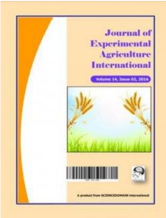

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Effect of Biodegradable Coatings on Post-harvest Quality of Papaya (cv. Golden) under Different Storage Conditions

Albert Einstein Mathias de Medeiros Teodosio[1*],
Graciele da Silva Campelo Borges[2], George Gérson Araújo da Silva[3],
Cristhian Alfredo Diaz Jopia[2], Marília Hortência Batista Silva Rodrigues[1],
Ana Paula Nunes Ferreira[1], Kalinne Passos dos Santos[1],
Jaína Geovana Figueiredo Lima Santos[1]
and Arthur Xavier Mesquita de Queiroga[1]

[1]Federal University of Campina Grande (UFCG), Pombal, PB, Brazil.
[2]Federal University of Paraiba (UFPB),João Pessoa, PB, Brazil.
[3]Federal Istitute of Rio Grande of Norte, Currais Novos, RN, Brazil.

Authors' contributions

This work was accomplished in collaboration among all of the authors. Authors AEMMT, CADJ, APNF, KPS, JGFLS and AXMQ performed the laboratory research, statistical analysis and preliminary sketch. While authors AEMMT, GGAS and MHBSR collaborated in the rewriting of the manuscript, improving the bibliographical revision with base in the obtained data. Authors AEMMT and GSCB collaborated in the development of the study and they accomplished corrections of the manuscript. In the end, all of the authors read and they approved the final manuscript.

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ABSTRACT

The objective of this study was to evaluate the post-harvest shelf-life of 'Golden' papaya fruits submitted to biodegradable coatings based on potato starch, associated or not with lemon grass essential oil under different storage conditions. The experiment was conducted in a completely randomized design with two factorial schemes, where the first one was 3x6, corresponding to three coating techniques: control (T1: no coating), 2% potato starch (T2), potato starch at 2 (T3) and six

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**Corresponding author: E-mail: albertemmt@gmail.com;*

evaluation periods (0, 4, 8, 12, 16 and 20 days), stored under refrigeration (10 ± 2°C for 12 days and then transferred to the condition 25 ± 2°C for an additional 8 days). In the second experiment, the 3x4 factorial scheme, represented by three coating techniques: (T1), (T2) and (T3) and four evaluation periods (0, 4, 8 and 12 days) stored in ambient condition 25 ± 2°C), with three replicates of two fruits per plot. The potato starch (T3) coating associated with lemon grass essential oil and refrigeration, controlled ripening and further enhanced its efficiency in the control of anthracnose.

Keywords: Carica papaya L.; potato starch; essential oil grass lemon; shelf-life.

1. INTRODUCTION

The cultivation of papaya has grown significantly in Brazil, however, presents a great challenge in the production of fruits with post-harvest quality. In 2016, the world production of papaya reached 12.85 million tons, Brazil being the second largest producer, with 11% of world production [1]. The papaya is a climacteric fruit, soon the ripening takes place rapidly after the fruit harvest, caused by the autocatalytic production of ethylene and increased respiratory rate, which makes these fruits perishable after harvesting [2,3]. Due to this high perishability, ripening control is essential for the increase in shelf-life after harvest, targeting the domestic market and fruit exports.

The cultivar Golden (*Carica papaya* cv. Golden) represents most of the fruits exported to the North American and European markets [4]. The cultivation of the papaya stands out for presenting a wide report of problems with phytopathogens, responsible for significant losses in the production [5]. Among the fungal diseases that cause losses and reduce the post-harvest quality of papaya fruits, the anthracnose caused by *Colletotrichum gloesporioides* and *Phoma caricae-papayae* caused by rot in the peduncle stan out.

The anthracnose is the most important, because it causes lesions in the bark that compromise the appearance, besides affecting the pulp, causing great damages in the commercialization, due to the appearance of the fruits one of the qualitative evaluation parameters most used by consumers [6].

Several methods are used to inhibit the development of these fungi, the most common being the use of synthetic fungicides due to the effectiveness of the control, however, the dosage and shelf life of these products are not always respected, nor is the active ingredient registered, thus offering irreparable risks and damages to the environment and consumer health [7,8]. Therefore, some studies have been carried out seeking the use of alternative methods such as the application of waxes and essential oils extracted from plants, thus constituting a viable and desirable alternative for the inhibition of post-harvest diseases, caused by the antifungal properties of the oil, which directly inhibits the pathogen, controlling diseases, and minimizing damage to the environment and public health [8,9]. However, the use of essential oils in the shelf-life of foods can be limited because of their application costs, intense aroma and the potential toxicity of some compounds that are present [10].

The main forms used to maintain fruit quality are the use of polymeric coatings, refrigeration, modified atmosphere and irradiation [11]. Starch is one of the most used biopolymers for the preparation of coatings, due to the lower cost and high availability. In addition to being biodegradable when released into the environment, thereby contributing to less pollution of nature [12,13].

Throughout its post-harvest life, the papaya when destined for export and domestic market, which has certification go through careful processes, initially in the harvest until its processing all the procedures are carried out with the fruits stored at room temperature, later after classified according to their quality are placed under refrigeration until arriving at their final destination, that is, to the consumer. In view of this, the use of biodegradable coatings associated with refrigeration aims at maintaining the appearance and the prolongation of post-harvest quality of the fruit in its logistic chain.

This context, the objective of this work was to evaluate the post-harvest shelf-life of 'Golden' papaya fruits submitted to biodegradable coatings based on potato starch, associated or not with lemon grass essential oil under different storage conditions.

2. MATERIALS AND METHODS

The experiment was developed in the Federal University of Paraíba (UFPB) in the Center of Technology and Regional Development in the laboratories of physiochemical analysis of foods, microbiology of foods and processing of foods. The papaya fruits ('Golden cultivar') used in this experiment were coming from orchards located on a farm in Mamanguape, Paraíba. The lemon grass essential oil (*Cymbopogon citratus*) was acquired from Ferquima Ind. and Ltd. at Vargem Grande Paulista, São Paulo, Brazil.

The harvest was carried out in the morning, where the fruits were in an area that had incidence of anthracnose contamination. Fruit maturation was determined visually, and those presented with the following characteristics were selected: fruit presenting color changes, whose first yellowing signs did not cover more than 15% of the bark. They were then packed in a single layer in cardboard boxes (640 x 480 cm) previously coated with the bubble bag to minimize impact and friction between them and transported to the laboratory where the fruits were selected for uniformity of size and color, discarding those with apparent defects or injuries due to transportation. Processing was started by washing the fruits with current drinking water and sanitizing with chlorinated solution at 100 ppm active chlorine for 2 minutes, allowing them to dry naturally on paper towel.

The potato starch solution was prepared at the concentration of 2% (w / v) [14]. By gelling the starch, the solution was heated to 70°C under constant stirring and then cooled to coat the fruits. For the incorporation of lemon grass essential oil to the coatings, 10 mL of lemon grass essential oil was used for each liter of the potato starch solution. The essential oil was mixed with Tween 40 (0.1% mL L-1) and glycerol (1.5% mL L-1) in order to emulsify and maximize the plastification properties of the coating [15].

The experiment was conducted in a completely randomized design with two factorial schemes, where the first one was 3x6, corresponding to three coating techniques: control (T1: no coating), 2% potato starch (T2), potato starch (0, 4, 8, 12, 16 and 20 days), stored under refrigeration (10 ± 2°C for 12 days and subsequently transferred for ambient condition of 25 ± 2°C for an additional 8 days). In the second one, we adopted the 3x4 factorial scheme, represented by three coating techniques: (T1), (T2) and (T3) and four evaluation periods (0, 4, 8 and 12 days) stored in ambient conditions (25 ± 2°C), with three replicates of two fruits per plot.

Then the coatings were applied to the fruits by immersing them for 1 minute, allowing the excess to drain on a grid and, after complete drying of the coatings, were placed and stored under two temperatures, averaging 25 ± 2°C and 10 ± 2°C and then 25 ± 2°C for further evaluation. At each evaluation period, the fruits were processed in a domestic centrifuge and evaluated for the following aspects:

Loss of fresh mass (PMF): determined by a gravimeter at a semi-scale analytical precision of ±0.01g. The results were expressed in percentage losses, employing the ratio before the mass loss and after each storage period.

Total titratable acidity (AT): measured according to the methodology recommended by the Adolfo Lutz Institute [16], using 10 grams of homogenized pulp diluted into 100 mL of distilled water, followed by titration with standardized solution of NaOH 0.1N, using the phenolphthalein turning point as indicator. The results were expressed in citric acid g by 100 g-1 of the sample.

Total soluble solids (SS): measured directly in the homogenized pulp through a digital refractometer (model PR-100, Palette, Atago Co., LTD., Japan), whose results were expressed in ºBrix [17].

Ratio*:* calculated between soluble solids and titratable total acidity (SS/ATT). The results were expressed in pure number to one decimal place.

Potential of Hydrogen (pH): measured by a digital peg for the direct reading of the homogenized pulp, according to IAL [16].

Total sugars and reducing: determined by the Lane and Eynon method, by titration, based on the reduction of copper by the sugar reducing groups, using Fehling's reagent, which is composed of solution A (crystalline copper sulphate in water) and a solution B (sodium and potassium tartrate and sodium hydroxide in water) with methylene blue indicator. The results were expressed as percentage of glucose 100 g-1, according to the IAL [16] methodology.

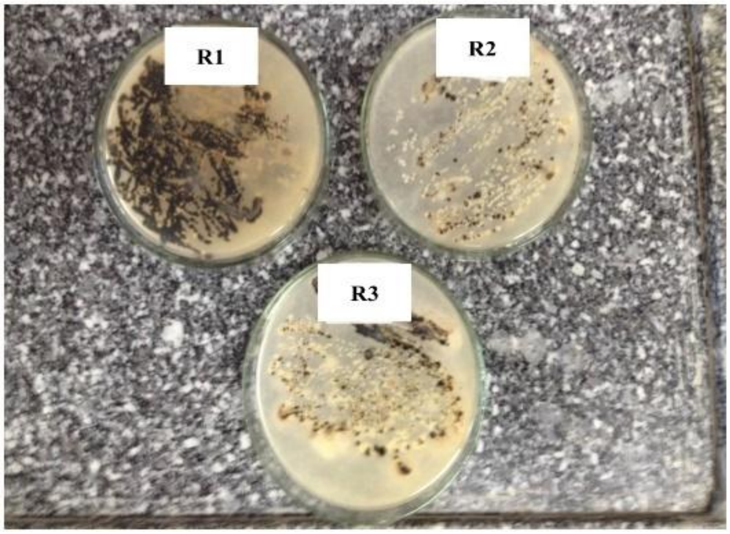

Fig. 1. *C. gloeosporioides* isolated in plates (different repetitions: R1; R2 and R3) contaminated papaya fruits from the João Pessoa Farm, UFPB, 2018

Isolation and identification of the fungus *C. gloeosporioides*: was isolated directly from "Golden" papaya fruits with disease symptoms and pathogen signals as shown in Fig. 1. Inside a laminar flow hood, using a sterile platinum loop on top of the anthracnose-injured fruit, was added to the petri dish containing the BDA culture medium. The BDA (potato, dextrose, agar) culture was prepared with 250g of potato, 20g of dextrose and 20g of agar per liter of water. 500 mg L-1 of ampicillin antibiotic was added to prevent bacterial contamination. 20 ml of culture medium was poured into Petri dish. They were then incubated at 25°C for five days.

Statistical analysis: The data were submitted for variance and regression analysis through the Sisvar Program [18].

3. RESULTS AND DISCUSSION

Starting with the variance analysis presented in Table 1, we can notice that there was significant interaction for all appraised variables between the factors under study (coating and storage time), demonstrating that the appraised factors interfere simultaneously in the post-harvest shelf life of the 'Golden' papaya fruits.

In relation to the data of loss of fresh mass, an increasing linear behavior was observed during the storage period and that in both conditions of temperature the greatest losses were registered for the fruits without application of coating (T1) 61,22% and 80, 3% (Fig. 2A and 2B). The lowest losses were observed in the treatment T3 (potato starch at 2% with essential oil of lemon grass at 1%) at the temperature when associated with refrigeration (Fig. 2A), which reached values below 30.86% at 12 days of storage and later transferred to 25 ± 2°C up to 20 days with 54.43%, whereas the fruits stored under ambient temperature the lowest values of fresh weight loss were obtained in treatment T2 (2% potato starch) with 56, 11% (Fig. 2B).

The T2 treatment promoted the greatest mass loss, while the T3 treatment presented the lowest, as shown in Fig. 2A. This result is associated with non-addition of glycerol and Tween 40 in the T2 treatment, which adhered to the fruit as a barrier, thus reducing water loss through transpiration. Similar results were observed by Azeredo et al. [15], when evaluating the quality of mango starch coated with cassava starch associated with essential oils and chitosan, found that when fruits were submitted to 24°C, they were covered with starch and associated with essential oil showed the lowest loss of mass in relation to the other coatings.

The use of the biodegradable coatings restrict the diffusion of water vapor and create a saturated atmosphere between the film and the surface of the fruit, reducing transpiration, however, due to the hydrophilic nature of the polysaccharide-based coatings, these generally do not constitute barriers to water vapor [19].

Table 1. Summary of the variance analysis of the mass loss (PM), pH, total soluble solids (SST), titratable acidity total (ATT), sugars total and sugar reducers, in function of different biodegradable coatings in the post-harvest shelf life of 'Golden' papaya fruits during storage to 10 ± 2°C for 12 days and later transferred for 25 ± 2°C (Refrigerated/Environment until 20 days and 25± 2°C (Environment), UFPB, 2018

Fruits stored in ambient condition (without cooling)								
FV	**GL**	**Medium square**						
		PM	**pH**	**SST**	**ATT**	**SS/AT**	**Sugars total**	**Sugar reducers**
Coatings (R)	2	371.29**	0.01*	9.47**	0.66**	10.80**	2.23**	4.13**
Times (T)	3	8335.6**	0.53**	141.91**	2.16**	16.28**	5.05**	10.56**
R x T	6	277.1**	0.03**	3.29**	0.46**	13.58**	0.34**	0.65**
Residue	24	0.29	0.07	0.01	0.01	0.83	0.01	0.007
CV (%)	-	1.50	0.97	1.08	7.65	14.44	3.33	3.25
Fruits stored under refrigerated/ambient								
FV	**GL**	**Medium square**						
		PM	**pH**	**SST**	**ATT**	**SS/AT**	**Sugars total**	**Sugar reducers**
Coatings (R)	2	41.19**	0.21**	14.16**	0.33**	2.47**	0.13**	0.61**
Times (T)	5	5227.99**	1.74**	132.61**	4.30**	7.65**	7.24**	13.42**
R x T	10	41.48**	0.10**	6.53**	0.63**	2.47**	0.22**	0.49**
Residue	36	1.74	0.004	0.01	0.01	0.09	0.008	0.009
CV (%)	-	4.97	1.20	1.43	6.78	5.50	2.39	3.23

*** significant (P <0,01); * significant (P <0,05); ns: not significant*

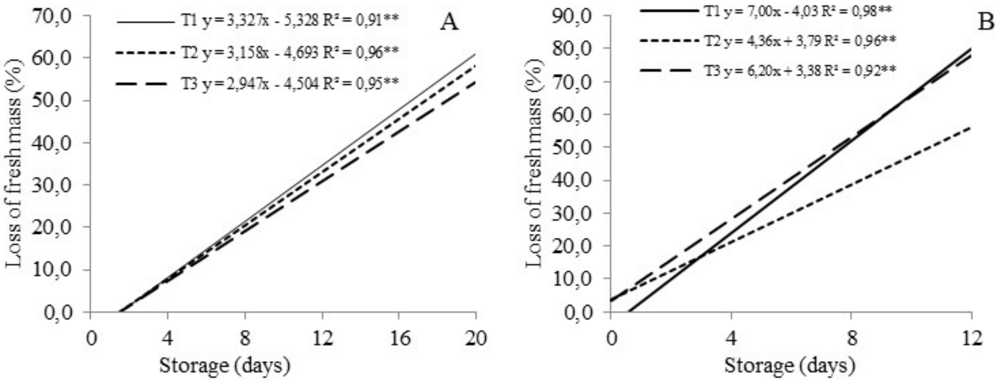

Fig. 2. Loss of fresh mass of papaya fruits submitted to different coatings (T1: no coating, T2: 2% potato starch and T3: 2% potato starch with 1% lemongrass essential oil), stored under refrigeration/ambient (A) and at room temperature (B) and as a function of storage, UFPB, 2018

In the pH of the 'Golden' papaya fruits stored under refrigeration, the data were not adjusted to a polynomial regression equation, with oscillation in mean values as a function of storage, decreasing in the first days of storage, followed by an increase with a subsequent decline from the 16th day of storage in the respective treatments T1 (5.0), T2 (5.1) and T3 (5.1), immediately afterwards an increase in mean pH values of 5.9, 5.9 and 6.3 for treatment T1, T2 and T3, respectively (Fig. 3A). This pH decline occurred due to the formation of organic acids and sugars in relation to respiration, increasing metabolism with increasing temperature.

It can be seen in Fig. 3B that the pH of the fruits presented quadratic behavior for all evaluated treatments with a minimum estimated of 5.09, 5.42 and 5.37 both at 6 days of storage for treatments T1, T2 and T3, respectively. Similar behavior was observed by Nunes et al. [20], evaluating the shelf-life of papaya, obtained values of pH 5.3 for the control, 5.4 for fruits treated with biofilm of 2% manioc starch and 5.4 for those of cassava starch biofilm 4% under a temperature of 10 ± 2°C during the period of 12 days.

The total soluble solids content is shown in Figs 4A and B. It was found that the treatments maintained in the two storage conditions presented increasing linear behavior with increase of 11.3 °Brix, 9.7 °Brix and 11.0 °Brix in treatments T1, T2 and T3, respectively, between the first and the last evaluation period, when stored under refrigeration, while fruits stored under ambient conditions increased 15.3 °Brix in the treatment T1, 13.7 °Brix in the T2 treatment and 15.3 °Brix for the T3 treatment. The treatments T2 and T3 delay fruit maturation due to a reduction in the respiratory rate, and when combined with refrigeration 10 ± 2°C, they have a higher efficiency in the reduction of soluble solids (Fig. 4A). At room temperature, 25 ± 2°C increased the soluble solids index with the storage period, that is, they reached a much faster maturation in treatments T1, T2 and T3 with estimated values of 15.9 °Brix, 15.2 °Brix and 13.9 °Brix (Fig. 4B). The use of refrigeration and the coating of potato starch reduces the respiratory activity, resulting in less hydrolysis of the accumulated reserve carbohydrates during plant growth and production of soluble sugars and, consequently, less ripening [21]. Ali et al. [2] also found that chitosan-coated fruits during refrigerated storage presented a small decrease in the concentration of soluble solids over the storage time.

The total titratable acidity of 'Golden' papaya fruits stored under refrigeration showed quadratic behavior for all evaluated treatments, where treatment T1 and T2 reached an estimated minimum of 0.74% and 0.56% at 2 and 6 days, respectively, increasing after this period, whereas T3 treatment presented a slight increase as a function of the evaluation periods, maximum of 1.24% at 20 days (Fig. 5A). However, the fruits stored in ambient conditions also presented a quadratic effect with a maximum estimated of 1.86% and 1.47% in treatments T1 and T2, respectively, at 12 days of storage. The T3 treatment stored in these same conditions showed an inversely proportional treatment with a maximum estimated of 1.18% at 8 days, reducing afterwards as a function of storage (Fig. 5B).

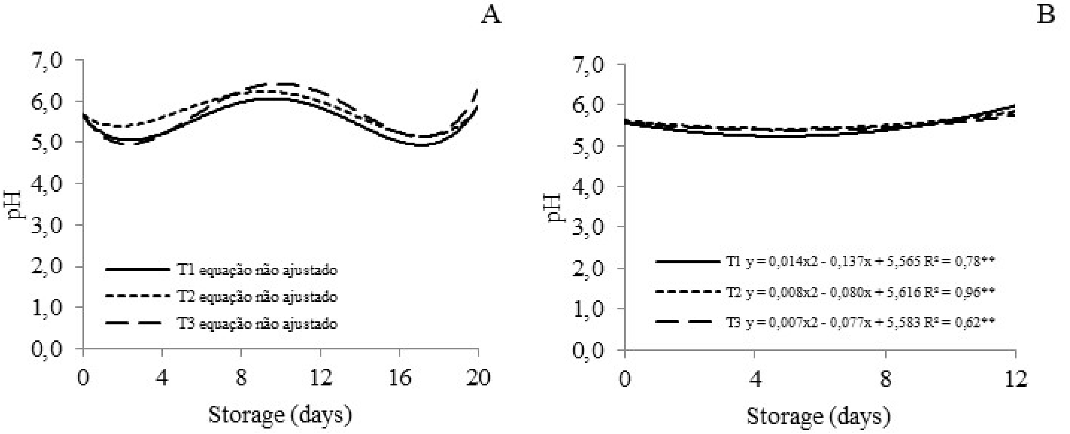

Fig. 3. pH of papaya fruits submitted to different coatings (T1: no coating, T2: 2% potato starch and T3: 2% potato starch with 1% lemongrass essential oil), stored under refrigeration/ambient (A) and at room temperature (B) and as a function of storage, UFPB, 2018

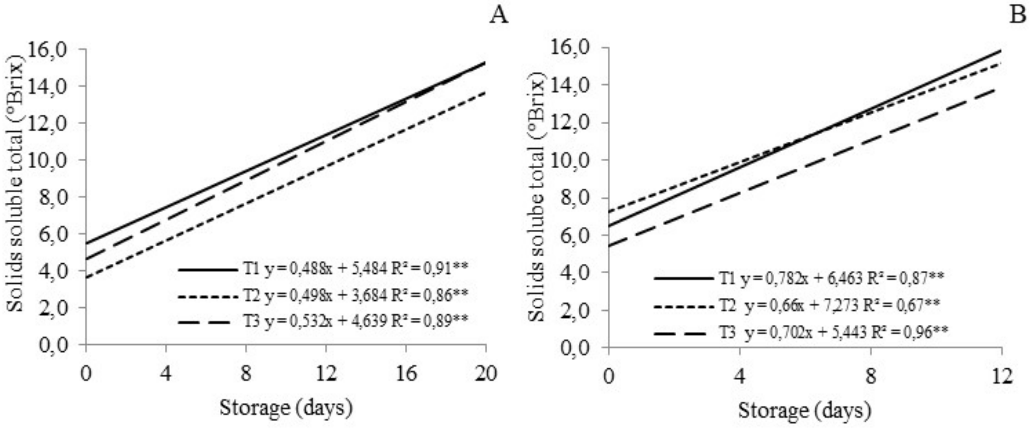

Fig. 4. Soluble solids total of papaya fruits submitted to different coatings (T1: no coating, T2: 2% potato starch and T3: 2% potato starch with 1% lemongrass essential oil), stored under refrigeration/ambient (A) and at room temperature (B) and as a function of storage, UFPB, 2018

The increase observed in the total titratable acidity is related with to the loss of water and the largest concentration of acids. This increase also the degradation of the pectin is associated and to the formation of galacturonic acids, as the cellular wall was degraded in the ripening process [20].

The ratio showed a quadratic behavior in treatments T1, T2 and T3, increasing as a function of storage with a maximum estimated of 6.03, 6.29 and 17.23 at 10, 11 and 20 days after this period, reduction of this ratio when stored under refrigeration (Fig. 6A). In Fig. 6B, it was verified that the ratio of the fruits stored without refrigeration presented a quadratic behavior with a maximum estimated of 6.84 and 10.05 at 7 and 6 days of storage, respectively, in treatments T1 and T2, reducing after this period, however T3 treatment increased until the end of the storage period, when it presented maximum of 8.10. Therefore, it can be stated that the T3 treatment maintained the highest degree of sweetness when compared to the other treatments at the end of the storage period in both storage conditions (Fig. 6A and B). Zillo et al. [10] evaluating the coat of carboxymethylcellulose associated with essential oil of rosemary (*Lippia sidoides*) in the shelf-life of papaya, also observed that the ratio increased during storage up to 9 days with a maximum of 11.50 when the treatment associated with essential oil.

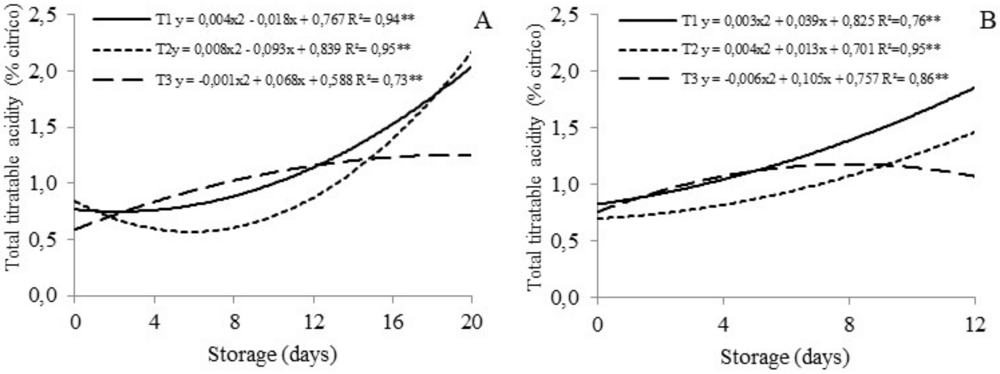

Fig. 5. Total titratable acidity of papaya fruits submitted to different coatings (T1: no coating, T2: 2% potato starch and T3: 2% potato starch with 1% lemongrass essential oil), stored under refrigeration/ambient (A) and at room temperature (B) and as a function of storage, UFPB, 2018

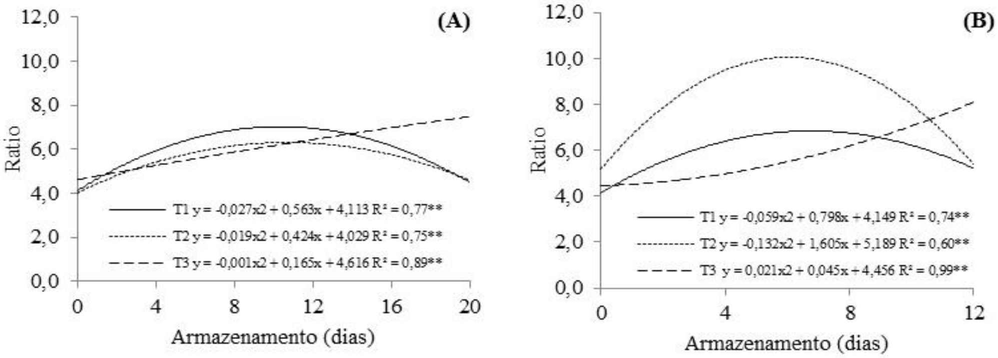

Fig. 6. Ratio of papaya fruits submitted to different coatings (T1: no coating, T2: 2% potato starch and T3: 2% potato starch with 1% lemongrass essential oil), stored under refrigeration/ambient (A) and room temperature (B) and as a function of storage, UFPB, 2018

Analyzing the total sugars content of the 'Golden' papaya fruits submitted to different biodegradable coatings, there was an increase as a function of the advance of the storage in all the treatments regardless of the conditions in which the fruits were stored. The treatments T1, T2 and T3 when stored under refrigeration showed a linear behavior with an increase of 41.81g.100g^{-1}, 47.94g.100g^{-1}, and 44.07 g.100 g^{-1}, respectively, between the beginning and the end of the storage period (Fig. 7A). The fruits stored without refrigeration presented similar behavior to those stored under refrigeration, with higher values in T1 and T2 treatments with an increase of 41.45g.100g^{-1} and 37.77g.100g^{-1}, whereas T3 treatment presented the smallest increase 21.82g.100g^{-1} in content of sugars (Fig. 7).

According to Chitarra & Chitarra [22], the increases in total sugars are associated with increases in soluble solids, although their measurement does not represent the exact content of sugars since other substances are also dissolved in the vacuolar sap (vitamins, phenolics, pectins, organic acids, etc.). However, among these sugars are the most representative, up to 85-90% of the soluble solids. Teodosio et al. [23], when evaluating the effect of biodegradable coatings based on microalgae and oil of the pomegranate seed in the post-harvest shelf-life of 'Golden' papaya under controlled temperature of 18°C for 15 days, verified that the fruits presented the highest total sugar contents at the end of the experimental period.

In the content of reducing sugars, there was an increasing linear behavior in both treatments evaluated in the two conditions of storage, however, when stored under refrigeration presented increase of 4.53g.100g^{-1}, 4.64g.100g^{-1} and 4.54 g.100g^{-1}, in the treatments T1 and T2 and T3, respectively, between the beginning and the end of the storage (Fig. 8A). The fruits stored under ambient conditions, ie, without refrigeration presented linear behavior with increase of T1 4.88g.100g⁻¹, T2 3.59g.100g⁻¹ and T3 3.05g.100g⁻¹ up to the last evaluated period (12 days), demonstrating than T1 values when compared to the other treatments (Fig. 8B).

This behavior observed in the content of reducing sugars can be associated to the fact that the papaya fruits are climacteric fruits, because they are harvested before the beginning of the storage, and only reach this phase, when they exceed the climacteric respiratory peak. In view of this, considerable changes are observed in the sugar content of the fruits, which increase after harvest and during storage [22]. Reis [24] evaluated post-harvest preservation of Tainung 1 papaya under refrigerated storage at 10°C for 21 days, and found that treatments (control, chitosan 2%, chitosan 4%) provided the highest contents of reducing and total sugars at the end of the storage period.

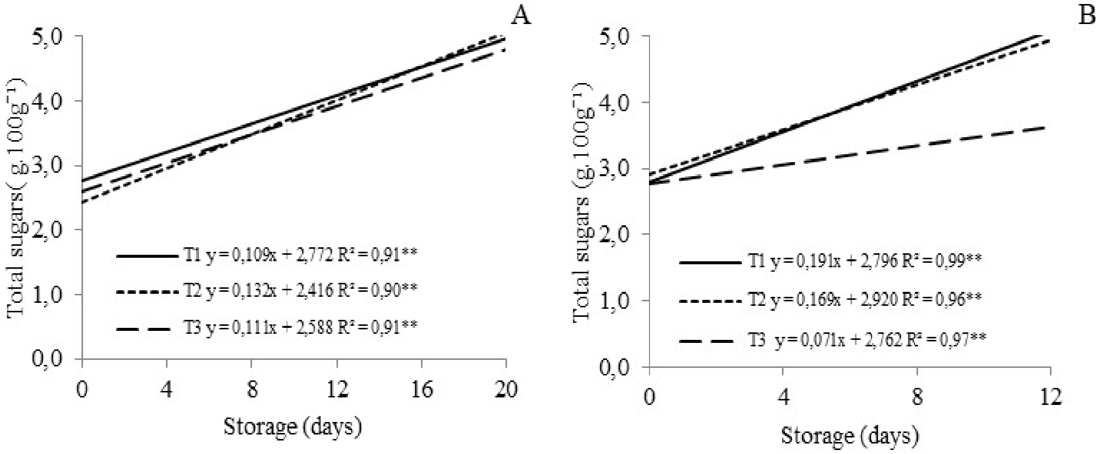

Fig. 7. Total sugars of papaya fruits submitted to different coatings (T1: no coating, T2: 2% potato starch and T3: 2% potato starch with essential lemon grass oil 1%), stored under refrigeration/ambient (A) and at room temperature (B) and as a function of storage, UFPB, 2018

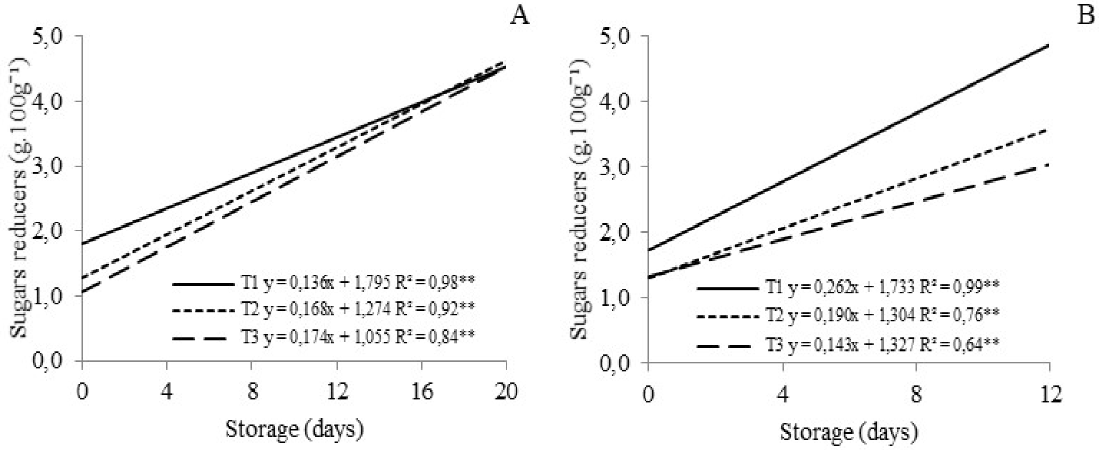

Fig. 8. Sugar reducers of papaya fruits submitted to different coatings (T1: no coating, T2: 2% potato starch and T3: 2% potato starch with 1% lemongrass essential oil), stored under refrigeration/ambient (A) and at room temperature (B) and as a function of storage, UFPB, 2018

3.1 Identification of the Fungus *C. gloeosporioides*

Its identification was made by means of slide preparations and light microscope observation, *C. gloeosporioides*, was identified based on their morphological characteristics in agreement with [25].

In relation to colouration, all isolates of *Colletotrichum* spp. were quite heterogeneous, varying from white to dark gray, indicating that such isolates are *C. gloeosporioides* (Fig. 1). Andrade et al. [26], characterized twenty-nine Colletotrichum cultures, isolated from papaya fruits. The characterization was based, among other aspects, on the conidial morphology, coloration and growth of the colonies. Based on the morphology of the conidia the 29 isolates were identified as *C. gloeosporioides*, most of the isolates conidial cylindrical and / or oblaced, in contrast to *C. acutatum*, isolated from strawberry, which presented fusiform conidia.

3.2 Presence of Visual Symptoms of Diseases in Post-harvest Fruits

During the 20 days of the experiment, the fruits were visually monitored, observing the development of symptoms characteristic of diseases that affect post-harvesting of fruits, considering that these fruits come from João Pessoa Farm, to which is described the high incidence of post-harvest diseases. The development of anthracnose in T1 treatment when stored at ambient conditions (25 ± 2°C) was observed, however, in T2 and T3 treatments, no evidence of anthracnose was observed. In view of this, it is possible to affirm that the use of potato starch associated with lemon grass oil was efficient and feasible in the control of fungal diseases, since the pathogen present in the fruit possibly remained in a latent state, not activating its factors of pathogenicity and thus not compromising the visual appearance of the fruit.

The efficiency of the control of the incidence of anthracnose in the coated fruits based on the potato starch was due to the interference of the film on the maturation process in the fruits, keeping them resistant for longer [22].

Oliveira et al. [9] when assessing the efficacy of chitosan associated with essential oil (*Cymbopogon citratus*) for the control of *Colletotrichum gloesporioides* in papaya, mango and guava, found that the inhibition of anthracnose development in the coated fruits was greater than that observed in fruits treated with synthetic fungicides.

The fruits stored under refrigeration (10 ± 2°C) did not present an anthracnose growth during the 12 days of storage; however, when submitted to a temperature of 25 ± 2°C for eight days, anthracnose development treatment T1, but in the treatments T2 and T3, ie, the fruits coated with potato starch, associated or not to the essential oil lemon grass did not develop any indication of the anthracnose.

Ribeiro et al. [27], evaluating the use of natural products in the control of anthracnose caused by *Colletotrichum gloeosporioides* in papaya fruits, observed that most of the natural products used against anthracnose in papaya were efficient in reducing the symptoms of the same, being verified the sizes of lesions significantly lower in relation to the control.

It is noteworthy that the treatment containing only potato starch fruits, totally controlled the anthracnose at both temperatures, showing that the potato starch coating, with or without the incorporation of lemon grass essential oil, formed a barrier preventing the development of symptoms of typical *C. gloeosporioides* diseases in papaya fruits, which increased post-harvest sanity.

4. CONCLUSION

The potato starch (T3) coating associated with lemon grass essential oil and refrigeration, controlled ripening and further enhanced its efficiency in the control of anthracnose.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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