respiratory rate of the fruits. This may have slowed down the metabolic reactions associated with the respiratory process and maturation, resulting in prolonged postharvest life of the mangoes.

Zapata et al. (2008) also found results similar to those of the present study while evaluating the impact of alginate and zein coating on the post-harvest life of tomatoes stored for 9 days at 20 °C. The authors observed a lower respiratory rate throughout the storage of tomatoes coated with alginate and zein compared to control tomatoes, without cover.

3.5 Microbiological analysis

Table 5 presents the data of three replicates on the count of mesophilic aerobic bacteria in the control treatment mangoes, and in the fruits treated with 0.5% cassava starch and 0.25% chitosan (T6) at the beginning and at the end of storage.

Table 5. Counts of mesophilic aerobic microorganisms, molds and yeasts, and total and thermotolerant coliforms in mangoes stored (start and end of the storage period) at 25 °C, for fruits without coating (control) and with coating containing 0.5% cassava starch and 0.25% chitosan.

Treatment	Repetition	Mesophilic Aerobic Microorganisms (UFC ¹)		Molds and Yeasts (UFC ¹)		Coliforms (NMP/g ²)			
		Start	End	Start	End	Total		Thermotolerant	
						Start	End	Start	End
Control	1	< 10	1.61 x 10 ³	< 10	7.96 x 10 ³	15	1.100	< 0.3	< 0.3
	2	< 10	< 10	< 10	< 10	< 0.3	240	< 0.3	< 0.3
	3	< 10	< 10	< 10	4.65 x 10 ³	< 0.3	93	< 0.3	9.2
Treatment (Chitosan 0.25% + Starch 0.5%)	1	< 10	< 10	< 10	< 10	< 0.3	93	< 0.3	< 0.3
	2	< 10	< 10	< 10	< 10	< 0.3	460	< 0.3	< 0.3
	3	< 10	< 10	< 10	5.5 x 10 ⁴	< 0.3	93	< 0.3	< 0.3

¹UFC: colony forming unit; ²NMP: Most Probable Number.

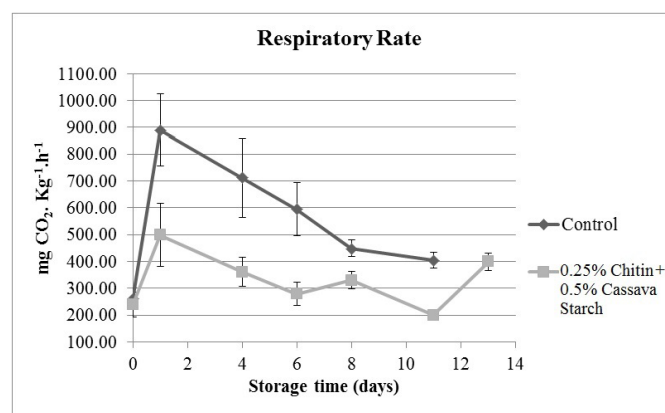


Figure 5. Respiratory rate of ‘Tommy Atkins’ mango fruits without any coating (control), and by mangoes having coating of 0.5% starch and 0.25% chitosan (Treatment T6), stored for 13 days at 25 °C. The bars represent the standard errors of the mean.

It was observed that at the end of storage there was an increase in mold and yeast counts in two replicates of the fruits of the control treatment, and in only one repetition of the fruits covered with 0.5% cassava starch and 0.25% chitosan. These results may indicate a possible antimicrobial effect exerted by the coating. In turn, both treatments showed counts below 10⁶ CFU/g, which is considered acceptable at any point in the post-harvest life of fruits, according to Rojas-Graü et al. (2007).

Table 5 also shows the increase of total coliform counts in all replicates of the fruits of the control treatment and in two replicates of the coating at the end of storage. However, the highest counts of coliforms were present in the fruits of the control, suggesting a probable antimicrobial effect of the coating on the counts of total coliforms. Although even with this increase, it was observed that the counts were low compared to the limits tolerated by Brasil (2001) according to the Resolution RDC No. 12, which determines the maximum tolerance of thermotolerant coliforms in 2 × 10³ CFU/g.

4 Conclusion

Overall, the results of the present study indicate that chitosan when used in the coating formulation, promotes an efficient O₂ barrier. In the mango fruits covered with 0.5% chitosan, this barrier reduced the rate of degradation of chlorophyll,

an O₂-dependent reaction, contributing to the mango peels remaining green even at the end of post-harvest shelf life. These formulations with coatings prevented the yellowing of the carotenoids in the peels of the mangoes, causing the trained panel members to classify the fruits as still immature even at the end of the post-harvest life, an undesirable fact in mangoes destined for the consumer market.

The most favorable results occurred when the mangoes were coated with formulations containing 0.25% chitosan and 0.5% cassava starch, which presented a post-harvest shelf life of an extra 3 days compared to the fruits of the control treatment which did not have any coating. These fruits also presented lower respiratory rates compared to the control treatment fruits, demonstrating that the coating actually produced a barrier to O₂, which was efficient in reducing the respiration rate of mango, consequently reducing the fruit ripening velocity and hence extending its post-harvest shelf life. The data suggested also the occurrence of an antimicrobial effect of this coating on the ‘Tommy Atkins’ mango fruits in relation to molds and yeasts and total coliforms.

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