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EFFECT OF CHITOSAN SALTS IN POST-HARVEST QUALITY OF MANGO 'TOMMY ATKINS'

Efecto de sales de quitosano en la calidad poscosecha del mango 'Tommy Atkins'

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ABSTRACT. Mango fruits have climacteric respiratory pattern and anthracnose disease affects their quality, so they have a short shelf life. Given the trends in fruit consumption without chemical residues, alternatives are sought to extend the post-harvest life, such as bioactive products. Chitin is an abundant biopolymer in the exoskeleton of crustaceans and mollusks. Chitosan have fungal properties and ability to form semi permeable films. The aim of this study was to evaluate the effect of chitosan lactate and acetate salts on post-harvest quality of mango 'Tommy Atkins'. Fruits were submerged in chitosan salts at 15 and 20 g $L^{-1}$and stored at 14 ± 1 °C for 12 days. At the storage end the incidence and severity were determined by anthracnose (%) and effectiveness of treatments, furthermore fresh mass loss (%), SST (°Brix), titratable acidity (%) and pH were determined. The incidence and severity of anthracnose decreased with the two salts, the acetate at 20 g L^{-1} exhibited 71,15 % effectiveness in disease control. The mass losses were greater than 5,53 % for the two salts without differences with the control. Lactate at two concentrations has a normal fruit ripening and with acetate, physiological process was delayed, evidenced in the maturity indicators and the presence of strange flavors.These results showed the potential of chitosan salts in the control of anthracnose and quality postharvest of fruits.

Key words: anthracnose, biopolymer, *Mangifera indica* L., ripening

RESUMEN. Las frutas de mango son de patrón respiratorio climatérico y la enfermedad por antracnosis afecta su calidad, por lo que la vida de anaquel es corta. Dadas las tendencias del consumo de frutas sin residuos químicos se buscan alternativas para alargar la vida poscosecha, como los productos bioactivos. La quitina es un biopolímero abundante en el exoesqueleto de crustáceos y moluscos. El quitosano tiene propiedades fungicidas y capacidad para formar películas semipermeables. El objetivo de este trabajo fue evaluar el efecto de sales de lactato y acetato de quitosano en la calidad poscosecha del mango 'Tommy Atkins'. Las frutas se sumergieron en las sales a 15 y 20 g L^{-1} durante cinco minutos y se conservaron a 14 ±1 °C durante 12 días. Al final del almacenaje se determinó incidencia y severidad por antracnosis (%) y efectividad de los tratamientos, además pérdidas de masa fresca (%), SST (ºBrix), acidez titulable (%) y pH. La incidencia y severidad por antracnosis disminuyeron con las dos sales, la efectividad del acetato a 20 g L^{-1} fue 71,15 % en el control de la enfermedad. Las pérdidas de masa mostraron valores superiores a 5,53 % para las dos sales sin diferencias al control. Con lactato a las dos concentraciones hubo maduración normal de las frutas y con acetato el proceso fisiológico fue atrasado, evidenciado en los indicadores de madurez y la presencia de sabor extraño. Estos resultados demostraron las potencialidades de las sales de quitosano en el control de la antracnosis y calidad poscosecha del mango.

Palabras clave: antracnosis, biopolímero, *Mangífera índica* L., maduración

INTRODUCTION

Chitosan is a natural polymer derived from the deacetylation process of chitin. Chitin is a biopolymer abundant in the exoskeleton of crustaceans and molluscs; it is also part of the structure of the cell wall of certain fungi and insects (1).

One of the most important properties of chitosan is its antimicrobial activity, several studies show the effect of the bioproduct in the control of pathogens that cause fungal diseases in fruits (2,3).

Another of the properties is the capacity to form semipermeable films, the little influence in the indicators of maturity and low toxicity for the human being. The application as a coating provides good results in terms of reducing the loss of fresh dough and improving the quality of the fruit, although they are closely related to the type of fruit, cultivate, storage

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conditions, combination with other post-harvest treatments and nature, molecular weight and degree of deacetylation of chitosan[A] (4).

During the commercialization of mango fruits (*Mangifera indica* L.) high losses occur and one of the main causes is the disease known as anthracnose, caused by the fungus *Colletotrichum* sp. (5). The post-harvest control is carried out by physical (hydrothermal) and chemical methods or the combination of both. The immersion of fruits in hot water 50 to 55 ºC for five minutes (min) and the applications of fungicides like imazalil and prochloraz are the most effective. Fungicides are applied according to market demands (6-8).

Mango fruits are highly perishable and have a short shelf life. In this sense, different postharvest treatments are recommended that allow the extension of storage time and reduce losses due to this cause[B] (9).

The mango cultivar 'Tommy Atkins' is originally from Florida, United States of America. It is currently among the best cultivars of the red type that is marketed as fresh fruit in the American and European markets. In Cuba it was introduced in the 1990´s (10) and there is a great interest in increasing the commercialization of fruit to the fresh market (border and export), but post-harvest losses are high, among other causes due to technological problems and high incidence of anthracnose (11).

Given the current trends of fruit consumption without chemical residues, other alternatives are sought for the control of post-harvest diseases and lengthen the shelf life, as is the application of bioactive products.

In Cuba, different studies show the potential of chitosan in agriculture (12,13). However, there are few related to the use of the bioproduct for the control of post-harvest diseases and extension of the shelf life of tropical fruits.

The objective of this work was to evaluate the effect of lactate and acetate salts of chitosan in the control of anthracnose and the indicators of maturity of mango fruits 'Tommy Atkins'.

MATERIALS AND METHODS

Evaluation of chitosan salts in the control of anthracnose and in the maturity indicators of mango fruits 'Tommy Atkins'

For the study mango fruits 'Tommy Atkins' were collected in a state of physiological maturity, from a plantation located in the Technological Scientific Unit of Base Alquízar, (UCTB, Alquízar), Artemisa province, which is located at 82 ° 32'N and 22 ° 47'W, at 11 m above the mean sea level, on a Ferrasoleutric soil with flat topography of slope 0 (14).

The 30 % lactate and acetate chitosan salts (w/w) were contributed by the Center for Research and Development of Drugs (CIDEM) of Cuba.

Chitosan with degree of deacetylation 77,7 % and molar mass 310 000 g mol^{-1} was used to obtain the two salts by spray drying and with the following properties:

Chitosan lactate molar mass 46.56 g mol-1 and pH 5,30.

Chitosan acetate molar mass 48.86 g mol-1y pH 5,55.

Fruits were washed with 0,1 % Tropicleaner® detergent, rinsed and immersed for five minutes. in:

- Chitosan lactate (LQ) 15 g L^{-1}
- Chitosan lactate (LQ) 20 g L^{-1}
- Chitosan Acetate (AQ) 15 g L^{-1}
- Chitosan Acetate (AQ) 20 g L^{-1}
- Control (without application)

Fruits were stored at 14 ± 1 °C, 80-85 % relative humidity (RH) for 12 days and determined at the end of storage:

- The incidence for anthracnose was established by the percentage of affected fruits, from the total fruit evaluated. Previously, the symptoms were identified by the disease (15).
- The severity of anthracnose damage was established through the percentage of the injured tissue of the total exocarp of the affected fruits with an arbitrary scale of five degrees, where: Grade 0 (fruits without damage), Grade 1 (fruits with 1 -10 % with affected exocarp), Grade 2 (fruits with 11-25 % with affected exocarp), Grade 3 (fruits with 26-35 % affected exocarp), Grade 4 (fruits with 36-50 % affected exocarp). The severity was determined according to the infection index (16) and the effectiveness of the treatments (17).

The following indicators of maturity were:

- Fresh mass (g): with a technical balance Alsep model EZ-5000 A (Error ± 0,01g) and loss of fresh mass calculated by the percentage of losses with respect to the initial weight.

[A] Contrera A. Efecto de tratamientos poscosecha novedosos en la calidad fisicoquímica, sensorial y nutricional de cítricos [Internet] [Tesis de Doctorado]. [España]: Universidad Politécnica de Valencia; 2010 [cited 2016 Sep 6]. Available from: https://riunet.upv.es/bitstream/handle/10251/8986/tesisUPV3420.pdf

[B] Brecht J, Sargent S, Mitcham E, Maul F, Brencht P, Menocal O. Manual práctico para el mejoramiento poscosecha del mango [Internet]. Orlando, Florida: National Mango Board; 2014 [cited 2016 Sep 6]. 5-67 p. Available from: http://WWW. edis.ifas.ufl.edu/pdffiles/hs/hs119000.pdf

- Firmness of the mesocarp: with a Lusa manual texturometer, model FT 40 (kgf), a 6 mm diameter metal cylinder was introduced on each side of the fruit and the average firmness was obtained.
- Total soluble solids (SST) (°Brix): with a COMECTA S.H FG-103 refractometer with temperature correction for the corresponding data at 20 °C.
- Titratable acidity: 5 mL aliquots of mesocarp extract were taken and titrated with 0,1N sodium hydroxide solution. Phenolphthalein was used as indicator. The results were expressed as citric acid percentage.
- The pH of the mesocarp was measured with a pH meter HANNA Hi 2210 with aliquots of 10 mL of mesocarp extract.

For each treatment, a sensory analysis was performed, eight panelists were used, who were previously explained the hedonic scale of four points related to taste A: I do not like it, B: I like little, C: I like it D: I like it much and the taste a: Little acid, b: Acid, c: Very acidic, d: Bad Taste (fermentation or ethanol).

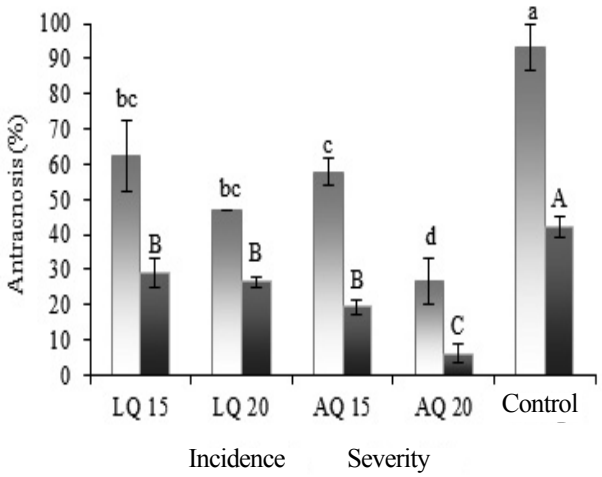
Experimental design

A completely randomized design was used. In each evaluation of anthracnose, three repetitions were performed (25 fruits x repetition) and the maturity indicators on 15 fruits individually (15 repetitions). The statistical processing of the results was carried out by means of an Anova of Simple Classification. The means were compared by the Tukey test ($p < 0.05$) (18). The statistical program STATISTICA Version 6.0 (19) was used.

RESULTS AND DISCUSSION

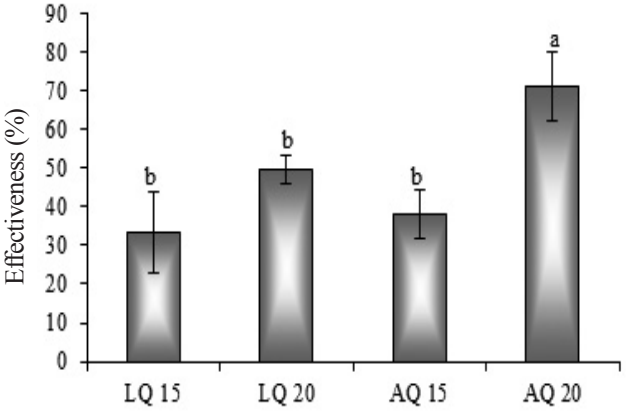
Evaluation of chitosan salts in the control of anthracnose and in the maturity indicators of mango fruits 'Tommy Atkins'

The immersion of the mango fruits in the two chitosan salts significantly reduced the incidence and severity of anthracnose compared to the control (Figure 1). With acetate 20 g L^{-1} there was a lower incidence and severity index, the effectiveness was higher than the rest of the treatments (Figure 2). In mango fruits previously inoculated with *C. gloeosporioides* and treated with chitosan at 0,5; 1,0; 1,5 and 2,0 % obtain similar results (20). Also in mango fruits 'Sanara' with chitosan at 0,05; 0,1; 0,2 and 0,4 % find lower incidence for anthracnose and lower concentrations are less effective (21).



The bars represent ± Standard Deviation (n = 3)
Capital letters and lowercase letters indicate different means with significant differences by Tukey test ($p < 0.05$)

Figure 1. Incidence and severity of anthracnose in 'Tommy Atkins' mango fruits after treatment with lactate (LQ) and chitosan acetate (AQ) at two concentrations (g L-1) and stored at 14 ± 1 ºC for 12 days



The bars represent ± Standard Deviation (n = 3)
Letras diferentes indican medias con diferencias significativas por la Prueba de Tukey ($p<0,05$)

Figure 2. Effectiveness of the lactate and chitosan acetate salts at two concentrations (g L-1) in the control of anthracnose in mango fruits 'Tommy Atkins' after being stored at 14 ± 1 ºC for 12 days

In vitro studies indicate the inhibitory action of different types and application form of chitosan in the development of the fungus *Colletotrichum* sp. isolated from several fruit species with typical anthracnose[C] lesions (22,23).

[C] Cechim F. Quitosana na indução de resistência e controle in vitro de mofo cinzento, podridão parda e podridão amarga [Tese de Doutor]. [Brasil]: Universidade Tecnológica Federal do Paraná; 2014.

Results previous to this study show that the antifungal activity of the salts of lactate and acetate of chitosan on the *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc isolated from mango and papaya fruits with anthracnose depends on the concentration used and shows the sensitivity of the fungus with its different stages of development (24,25), so that the application in fruits can cause a delay in the stages of infection, colonization of the tissues and expression of the disease in the exocarp.

The effectiveness of the two salts of chitosan in the control of anthracnose in fruits may be due to the pH of each of the salts, for lactate 5,30 and acetate 5,55. The antimicrobial activity of chitosan depends on factors such as pH, which help accentuate the positive charge of the amino groups in the biopolymer chain (26).

Other mechanisms of chitosan action influence the antimicrobial activity, among these the positive charge exhibited by the amino group on carbon C_2, which can interact with the cell walls of negatively charged microorganisms and cause cell disorganization and cell death (27), as well as the degree of deacetylation, polymerization and concentration (28,29) and the effect on the activation of the defense mechanisms of fruits (30). In the same way, it is given by the type of microorganism, physical state of the chitosan, environmental factors, and complementary methods of control and chemical composition of the substrate (31).

Table I shows the indicators of fruit maturity after treatment with lactate and chitosan acetate. The acetate salt 15 g L^{-1} showed a higher mass loss value that did not differ from lactate 15 g L^{-1}, likewise there were no differences between lactate 15 g L^{-1}, the two salts at 20 g L^{-1} and the witness. In general, the two salts did not have an effect in the reduction of mass losses. These results do not coincide with those obtained in mango fruits 'Tommy Atkins' with chitosan 1 % and conserved at 14 ºC for 15 days, which show lower percentage of mass losses than those stored at 20 ºC (32), but with what other authors mention that chitosan does not reduce losses of fresh mass in mango fruits 'Ataulfo', 'Tommy Atkins' and 'Jinhwang' (33-35).

In general, edible coatings based on polysaccharides are an ineffective barrier to moisture, due to their hydrophilic nature. For this reason, coatings intended for fruit incorporate lipids, plasticizers capable of controlling the weight loss of fruits (36). Likewise, the water vapor permeability of chitosan films depends on the degree of deacetylation and molecular weight (37).

In this study no symptoms of dehydration or wilting were observed in the fruits treated with lactate and chitosan acetate. In fruits of orange 'Valencia' waxed with FreshSealP® based on chitosan at 2,5 % during refrigerated storage at 4 °C for eight weeks plus 6 days at 20 °C, have greater mass losses and do not show symptoms of dehydration or withering. This behavior in terms of mass losses can be attributed to the fact that chitosan-based films have higher water vapor permeability than edible covers based on carnauba, candelilla and other polisácaridos (38).

The positive effect of the application of chitosan on the decrease of mass losses in different fruits is the result of the dense structure of the film that acts as a barrier to gases and reduces the rate of respiration with the consequent decrease in the maturation process (39,40).

The fruit mesocarp firmness with chitosan acetate at the two concentrations showed significantly higher values than the control, at 15 g L^{-1} there were no differences with the lactate salt at the two concentrations. Fruits treated with lactate salt showed less firmness without differences in control. In mango fruits 'Bocado' when they use chitosan among other coatings, they obtain greater retention of firmness as a result of the delay in the degradation of insoluble protopectins to soluble pectins (41).

Table I. Indicators of maturity in mango fruits 'Tommy Atkins' after treated with lactate and chitosan acetate at two concentrations (g L^{-1}) and stored at 14 ± 1 °C for 12 days

	INDICATORS OF MATURITY				
Treatments	Mass losses (%)	Firmness (kgf)	SST(°Brix)	Acidity (%)	pH
LQ 15	6,54^{a}	5,55^{bc}	12,71^{a}	0,13^{b}	4,23^{a}
LQ 20	5,26^{b}	4,49^{bc}	12,71^{a}	0,13^{b}	4,13^{a}
AQ 15	5,69^{ab}	7,57^{ab}	10,71^{b}	0,19^{a}	3,94^{b}
AQ 20	5,53^{b}	9,4^{a}	10,12^{b}	0,16^{a}	3,94^{b}
Control	5,68^{b}	3,51^{c}	12,91^{a}	0,12^{b}	4,14^{a}
CV (%) / ESx	13,26/0,08	27,2/0,16	3,15/0,04	12,4/0,02	1,46/0,01

The data shows the average of 15 replicas. Averages with equal letters in the same column do not differ from each other by the Tukey test ($p<0.05$) LQ (Chitosan Lactate); AQ (Chitosan Acetate)

Chitosan creates a modified atmosphere that reduces alterations in the pectic compounds that form the cell wall of the host, maintains the firmness of the fruit for a longer time and reduces vulnerability to attack by pathogens (42).

The effectiveness of an edible film or coating also depends on the characteristics of the fruit (type of cuticle and transpiration rate), the production of phenolic compounds, natural nutrients, chemical or nutritional composition of the substrates and environmental conditions (43,44).

Fruits with chitosan acetate at the two concentrations presented lower values of SST, high content of titratable acids in correspondence to low pH with significant differences to the control. This can be the result that the chitosan creates a barrier to O2, modifies the internal atmosphere, it reduces O_2 and CO_2, inhibiting the respiration of the fruit and as a consequence there is a delay in ripening (45).

In the fruits with lactate, the SST were higher, the acidity low and pH high without differences to the control, the application of the salt of lactate did not influence the indicators of maturity that evolved in correspondence to the period of cold-storage storage and they are very related to the flavor of the fruits, so the ripening was normal.

The contradictions found regarding the external and internal quality indicators of the fruit treated with two salts and a single chitosan may be due to the differences in the physical and chemical properties of the two salts, which depend on the nature of the contraction. The counterion associated to chitosan in its saline form manages to shield to a variable extent the positive charges of this, among other reasons for steric effects, likewise the relationship between the pH of the dilutions of the salts and the natural pH of the fruit (46, 47). The application of medium molecular weight chitosan dissolved in 0,5 % acetic acid shows the best results in the delay of maturation and extension of the shelf life of mango fruits 'Nam Dok Mai' (48). Other authors report that mango fruits submerged in chitosan have a normal maturation process (32, 33).

The sensory analysis showed that the largest number of panelists tasted in the degree of the C scale (I like it) for the fruits with chitosan lactate at the two concentrations and the control (Table II). With acetate they were in degrees A (I do not like it) and B (I do not like it), they were also classified as low acid fruits at 15 g L^{-1} and acid at 20 g L^{-1}, in turn they had a bad taste as fermented or ethanol.

The application method, the concentration and the immersion time of the fruits in the acetate salt, which was also observed more dense and viscous than the lactate, could impede the exchange of gases, affect the physiological processes such as respiration and transpiration and cause a high concentration of volatile compounds such as ethanol and acetaldehyde, substances that are related to the presence of bad tastes. Similar criteria corroborate with the use of chitosan at concentrations higher than 1,5 % in papaya fruits (*Carica papaya* L.) (49).

Table II. Sensory analysis in 'Tommy Atkins' mango fruits treated with lactate salts and chitosan acetate at two concentrations (g L^{-1}) and stored at 14 ± 1 °C for 12 days

Treatments	Sensory analysis (Nu of panelists)							
	Taste				Flavor			
	A	B	C	D	a	b	c	d
LQ 15	-	1	7	-	5	3	-	-
LQ 20	-	4	4	-	6	2	-	-
AQ 15	5	3	-	-	6	1	1	4
AQ 20	2	4	2	-	3	5	-	5
Control	-	2	6	-	7	1	-	-

A: I do not like it, B: I like little, C: I like it, D: I like it a lot
a: Little acid, b: Acid, c: Very acid, d: Bad Taste
LQ (Chitosan Lactate); AQ (Chitosan Acetate)

These results are the first studies on the application of the lactate and acetate salts of chitosan obtained by spray drying, under the conditions of mango 'Tommy Atkins' production in Cuba and show the practical application of these in the process of conditioning fruits for commercialization, with effects on fruit quality parameters similar to those found when chitosan is applied in dilutions of acetic and lactic acids.

The application of chitosan salts in mango fruits will contribute to the design of a new post-harvest technology, which will allow the reduction of losses caused by anthracnose, lengthen the shelf life and increase quality commercialization without chemical residues, given the current trends in the consumption of healthy and harmless fruits, it is important to recommend the lactate salt of chitosan and continue the study of other formulations or combinations with other organic, inorganic, biological and natural compounds, as well as with different post-harvest treatments to achieve better results in the quality parameters.

CONCLUSIONS

- Lactate and acetate salts of chitosan contribute to a lower incidence and severity of anthracnose damage in mango fruits 'Tommy Atkins', acetate salt being the most effective in controlling the disease.

- The salts of lactate and acetate of chitosan do not favor the reduction of mass losses in mango fruits 'Tommy Atkins', the lactate salt of chitosan allows the normal maturation of fruits and with chitosan acetate the physiological process is delayed , which is evident in the indicators of maturity and the presence of bad tastes.

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