

The preservative effect of bee wax and calcium chloride coating on the quality and firmness of graviolas (*Annonamuricata* L.)

Abstract

The ripening of soursop is marked by significant changes in the firmness of the fruit, with pulp softening being the main cause of quantitative and qualitative losses during commercialization. To minimize this problem, fruits of the 'Morada' soursop were immersed in solutions of distilled water (control), beeswax (3%), CaCl_2 (3%) and the combination of beeswax + 3% CaCl_2 for a period minutes and then stored at 15°C for 16 days. Analyses of physical-chemical and biochemical quality were investigated at four-day intervals. Considering the immersion solutions using isolated beeswax (3%) and CaCl_2 (3%) resulted in satisfactory quality aspects in relation to the control, but it is in the combined use (beeswax + 3% CaCl_2) that results were observed significant ($p < 0.05$) such as less loss of fresh weight (6.93%), greater firmness (32.18 N), better balance of soluble solids (11.98°Brix), titratable acidity (0.82 g. 100 g⁻¹ citric acid), SS / AT (14.60), pH(5.58) and less degradation of starch (8.98 g.100g⁻¹) and total pectin (0.25 g.100g⁻¹), in addition to less activity of the enzymes amylase, pectinamethylesterase and polygalacturonase in relation to the other treatments, especially the control. These results indicate that the combined use of 3% beeswax + 3% CaCl_2 preserves the physical-chemical quality and firmness of the pulp by reducing the activity of enzymes associated with the cell wall, thus suggesting a delay in fruit ripening.

Keywords: *annonamuricata* L, ripening, post-harvest, softening of the pulp, coating, immersion solution

Volume 8 Issue 2 - 2020

Edmarcos Xavier dos Santos,¹ Ricardo Patrese Jorge Repolho,¹ Alex Guimarães Sanches,² Keila Sousa Lima¹

¹Agronomist Engineers

²Plant Production Department, Paulista State University, Brasil

Correspondence: Alex Guimarães Sanches, Plant Production Department, Paulista State University, Access Way Professor Paulo Donato Castelane Castellane S/N - Vila Industrial, 14884-900, Jaboticabal, São Paulo, Brazil, Email: alexsanches.eng@gmail.com

Received: February 13, 2020 | **Published:** April 29, 2020

Introduction

Soursop (*Annonamuricata* L.) is a tropical fruit of the Annonaceae family, with its center of origin in Central and North South America. Its fruit, the graviola, has a compound berry and peel with soft spikes when ripe.¹ Brazil is the world's largest producer of soursop and cultivation/consumption grows according to its own commercial demand,² of vitamin and sensory properties (carbohydrates, B vitamins, minerals, flavor and aroma) and therapeutic (diuretic, astringent, anti-inflammatory, anti-rheumatic, anti-cancer, among others).³

However, the sale of fresh fruit is hampered by physical factors such as the unevenness in the size and weight of the fruits and the distance from consumer markets⁴ and, mainly, by the ripening pattern classified as climacteric, which accelerates perishability and reduced shelf life when stored at room temperature.^{5,6} The refrigerated storage of the fruit, on the other hand, is limited to temperatures below 13°C, as it is affected by cold injuries.⁷

In view of these difficulties in the post-harvest handling of soursop, the need arises for the combined use of post-harvest conservation technologies that allow their storage for a longer period. The covering of fruits with biofilms, like the bee era, has proven to be an efficient strategy in terms of conservation (better physical-chemical and sensory quality, less loss of mass, reduction of physical damage, greater firmness).^{8,9,10,11,12} In turn, calcium is directly related to post-harvest quality,¹³ since it acts in the regulation of fruit softening, forming bridges between pectic acids and polysaccharides that provide resistance to the structure of the wall and cell membrane¹⁴ and hinder the activity of hydrolytic enzymes, responsible for the loss of firmness

in fruits, especially in soursop. In an attempt to delay ripening and senescence, reduce breathing, prolong life, maintain firmness and quality during storage, calcium chloride has been used in fruits such as peaches,^{15,16} pear,¹⁷ pitanga,¹⁸ fig,¹⁰ banana,¹⁹ among others. Thinking about the market potential, consumption and preservation of the graviola's useful life, the objective of this work was to evaluate the efficiency of the coating with beeswax associated or not with calcium chloride on the physical-chemical quality and the control of fruit softening over 16 days of cold storage (13°C).

Material and methods

Plant material

Soursop 'Morada' were harvested at physiological maturity in a commercial orchard, free from pests, diseases and physiological defects and transported in plastic boxes to the Product Technology Laboratory of the Federal University of Pará, Campus Altamira, PA. In the laboratory, the fruits were washed in running water, cleaned in sodium hypochlorite solution 5 mg.L⁻¹ for five minutes and then dried on benches at room temperature.

Preparation of immersion solutions

The coating with 3% beeswax (w/v) followed the methodology described by Rodrigues et al. (2014).²⁰ For that, 3g of beeswax was weighed, placed in a beaker and taken to a water bath at 100°C for 10 minutes until liquefaction. Then 5% (v/v) of Tween 80, 0.3% (v/v) of sunflower oil and 1000 ml of distilled water heated to 70°C were added. The components were homogenized in ultra turrax for approximately 2 min at speed 10 and then the emulsion was filtered

through TNT (non-woven fabric). The pH of the solution is adjusted to 5.6 with 1 mmol L^{-1} NaOH using pH meter. When the solution reached room temperature ($\pm 25^{\circ}\text{C}$) the fruits were immersed. The 3% (w/v) calcium chloride solution was prepared by dissolving 3 g of CaCl_2 in 100 ml of distilled water. The solution was stirred for 30 min using a magnetic stirrer (model SP 18420-26 Barnstead Thermolyne 2555 Kerper Boulevard Dubuque, USA) and 0.2 mL of Tween 20 (Sorbitan Polyoxyethylene Monoleate, Sigma-Aldrich) was added to the solution to improve plasticity. For the combined treatment (beeswax + calcium chloride), the 3% CaCl_2 solution was incorporated with the 3% beeswax solution, stirred for 30 min and the pH of the solution adjusted to 5.6 with 1 mmol L^{-1} NaOH using pH meter. In each solution, the fruits were immersed for a period of three minutes and then stored in a cold chamber ($13\pm 1^{\circ}\text{C}$) and relative humidity of $95\pm 3\%$ for a period of 16 days, with evaluations at intervals of four days.

Physical-chemical and biochemical analysis

Mass loss was determined with the aid of a semi-analytical balance with an accuracy of 0.1g, calculating the difference between weight at the beginning of the experiment and after each day of evaluation, and the results were expressed as a percentage (%). The firmness of the fruit was determined using a texturometer (Stevens-LFRA Texture Analyzer) with a tip 8 mm long and 4 mm in diameter and a penetration distance of 20 mm. The readings were taken in the equatorial region on both sides of the fruit and the results expressed in Newton (N).

The soluble solids content was determined by refractometric reading with Abbe digital refractometer, brand ATAGO - N1, according to the methodology described by AOAC²¹ and the results expressed in Brix degrees ($^{\circ}\text{Brix}$).

The titratable acidity was determined according to the methodology recommended by AOAC²¹ using 5 grams of homogenized pulp and diluted in 100 mL of distilled water, followed by titration in standard solution of 0.1 N NaOH and phenolphthalein as the point indicator turning point and the results are expressed in (g.citric acid.100g⁻¹ pulp). The ratio (SS/AT) was determined by the difference between soluble solids and titratable acidity. The pH was determined using 5g of pulp homogenized in 100 mL of distilled water with the aid of a digital pot (Digimed DM 20) calibrated with buffer solutions of pH 4.0 and 7.0.²¹

The starch content was quantified by washing the sample (10g) with distilled water and centrifuging at 11,000 rpm for 10 minutes, discarding the supernatant. The sample was transferred to a condenser flask with 75 mL of water and 95 mL of hydrochloric acid, being kept at reflux for 2 hours. Then, the solution was cooled and neutralized with 20% sodium carbonate. The sample was filtered in a 200 mL flask and measured with distilled water.²¹ From this extract, a spectrophotometer (Shimadzu model UV-1650P) was read at 510nm as proposed by Miller GL, et al (1959),²² The results were multiplied by 0.9, which corresponds to the yield of the transformation of sucrose into glucose during hydrolysis. The extraction and quantification of the total pectin content (g.100g⁻¹) were carried out following the recommendations of McReady PM, et al (1952),²³ and Blumenkrantz N, et al (1973).²⁴

In determining amylase activity (μmol of maltose g⁻¹min⁻¹), extraction and quantification followed the method described by Bernfeld P, et al (1955),²⁵ modified by Khader SESA, et al (1992).²⁶ The extraction and determination of pectinamethylesterase activity (PME, UAE g⁻¹ min⁻¹) followed the recommendation of Jen

JJ, et al (1984).²⁷ The demethylation rate of the extract was measured by titration with 0.025 N NaOH, and the pH was maintained at 7 for 10min. A unit of enzymatic activity (UAE) was defined as the amount of the enzyme capable of catalyzing the pectin demethylation corresponding to the consumption of 1nmol of NaOH for 10min. The extract for the determination of polygalacturonase (PG, UAE g⁻¹ min⁻¹) was based on the methodology described by Jen JJ, et al (1984).²⁷ The extract was incubated with 0.25% polygalacturonic acid solution, in 37.5 mM sodium acetate buffer, pH 5.0 at 30°C, for 3 hours. The reaction was stopped in a boiling water bath, and the reducing groups released were determined by the DNS technique.²² 1 UAE of PG was admitted as the amount of enzyme capable of catalyzing the formation of 1 nmol of reducing groups per minute.

Experimental design and statistical analysis

The experiment was conducted in a completely randomized design under a 4x5 factorial arrangement, with four treatments (control, beeswax, calcium chloride and beeswax+calcium chloride) and five storage times (0, 4, 8, 12 and 16 days) with five repetitions and the experimental portion composed of a fruit. The data were submitted to analysis of variance and the means compared by the Tukey test ($p < 0.05$) of probability, with the aid of the statistical program Agrostat.

Results

The combination of beeswax and calcium chloride (CaCl_2) resulted in fruits with less loss of fresh mass (6.93%) and greater firmness (32.18N) compared to the other treatments ($p < 0.05$) over 16 days, especially in control fruits where the loss of mass reached 17.11% and the firmness was less than 10N for the same storage period (Figures 1), respectively.

There was an increase in soluble solids (SS) (Figure 2A) and a reduction in the starch content (Figure 2B) with the storage time and in all treatments. Fruits treated with the combination (beeswax + CaCl_2) resulted in less SS accumulation (11.98 $^{\circ}\text{Brix}$) and higher starch content (8.98 g.100 g⁻¹) after 16 days differing ($p < 0.05$) of the other treatments, especially of the control fruits that presented higher SS (15.21 $^{\circ}\text{Brix}$) and starch degradation (4.27 g.100g⁻¹).

The titratable acidity (TA) increased in the initial days of storage in all treatments. In the control fruits, the maximum peak was observed on the 8th day (0.93 g.100 g⁻¹ citric acid) while on those treated with wax and calcium chloride it was on the 12th day (0.87 and 0.83 g.100 g⁻¹ citric acid), respectively. The combined treatment of beeswax + calcium chloride favored greater stability in the AT content over 16 days (0.82 g.100 g⁻¹ citric acid) differing ($p < 0.05$) in relation to the other treatments (Figure 2C). Fruits treated with beeswax + CaCl_2 showed, over 16 days, the lowest ratio of SS/AT ratio (16.61), differing ($p < 0.05$) in relation to fruits treated with wax (18.89) and CaCl_2 (19.81), mainly in the control fruits (17.71) whose peak of relation was on the 12th day (Figure 2D).

The pH values remained unchanged between day zero (4.98) and day 8 (5.03) with no significant difference ($p > 0.05$) between treatments. After this period, a significant increase ($p < 0.05$) is noted until the 16 day of storage when the treatment averages corresponded to 5.98(control), 5.79(calcium chloride), 5.74(wax bees) and 5.58(beeswax + CaCl_2) (Figure 2E). The total pectin content (Figure 2F) decreased more significantly after the 4 day of storage, but it

remained significantly ($p < 0.05$) higher ($0.25 \text{ g} \cdot 100 \text{ g}^{-1}$) in the fruits treated with beeswax + CaCl_2 after 16 days in relation to treatments with beeswax, CaCl_2 and control (0.16 , 0.14 and $0.09 \text{ g} \cdot 100 \text{ g}^{-1}$), respectively.

Amylase activity (Figure 3A) increased in all treatments corroborating the reduction in starch content (Figure 2B). In the fruits of the control treatment, the greatest activity occurred on the 12 day ($14.96 \mu\text{mol}$ of maltose $\text{g}^{-1} \text{ min}^{-1}$) with subsequent reduction. The fruits treated with beeswax, CaCl_2 and their combination did not differ between themselves ($p > 0.05$) until the 8 day of storage, however, after this period the amylase activity remained significantly lower ($p < 0.05$) in fruits immersed in solution of beeswax + CaCl_2 until the 16 day ($11.45 \mu\text{mol}$ of maltose $\text{g}^{-1} \text{ min}^{-1}$) in relation to the others, suggesting less starch degradation (Figure 2B).

During storage, pectinamethylesterase (PME) activity increased significantly after the 8th day of storage (Figure 3B). In the fruits of the control treatment there was an increase about four times between day 0 ($1000.87 \text{ UAE g}^{-1} \text{ min}^{-1}$) and the 16th day ($41000.67 \text{ UAE g}^{-1} \text{ min}^{-1}$) differing ($p < 0.05$) of the other treatments, especially those immersed in the combined solution of beeswax + CaCl_2 whose activity was twice less ($2056.94 \text{ UAE g}^{-1} \text{ min}^{-1}$) compared to the control at the end of storage. Regarding the activity of polygalacturonase (PG) (Figure 3C), the most significant increase occurred from the 8 day on, coinciding with the reduction in the total pectin content (Figure 2F). During storage, the fruits of the combined treatment of beeswax + CaCl_2 was characterized by lower PG activity ($28.77 \text{ UAE g}^{-1} \text{ min}^{-1}$) compared to the other treatments ($p < 0.05$).

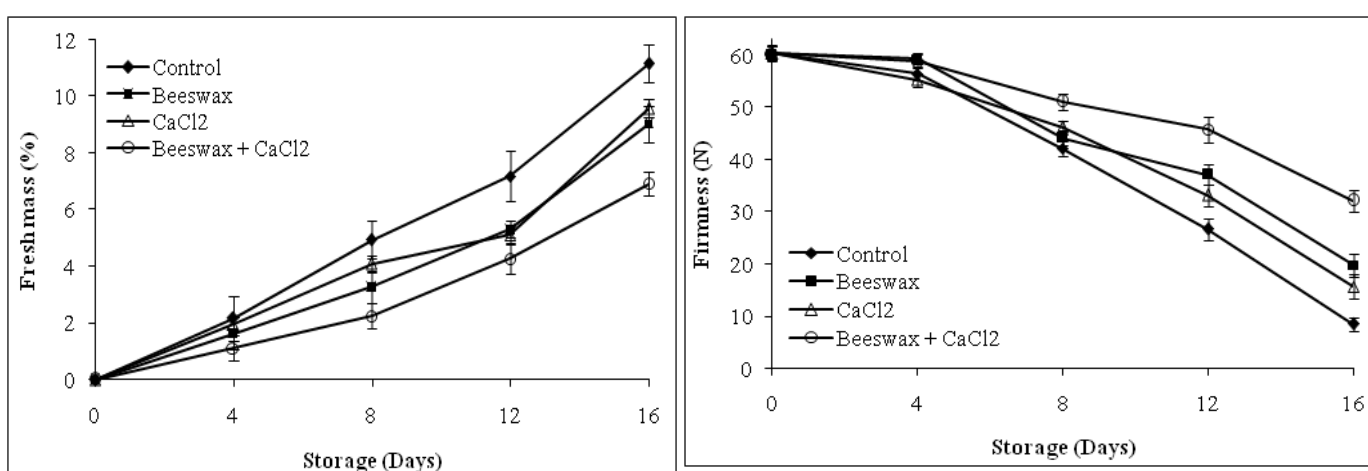
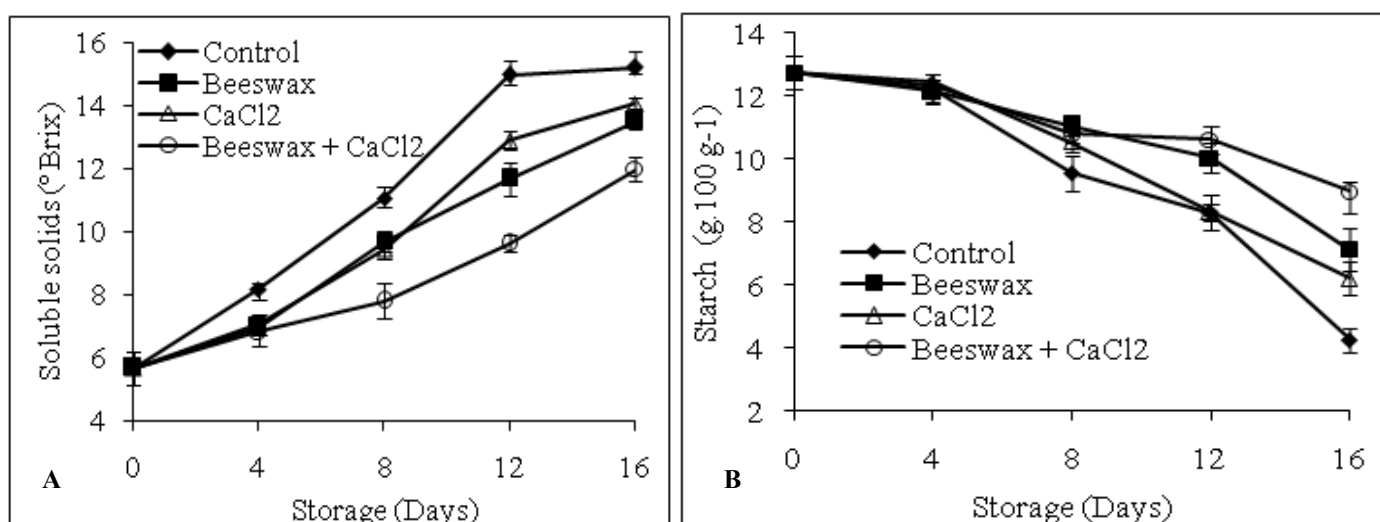


Figure 1 Fresh mass (A) and reduced firmness (B) in ourisop treated with beeswax and calcium chloride with subsequent storage at 13°C for 16 days.



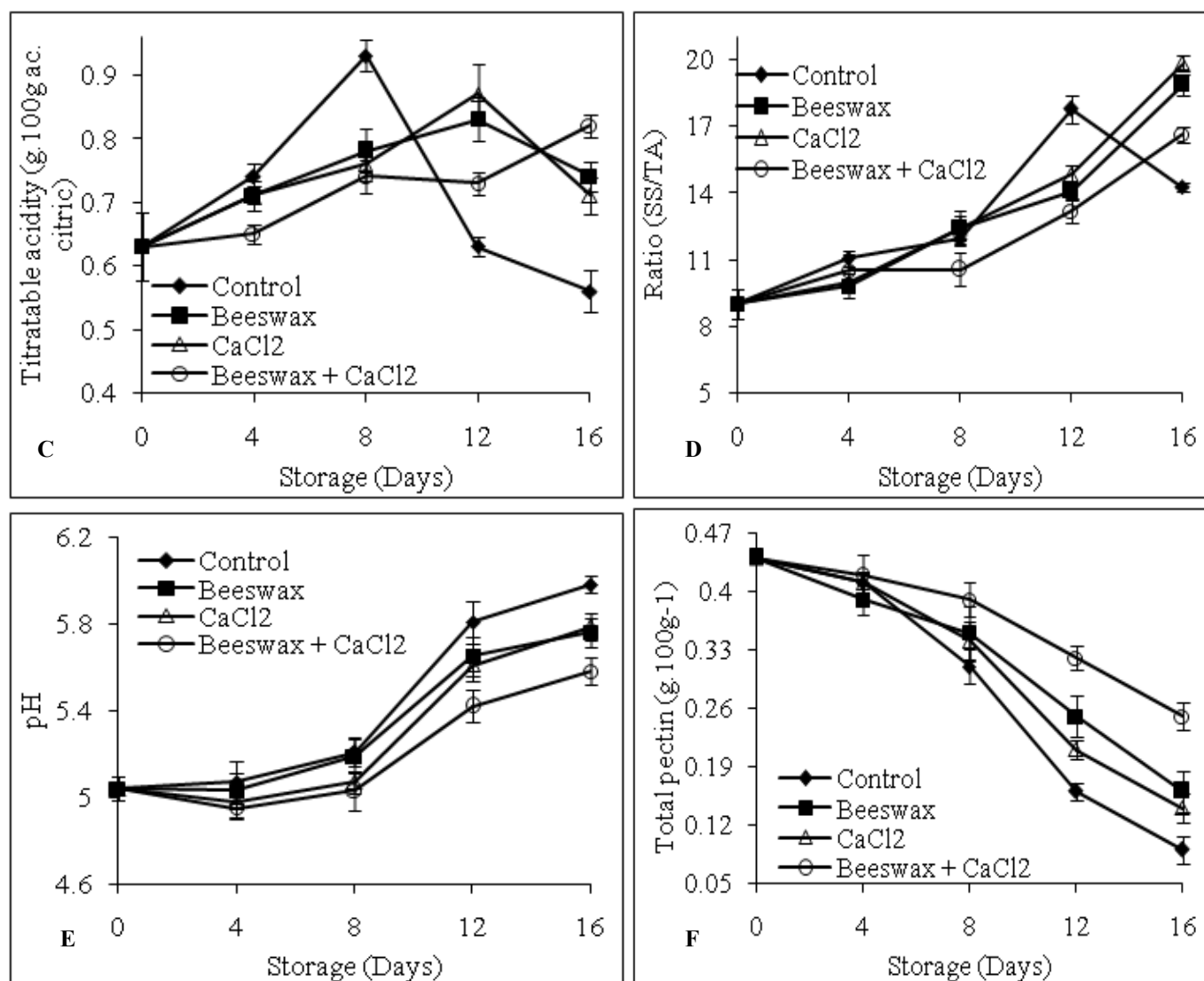


Figure 2 Soluble solids (A), starch (B), titratable acidity (C), pH (D), ratio SS / AT (E) and total pectin (F) in soursop treated with beeswax and calcium chloride with subsequent storage at 13°C for 16 days.

Discussion

The lower loss of fresh mass and firmness observed in fruits treated with beeswax + CaCl₂ (Figure 1A and B) may be associated with the physical barrier formed by the biofilm that reduced gas exchange (sweating and breathing) with the external environment, controlling the loss of moisture and the action of CaCl₂, since this cation binds to the pectic components of the cell wall, mainly in the middle lamella, making it more resistant to water deficit, in addition to preserving the firmness of the fruits. In this sense, some studies show that the use of CaCl₂ as a post-harvest treatment helped to reduce weight loss through its action on the cell wall in terms of preserving firmness.^{28,10,29} A similar study using beeswax (10%) associated with CaCl₂ (4%) also resulted in less mass loss and maintenance of firmness in pine cones over 12 days of storage at 10°C.³⁰

The increase in the SS content (Figure 2A) with the storage time

may be associated with the loss of mass (Figure 1A) that concentrates the sugars in the pulp as it loses water due to perspiration or how much it ripens through the solubilization of pectic substances, as in the case of starch whose content reduced over 16 days (Figure 2B). Thus, the preservation of the fruits favored by the film with beeswax associated with CaCl₂ reflects the delay in their ripening through the lower accumulation of SS (Figure 2A) and starch degradation (Figure 2B). These results corroborate the findings of Eshetu et al,¹¹ in a study with mangos and Sanches et al,¹⁸ in pitangas where concentrations of 2 and 1% of beeswax and CaCl₂, respectively, resulted in delayed ripening and synthesis of carbohydrate metabolism due to the preservation of starch and less accumulation of SS.

Regarding the titratable acidity (Figure 2C), the increase in the initial days of storage is a result of the synthesis of organic compounds related to the ripening of the fruits, while the observed

reduction occurs due to the conversion of acids into sugars that serve as a substrate for respiratory metabolism. The combined treatment of beeswax + CaCl_2 , in addition to maintaining a stable acidity, favored lower contents than that of 16 days of storage, indicating, in this sense, delay in the ripening of the fruits due to less synthesis and consumption of organic acids. This can be corroborated through the SS/AT ratio whose better fruit flavor ratio, that is, the balance of sugars and acids in the pulp, resulted in fruits in a less advanced stage of ripeness (Figure 2D). The increase in pH (Figure 2E), especially

after the 4th day of storage is indicative of the advancement in fruit ripening/senescence, so the combination of beeswax + CaCl_2 delayed these processes in view of more acidic pH values. Research using beeswax as a coating on papaya, orange and mango^{9,31,11} and calcium chloride in pear and cherry,^{15,18} respectively, also demonstrated lower consumption of organic acids that allowed the maintenance of pH at low levels during storage, in addition to preservation in the taste ratio (SS/AT).

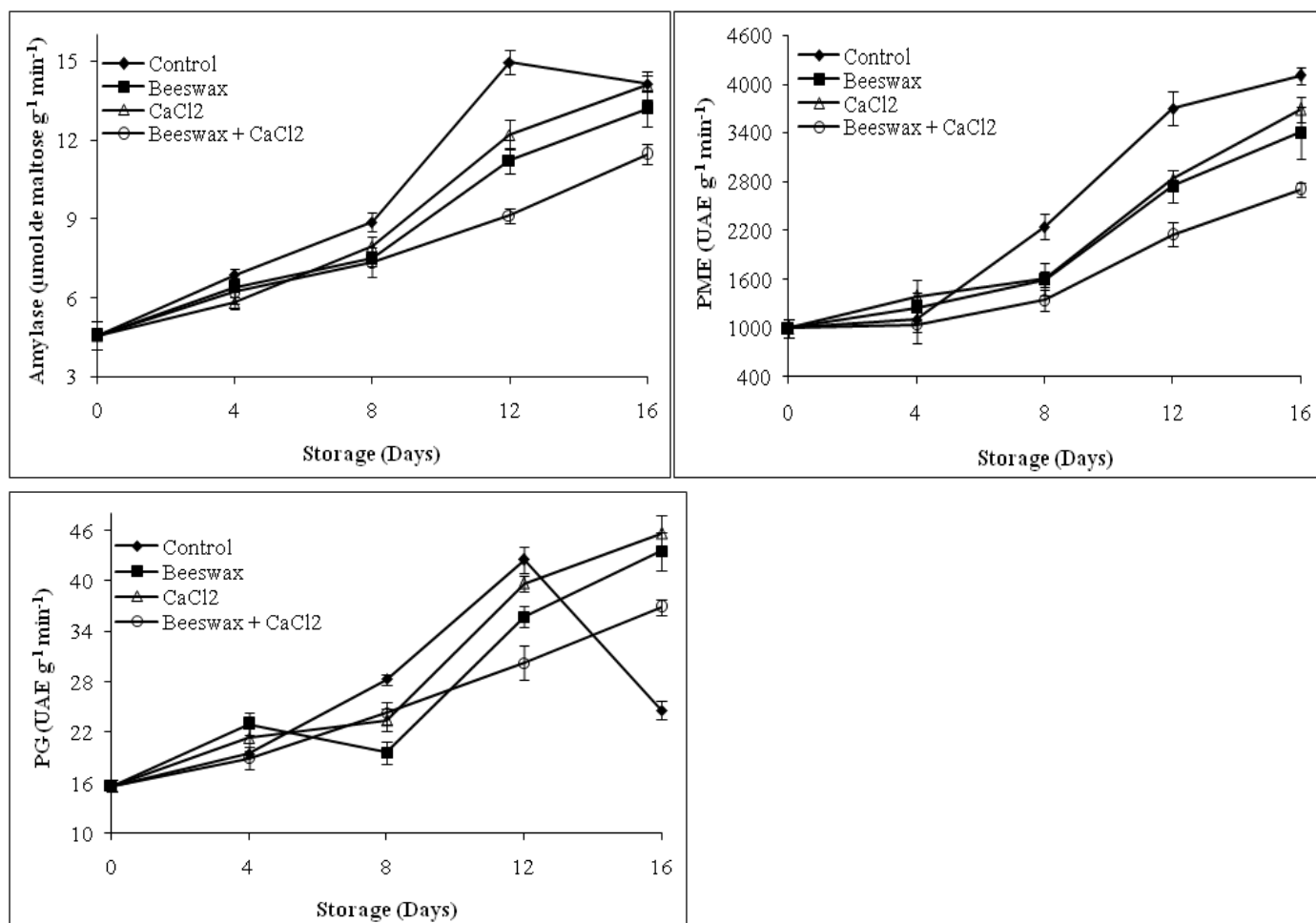


Figure 3 Activity of amylase (A), pectinamethylesterase (B) and polygalacturonase (C) in soursop treated with beeswax and calcium chloride with subsequent storage at 13°C for 16 days.

Pectins constitute a heterogeneous group of polysaccharides, containing acid sugars such as galacturonic acid, and neutral sugars, such as rhamnose, galactose and arabinose. On the wall, pectins are large and complex molecules, responsible for the physical structure that provides mechanical resistance in fruits and vegetables.³² In this study, the reduction in the total pectin content (Figure 2F) is linked to the ripening of the fruits where the pectic substances that constitute the cell wall were solubilized by the enzymatic action of pectinamethylesterase (PME) and polygalacturonase (PG) (Figures 3B and C), respectively. The total pectin content also decreased during the storage of soursop^{33,34,35} in all cases there was direct action by PME and PG on the degradation of pectin.

Amylase is one of the main enzymes involved in the degradation of starch in fruits so that its action releases oligosaccharides and, subsequently, sugars (maltose, glucose and glucose-1-phosphate) to the cellular medium.^{36,35} The increase in amylase activity in this study (Figure 3A) corroborates the reduction in starch content (Figure 2B) and sugar synthesis (Figure 2A), that is, SS. The lower amylase activity during storage of graviolas treated with beeswax + CaCl_2 reinforces the hypothesis about the delay in ripening. Increases in amylase activity during soursop ripening have been observed by Paull et al.³⁷. During the storage of 'Crioula' graviolas at 23°C, the period of greatest amylase activity coincided with the greatest degradation of starch, suggesting that the enzyme acts in the first line of starch degradation in these fruits.³⁴

In the cell wall, pectins are secreted in methyl esterified form, then de-esterified by pectinamethylesterase (PME), becoming available for intermolecular cross-linking with the Ca_2^+ ion. In turn, polygalacturonase (PG) is more active in the degradation of demethylated than methylated pectins.^{38,39} In this way, PME, by catalyzing the demethylation of the C6 carboxylic group of galacturanosyl residues, can play an important role in determining the extent to which pectin will be accessible for degradation by PG, being involved in the softening process.⁴⁰ In practice, the visible post-harvest effect of these enzymes is linked to loss of firmness, changes in pulp viscosity and juice yield (Perfeito, 2014). In this study, the increase in PME and PG activities (Figure 3B and C) indicates their action on cell wall pectin degradation (Figure 2F) resulting in a reduction in fruit firmness (Figure 1B) during storage. However, the physical barrier promoted by the coating with beeswax + CaCl_2 reduced metabolic processes, especially the activity of pectin hydrolysis enzymes (PME and PG), delaying fruit softening.

Conclusion

The combined treatment of beeswax + CaCl_2 delayed the ripening of the soursop due to the preservation of quality attributes and firmness through the lower activity of enzymes associated with the cell wall during storage.

Funding

None.

Acknowledgements

None.

Conflicts of interest

The authors declare that there was no conflict of interest.

References

1. Zacaroni AB, Junqueira NTV, Sussel AAAB, et al. Agronomic performance of soursop (*Annona muricata* L.) on different rootstock species. *Cadernos de Agroecologia*. 2014;9(3):116–120.
2. Junqueira NTV, Junqueira KP. Major diseases of Annonaceae in Brazil: description and control. *Revista Brasileira de Fruticultura*. 2014;36:55–64.
3. Bento EB, Monteiro AB, Lemos ICS, et al. Ethnopharmacological comparative study in the region of the Araripe of *Annona muricata* L. (Graviola). *Revista Cubana de Plantas Medicinales*. 2016;21(1):9–19.
4. Lemos EEP. The production of annona fruits in Brazil. *Revista Brasileira de Fruticultura*. 2014;36(1):77–85.
5. Lima MAC, Alves RE, Filgueiras HAC, et al. Respiratory behavior and postharvest quality of 'Morada' soursop (*Annona muricata* L.) at room temperature. *Revista Brasileira de Fruticultura*. 2003;25(1):49–52.
6. Sanches AG, Silva MB, Moreira EGS, et al. Soursop maturation after controlled atmosphere storage. *Journal of Neotropical Agriculture*. 2016;3(2):54–61.
7. Silva AVC, Muniz EM. Quality of atemoya harvested at two stages of maturation. *Rev. Caat*. 2011;24:9–13.
8. Santos AA. Efficiency of bio-organic coating in post-harvest mango conservation. *Cadernos de Agroecologia*. 2016;10(3):1–4.

9. Mukdisari Y, Suketi K, Widodo WD. Fruit coating with chitosan and beeswax to extend papaya shelf life. *Journal of Tropical Crop Science*. 2016;3(3):1–6.
10. Oliveira MAJ, Souza JMA, Silva MS, et al. Calcium chloride postharvest application on Roxo de Valinhos fig fruits. *Revista de Ciências Agrárias*. 2018;41(4):241–250.
11. Eshetu A, Ibrahim AM, Forsido SF, et al. Effect of treatments with beeswax and chitosan on the quality and shelf life of selected mango cultivars (*Mangifera indica* L.). *Heliyon*. 2019;5(1):11–16.
12. Baswal AK., Dhaliwal HS, Singh Z, et al. Influence of carboxy methylcellulose, chitosan and beeswax coatings on cold storage life and quality of Kinnow mandarin fruit. *Scientia Horticulturae*. 2020;260:1–10.
13. Arah IK, Ahorbo GK, Anku EK, et al. Postharvest handling practices and treatment methods for tomato handlers in developing countries: A Mini Review. *Advances in Agriculture*. 2016;64(3):1–8.
14. Lara I. Preharvest sprays and their effects on the postharvest quality of fruit. *Stewart Postharvest Review*. 2013;3(5):1–9.
15. Sohail M, Ayub M, Khalil AS, et al. Effect of calcium chloride treatment on post harvest quality of peach fruit during cold storage. *International Food Research Journal*. 2015;22(6):2225–2229.
16. Sharma SK, Pratima P. Effect of pre-harvest gibberellic acid and calcium application on post-harvest behaviour of subtropical peaches. *Current Agriculture Research Journal*. 2018;6(1):78–84.
17. Kumar S, Thakur KS. Influence of post-harvest calcium chloride treatments on physico-chemical and sensory attributes of pear cv. 'Bartlett' under ambient storage conditions. *Applied Biological Research*. 2017;19(3):270–279.
18. Sanches AG, Silva MB, Moreira EGS, et al. Extension of the service life of pitangas subjected to calcium chloride treatment. *Acta Iguazu*. 2017;6(1):45–58.
19. Santos EX, Santana PJA, Sanches AG, et al. Postharvest technologies in the ripening of 'Maçã' bananas stored in ambient condition. *Amazonian Journal of Plant Research*. 2019;3(1):290–297.
20. Rodrigues DC, Cáceres CA, Ribeiro HL, et al. Influence of cassava starch and carnaúba wax on physical properties of cashew tree gum-based films. *Food Hydrocolloids*. 2014;38:147–151.
21. Association of Official Analytical Chemistry. Official methods of analysis of the Association of Official Analytical Chemistry. Washington: AOAC; 2012.
22. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Analytical Chemistry*. 1959;31:426–428.
23. McReady PM, McComb EA. Extraction and determination of total pectin materials. *Analytical Chemistry*. 1952;24(12):1586–1588.
24. Blumenkrantz N, Asboe-Hansen G. New method for quantitative determination of uronic acids. *Analytical Biochemistry*. 1973;54(2):484–489.
25. Bernfeld P. Enzymes in carbohydrate metabolism. In: Colowick SP, Kaplan NO, editors. *Methods in Enzymology*. New York: Academic Press; 1955. p.149–150.
26. Khader SESA. Effect of gibberellic acid and vapor guard on ripening amylase and peroxidase activities and quality of mango fruits during storage. *Journal of Horticultural Science*. 1992;67:855–860.
27. Jen JJ, Robinson MLP. Pectolytic enzymes in sweet bell peppers (*Capsicum annuum* L.). *Journal of Food Science*. 1984;49:1045–1087.

28. Sanches AG, Costa JM, Silva MB, et al. Chemical treatments for maintaining post-harvest quality in pitanga fruits (*Eugenia uniflora* L.). *Nativa*. 2017;5(4):257–262.
29. Garcia LGC, Silva EP, Silva Neto CM, et al. Effect of the addition of calcium chloride and different storage temperatures on the post-harvest of jaboticaba variety Pingo de Mel. *Food Science and Technology*. 2019;39(1):261–269.
30. Suresh GJ, Jagadeesh L. Effect of post-harvest immersion treatment of calcium chloride and wax on post-harvest behavior of pinecone fruits. *Acta Horticulture*. 2019:1241;541–548.
31. Mazemir S, Abera S, Solomon WK. Effect of bee wax and linseed oil coatings and frequency of dipping on the biochemical and organoleptic quality of fresh orange juice (*Citrus sinensis* cv. Valencia). *Journal of Postharvest Technology*. 2017;05(2):17–28.
32. Taiz L, Zeiger E, Moller I, et al. Plant physiology and development. 6th edition. Porto Alegre: Artmed; 2017. p.888.
33. Aziz PA, Yusof S. Physico-chemical characteristics of soursop fruit (*Annona muricata*) during growth and development. *Asean Food Journal*. 1994; 9(4):147–150.
34. Lima MAC, Alves RE, Filgueiras H.A.C. Changes related to softening of soursop during postharvest maturation. *Pesquisa Agropecuária Brasileira*. 2006;41(12):1707–1713.
35. Lima MAC, Alves RE, Filgueiras HAC. Respiratory behavior and softening of soursop fruit (*Annona muricata* L.) after postharvest treatments with wax and 1-methylcyclopropene. *Ciência Agrotecnica*. 2010;34(1):155–162.
36. Irving DE, Shingleton GJ, Hurst PL. Starch degradation in buttercup squash (*Cucurbita maxima*). *Journal of the American Society for Horticultural Science*. 1999;124(6):587–590.
37. Paull RE, Deputy J, Chen NJ. Changes in organic acids, sugars, and headspace volatiles during fruit ripening of soursop. *Journal of the American Society for Horticultural Science*. 1983;108(6):931–934.
38. Kayshap DR, Vohra PK, Chopra S, et al. Applications of pectinases in the commercial sector: a review. *Bioresource Technology*. 2001;77(3):215–227.
39. Jayani RS, Saxena S, Gupta R. Enzimas pectinolíticas microbianas: uma revisão. *Process Biochemistry*. 2005;40:2931–2944.
40. Ali ZM, Chim LH, Lazan H. A comparative study on wall degrading enzymes, pectin modifications and softening during ripening of selected tropical fruits. *Plant Science*. 2004;167:317–327.