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Edible coatings based on cassava starch, salicylic acid and essential oils for preservation of fresh-cut mango

Recubrimientos comestibles a base de almidón de yuca, ácido salicílico y aceites esenciales para la conservación de mango fresco cortado

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ABSTRACT

Keywords:
Chitosan
Cinnamaldehyde
Thymol
Tommy Atkins

Mango has a short shelf-life after harvesting. The use of edible coatings on the elaboration of minimally processed mango is an alternative for its commercialization. In the present work edible coatings based on chitosan, starch-salicylic acid and starch-cinnamaldehyde-thymol were applied to fresh cut-mango. Weight loss, soluble solids, titratable acidity, color and microbiological analyses were studied along storage for 12 days at 8 °C and 90% relative humidity. Titratable acidity was the highest for mangoes coated with chitosan and the lowest was for starch-salicylic acid coating. Regarding instrumental texture, fruit coated with chitosan showed a higher penetration force compared to fruit coated with starch and uncoated samples. Microbiological results showed that all coated mangoes inhibited growing of fungi and yeast whereas uncoated samples showed an increase of both microorganisms along 12 days of storage period.

RESUMEN

Palabras clave:
Quitosano
Cinamaldehído
Timol
Tommy Atkins

El mango se caracteriza por presenta un tiempo de vida útil corto luego de la cosecha. El uso de recubrimientos comestibles en la elaboración de mango mínimamente procesado es una alternativa para su comercialización. En este estudio, se aplicaron películas de quitosano, almidón de yuca–ácido salicílico o almidón de yuca–cinamaldehído–timol a mango Tommy Atkins cortado con posterior almacenamiento de 12 días a 8 °C y 90% de humedad relativa. Se analizó la pérdida de peso, sólidos solubles, acidez titulable, textura instrumental, color y análisis microbiológico del mango. Los resultados mostraron que la mayor y menor acidez titulable se obtuvo para las frutas recubiertas con quitosano y almidón-ácido salicílico, respectivamente. En cuanto a textura instrumental, la fruta recubierta con quitosano tuvo una mayor fuerza de penetración que la fruta tratada con almidón y el control. A nivel microbiológico, todas las películas inhibieron el desarrollo de hongos y levaduras mientras el control presentó un incremento durante 12 días de almacenamiento.

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Mango (*Mangifera indica* L.) is one of the five tropical fruits with the highest consumption worldwide (Caballero *et al*., 2015). Tommy Atkins is the most exported variety in Ecuador with 65% of total mango exports. Tommy Atkins mango has high resistance to handling; therefore, it has potential for the development of minimally processed products (Chiumarelli *et al.,* 2011).

Refrigerated minimally processed mango is a good option on the market; and it responds to the need of a modern world where less time is available to prepare food (González-Aguilar *et al.,* 2008). Industrialization of mango could contribute to the development of Ecuadorian agroindustry, through the creation of small and medium-sized enterprises (Cedeño and Cerón, 2018), and its contribution to GDP, which currently represents 7%, and is lower compared to Colombia (10%), Chile (13%), and Uruguay (12.4%) (Fiallo, 2017).

Postharvest mango losses vary between 20 and 50% (Dávila, 1998; Singh *et al.,* 2013). The most common problems of mango agroindustry are weight loss, mechanical damage and attack of bacteria and fungi. Physicochemical properties such as color, firmness, among others, are affected by metabolic disorders caused by cutting (Tovar *et al.,* 2001). Therefore, it is necessary to use techniques to preserve the quality attributes of minimally processed mango.

Fresh and minimally processed fresh-cut products are naturally contaminated by microorganisms of several sources, including the farm environment, post-harvest handling and processing (Abadias *et al.,* 2008). The microflora associated with raw fruits mostly includes yeasts and moulds (Burnett and Beuchat, 2000; Tournas, 2005).

Edible coatings are an alternative to preserve the quality and freshness of minimally processed products and prolong their shelf-life. The application of coatings creates a semipermeable gas and water vapor barrier that reduces the speed of respiration and dehydration of the coated products and creates conditions similar to foods subjected to modified atmospheres (Chiumarelli *et al.,* 2011). The most common polymers in the preparation of edible coatings are proteins, polysaccharides and lipids. Among the polysaccharides, cassava starch has been widely used due to its availability and relative low cost (Santacruz *et al*., 2015; Souza *et al*., 2012; Kampeerapappun *et al*., 2007; Flores *et al*., 2007). Edible coatings based on only polymers or in combination, have been applied to different fruits, e.g., cassava starch in fresh-cut pineapple (Bierhals *et al*., 2011), cassava starch and citric acid in fresh-cut Tommy Atkins mango (Chiumarelli *et al*., 2010), mixtures of starch and chitosan in guava (Bezerra *et al*., 2015), ascorbic acid and N-acetyl-cysteine in bananas (Palacín, 2012), and modified cassava starch in tomato (Hernández *et al*., 2011).

Chitosan has achieved considerable interest in the industry due to its biodegradability, biocompatibility and non-toxicity properties (Dash *et al*., 2011). Chitosan solutions exhibit good coating formation capacity and antimicrobial activity, making them potentially useful for antimicrobial biopolymer development (Dutta *et al*., 2009). The antimicrobial effect of chitosan could be the result of changes in cell permeability produced by the electric charge of chitosan (Devlieghere *et al*., 2004), molecular weight and degree of deacetylation (Zheng and Zhu, 2003). The pH and type of acid where chitosan is dissolved, as well as storage conditions may also influence antimicrobial properties (Begin and Van Calsteren, 1999; Leceta *et al*., 2013).

Edible coatings can be used with food additives acting against enzymatic browning, microbial growth and texture loss. The use of essential oils (EO) or active ingredients of essential oils such as carvacrol, carvone, cinnamaldehyde, citral, p-cimene, eugenol, limonene, menthol and thymol have been particularly prominent because they extend the shelf-life of food (Sung *et al*., 2013). Perdones *et al*. (2012) reported minimal changes in the physicochemical and microbiological characteristics of strawberries coated with chitosan and lemon EO for 15 days in storage at 4 °C compared to uncoated fruits or fruits coated only with chitosan. Another additive used together with edible coatings is salicylic acid. It delays the ripening of fruits, probably due to the inhibition or action of ethylene biosynthesis (Srivastava and Dwivedi, 2000). Salicylic acid has been used to control the aging by cooling of pears (Asghari *et al.,* 2007), strawberries (Babalar *et al.,* 2007), grapes (Asghari *et al.,* 2009) and fresh-cut Sindrhi mangoes (Moradinezhad, 2020). There are no studies of the use of cassava starch with the addition of either cinnamaldehyde or thymol for the preservation of fresh-cut Tommy Atkins mango.

The present work aimed to study the use of edible coatings based on cassava starch together with salicylic acid, cinnamaldehyde and thymol, as well as chitosan, for the preservation of fresh-cut Tommy Atkins mango stored in refrigeration conditions. Analyses of instrumental texture (penetration force), titratable acidity, color and microbiological analysis (fungi and yeast) were performed on stored mango.

MATERIALS AND METHODS

The chitosan (molecular weight 149 kDa, deacetylation degree 95%) was donated by the Public University of Navarra (Pamplona, Spain). Tommy Atkins mangoes were purchased at a local market in the city of Manta, Ecuador. Mangoes with a degree of ripening of two (Báez, 1998) were selected according to the size and without damage.

The selected mangoes were washed, manually peeled and cut into 8.0x1.5 cm slices. These slices were immersed in the corresponding coating solution (chitosan, C; starch+salicylic acid, SSA; starch+cinamaldehyde+thymol, SCT) and dried at room temperature (approx. 25 °C). Mango samples with no coating were used as control samples. The mango slices (approx. 100 g) were then placed on polyurethane trays and coated prior to storage at 8 °C and 90% relative humidity.

Coating preparation

Coating based on either starch or chitosan were prepared by the casting technique. The chitosan coating was prepared using a chitosan solution 1% (w/v), using citric acid solution 1% (w/v) as solvent. Tween 20 at 1% (w/v), glycerol 0.5% (w/v) and glucose 0.5% (w/v) were added to the solution before homogenization with an ultraturrax (Politron, Switzerland) at 11,000 rpm for 4 min.The starch coating was prepared according to Santacruz *et al.* (2015). A solution of cassava starch 0.5% (w/v) was heated to 90 °C for 5 min. Tween 20 at 1% (w/v), glycerol 0.5% (w/v) and salicylic acid 2 mmol L^{-1} were added to the hot solution. Once the solution reached room temperature, glucose 0.5% (w/v), cinnamaldehyde 0.15% (w/v) and thymol 0.15% (w/v) were added. Finally, the solution was homogenized as described previously.

Physical-chemical characterization

Weight loss. It was calculated by weighting the fruit at 0 day and after each storage time. Measurements were performed in triplicate and the results were reported as percentage of weight.

Instrumental texture. Penetration analyses were performed according to Castro *et al.* (2014). Analyses were performed using a Shimadzu texturometer (EZ LX Model, Japan). A stainless-steel probe of 3 mm diameter and 8 cm length was used. The probe was introduced into the fruit at 15 mm depth with a velocity of 10 mm s^{-1}. The maximum force (penetration force) resulting from three measurements was reported.

Soluble solids. The fruit was disintegrated using a domestic blender, followed by a filtration on a piece of cloth. The filtered juice was analyzed by a digital refractometer (KRÜSS, Germany) according to the AOAC method (1990), the results of three measurements were reported as °Brix.

Titratable acidity. Titratable acidity was determined by titration with 0.01 M NaOH solution according to the AOAC method (1984), the results of three measurements were reported as percentage of citric acid.

Color analysis. The color of mango pulp was determined using a Konic Minolta (Japan) colorimeter in a L*, a*, b* scale. Color measurements were expressed based on the chromaticity parameters a* (green [-], red [+]) and b* (blue [-], yellow [+]). Measurements were made in triplicate.

Microbiological analysis. Fungi and yeasts counting were performed on mango samples at 0, 4, 8, and 12 days of storage. 10 g of sample were used to mix with 90 mL of KCl solution 0.1% (w/v). The inoculum was prepared by mixing 1 mL of the previous solution with 9 mL of distilled water. Counting of fungi and yeasts were made according to NOM-111-SSA1-1994 (Norma Oficial Mexicana, 1994). Three repetitions were performed for each sample.

Statistical analysis. The results were analyzed by means of ANOVA and a Tukey test, using a significance of 5% by the statistical package InfoStat, Professional Version 2016. Measurements of the previous analyses were performed in triplicate along 12 days of storage.

RESULTS AND DISCUSSION

Instrumental texture. The results of the instrumental texture revealed that the maximum penetration force decreased for coated and uncoated mango samples along the 12 days of storage with values ranging from 6.0 to 0.48 N (Figure 1).

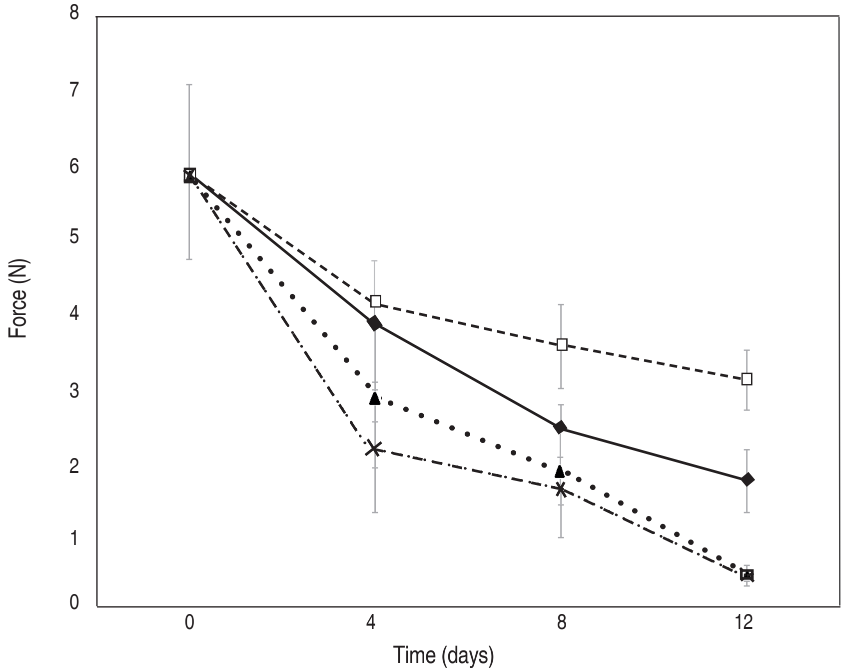

Figure 1. Maximum penetration force on fresh-cut Tommy Atkins mango with and without edible coating for 12 days at 8 °C and 90% relative humidity. □ chitosan (C), ▲ cassava starch+ salicylic acid (SSA), **X** cassava starch+cinamaldehyde+thymol (SCT) and ♦ uncoated

There was no statistically significant difference in penetration force between the samples at 4 day of storage. However, after 8 and 12 days of storage, penetration force was higher for samples coated with chitosan, while the lowest penetration force was recorded for mango samples coated with salicylic acid (SSA) and cinamaldehyde+thymol (SCT). The penetration force for the chitosan-coated (C) mango sample is maintained probably due to the reduced respiration rate (Cissé, 2015). Similar results were found by Zhu *et al.* (2008), using chitosan at different concentrations in 'Tainong' cv mangoes.

Weight loss. There was no difference on the weight loss of both samples coated with starch (SSA and SCT) during the whole storage time ($P<0.05$). Uncoated sample had the lowest weight loss followed by samples coated with chitosan. Castro *et al.* (2017) showed lower weight loss for papayas coated with chitosan and uncoated fruits compared to papayas coated with starch. The presence of compounds like cinnamaldehyde, thymol or salicylic acid into the coating, could accelerate a weight loss by osmotic dehydration (Vega *et al.,* 2007).

Soluble solids. The results showed no significant difference in soluble solids content between the uncoated and coated mango samples along storage ($P<0.05$). Samples had a soluble solids content of approximately 9.5 °Brix at 0 day and after 12 days of storage had a value of 8.5 °Brix (Table 1). A decrease in soluble solids content was found by Bueno *et al.* (2005), in minimally processed pineapple stored at 5 °C. The reduction of soluble solids is probably due to the respiratory process which may lead to high consumption of organic substrates, i.e. sugars (Kader *et al.*, 2002).

Titratable acidity. The results showed that fruits coated with chitosan and uncoated samples presented a significant difference compared to samples treated with SSA and SCT (Table 1). Acidity values were higher for uncoated sample with value of 0.45% for 0 day, reaching 0.85% for 12 day, followed by chitosan treatment with 0.45% for 12 day and 0.65% for 12 day of storage. The lowest values were for samples treated with salicylic acid, which presented values of 0.45% for 0 day and 0.49% for 12 day. Samples treated with cinamaldehyde+thymol, showed acidities of 0.45% for 0 day and 0.44% for 12 day of storage.

Table 1. Titratable acidity, soluble solids and weight of fresh-cut Tommy Atkins mango coated with either chitosan (C) or starch+salicylic acid (SSA), starch+cinnamaldehyde+thymol (SCT), stored for 12 days at 8 °C and 90% relative humidity.

Treatments	Days								
	4			8			12		
	Acidity	°Brix	Weight Loss	Acidity	°Brix	Weight loss	Acidity	°Brix	Weight loss
SCT	0.45^a	7.85^a	1.52^a	0.45^a	9.05^a	3.72^a	0.44^a	8.80^a	5.52^a
SSA	0.45^a	8.80^a	1.86^a	0.46^a	8.85^a	3.99^a	0.49^a	8.85^a	5.71^a
Uncoated	0.45^a	9.20^a	1.16^b	0.52^b	8.65^a	3.08^b	0.85^c	8.40^a	4.87^b
C	0.45^a	7.20^a	0.99^c	0.51^b	7.60^a	2.30^c	0.65^b	8.80^a	3.99^c

Acidity: percentage of citric acid. Weight loss: percentage of weight. The values correspond to the average of 3 replicates. Within each column, different letters (superscripts) correspond to statistically different values (P<0.05).

High values of titratable acidity of uncoated sample may be due to the production of organic acids by growing of microorganisms (Russo *et al.*, 2014). Similar behavior was found by Bueno *et al.* (2005) for minimally processed pineapple stored at 5 °C. Low values of titratable acidity for fruits treated with starch edible films is another indicator of ripening, as organic acids are also used during respiration (Freire *et al.*, 2005). Additionally, starch edible coating may reduce the level of O_2 inside the packages of mango slices, leading to minimal effect on the physical and chemical changes of mango during storage in low O_2 atmospheres (Freire *et al.*, 2005; Rathore *et al.*, 2007). The chitosan treatment showed greater values of acidity in relation to the other treatments throughout the storage period. Chitosan films are more selectively permeable to O_2 than to CO_2, maintaining the conditions of the coated fruit similar to an uncoated sample and promoting the production of metabolites that lead to an increase of acidity (Kweon *et al.*, 2001).

Color. Table 2 shows the results of color of mango slices during storage. There was not statistically difference for L*, a* and b* values between coated and uncoated samples. The only exception was the L* value of the sample treated with SSA after 12 days of storage, which was smaller and statistically different to the other samples. At those days of storage, L* value decreased by 10% for SSA samples and by 7.9% for SCT samples, while the uncoated sample increased 9%. The decrease in the L* value means that mango pulp became less bright during storage. There was not difference between a* and b* values among samples.

Table 2. Changes in L*, a* and b* values of fresh-cut Tommy Atkins mango coated with C, SSA or SCT, stored for 12 days at 8 °C and 90% relative humidity.

Treatments	Days								
	4			8			12		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
SCT	67.3^a	-3.4^a	60.6^a	63.4^a	-4.3^a	58.1^a	62.0^b	-2.9^a	55.6^a
SSA	67.3^a	-3.4^a	60.6^a	62.4^a	-2.1^a	62.1^a	60.6^a	-0.5^a	61.1^a
Uncoated	67.3^a	-3.4^a	60.6^a	68.5^a	-1.9^a	66.8^a	65.2^b	-2.8^a	59.2^a
C	67.3^a	-3.4^a	60.6^a	73.5^a	-4.9^a	62.6^a	73.4^b	-4.5^a	62.2^a

The values correspond to the average of 3 replicates. Within each column, different letters (superscripts) correspond to statistically different values (P<0.05).

Other authors (Robles *et al*., 2013) used antioxidant edible coatings for Kent mango cubes. They found at the end of storage that coated samples lost only 2.5% of the initial L* value compared to 7% loss in samples with no edible coating. Edible coatings based on polysaccharides and antioxidants have been used to delay browning in freshly cut apples maintaining L* values throughout storage (Lee *et al*., 2003; Fontes *et al*., 2008). Chiumarelli *et al.* (2011) reported that cassava starch coatings offer effective maintenance of color characteristics in cut mango samples due to the combined effect of the coating and citric acid. Although edible polysaccharide coatings such as starch are a good gas barrier (Dussan *et al*., 2014), in the present study, SSA or SCT did not achieve an effective browning delay.

Microbiological analysis. The results of the microbiological analysis (Table 3) show that all the treatments inhibited the development of fungi and yeasts in comparison with the uncoated samples, which showed an increase in the colonies from 1 log CFU g^{-1} at 0 day to 1.8 log CFU g^{-1} at 12 day of the study. Studies show chitosan at concentrations lower than 1% affects the sporulation of *Botrytis cinerea* and *Penicillium expansum* (Liu *et al*., 2007). The effect of chitosan on the germination of *Rhizopus stolonifer* spores has been previously reported at concentrations ranging from 1 to 2 mg mL^{-1} (Hernández *et al*., 2007; Hernández *et al*., 2008). Besides, Badawy and Rabea (2009) reported that chitosan applied in concentrations of 2 to 4 mg mL^{-1} can control *Botrytis cinerea* infections in tomato fruits. Bautista *et al.* (2003) showed that chitosan coatings control anthracnose in papaya fruits and inhibits the growth of fungi such as *Fusarium oxysporum*, *R. stolonifer, Penicillium digitatum* and *C. gloeosporioides* at 3% (Bautista *et al*., 2003; Bautista *et al*., 2004). The efficacy of cinamaldehyde in inhibiting the growth of fungi of the genera *Aspergillus* and *Penicillium* has been demonstrated by several authors. López *et al.* (2007a) found that *P. islandicum* and *A. flavus* were completely inhibited by cinamaldehyde-fortified in vapor phase. Tunc *et al.* (2007) found that cinamaldehyde is one of the strongest growth inhibitors of *Penicillium notatum*. Antimicrobial polypropylene films incorporating 2% cinamaldehyde also showed complete inhibition of *A. flavus, Penicillium comuna, P. expansum, Penicillium nalgiovense, Penicillium roqueforti, and P. islandicum* (López *et al*., 2007b). Plotto *et al.* (2003) reported that carvacrol, thymol, and citral compounds showed inhibition of mycelial growth of *Botrytis cinerea*, *Alternaria arborescens*, and *Rhizopus stolonifer*. Essential oils may affect stages of fungal development such as germination of spores and development of mycelium.

Table 3. Fungi and yeast growing on fresh-cut Tommy Atkins mango coated with C, SSA or SCT stored for 12 days at 8 °C and 90% relative humidity.

Treatments	Days			
	7	14	21	28
	(log CFU g^{-1})			
SCT	1.00^{a}	nd	nd	nd
SSA	1.00^{a}	nd	nd	nd
Uncoated	1.00^{a}	nd	nd	nd
C	1.00^{a}	1.2^{a}	1.4^{a}	1.8^{a}

The values correspond to the average of 3 replicates. Within each column, different letters (superscripts) correspond to statistically different values (P<0.05). nd: not detected.

CONCLUSIONS

The use of edible chitosan-based coatings reduces textural and weight loss changes in mango slices stored in refrigeration, compared to coated samples with starch and uncoated samples. There was no difference in soluble solids between coated and uncoated samples during the whole storage, whereas differences in color were observed after the 8 day. The starch-based coatings reduce the changes of acidity during storage. Chitosan and starch-based coatings inhibits the growth of fungi and yeasts on mango slices. Further studies could examine the solubilization of chitosan in other acids, as well as sensory analysis of coated samples for consumer acceptability

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