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# 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 15ºC for 16 days. Analyzes 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 (11.98ºBrix), 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.100$g^{-1}$) and total pectin (0.25 g.100$g^{-1}$), 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

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## 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 gaviola, 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 markets[4]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 13ºC, 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 membrane[14] 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 (13ºC).

## 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 100ºC 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 70ºC 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 1mmol $L^{-1}$ NaOH using pH meter. When the solution reached room temperature (±25ºC) 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±1ºC) and relative humidity of 95±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 byAOAC[21] and the results expressed in Brix degrees (ºBrix).

The titratable acidity was determined according to the methodology recommended byAOAC[21] using 5grams 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.100$g^{-1}$ 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 byMiller 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.100$g^{-1}$) were carried out following the recommendations ofMcReady PM, et al (1952),[23]andBlumenkrantz N, et al (1973).[24]

In determining amylase activity (µmol of maltose $g^{-1}min^{-1}$), extraction and quantification followed the method described byBernfeld P, et al (1955),[25] modified byKhader SESA, et al (1992).[26]The extraction and determination of pectinamethylesterase activity (PME, UAE $g^{-1}$ $min^{-1}$) followed the recommendation ofJen 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 30ºC, 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 Agroestat.

## 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 ºBrix) 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 ºBrix) and starch degradation (4.27 g.100$g^{-1}$).

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 12$^{th}$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 g.100 $g^{-1}$) 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 g.100 $g^{-1}$), 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 µmol of maltose $g^{-1}$ $min^{-1}$) 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 µmol of maltose $g^{-1}$ $min^{-1}$) 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 UAE $g^{-1}$ $min^{-1}$) and the 16$^{th}$ day (41000.67 UAE $g^{-1}$ $min^{-1}$) 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 UAE $g^{-1}$ $min^{-1}$) 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 UAE $g^{-1}$ $min^{-1}$) compared to the other treatments ($p<0.05$).

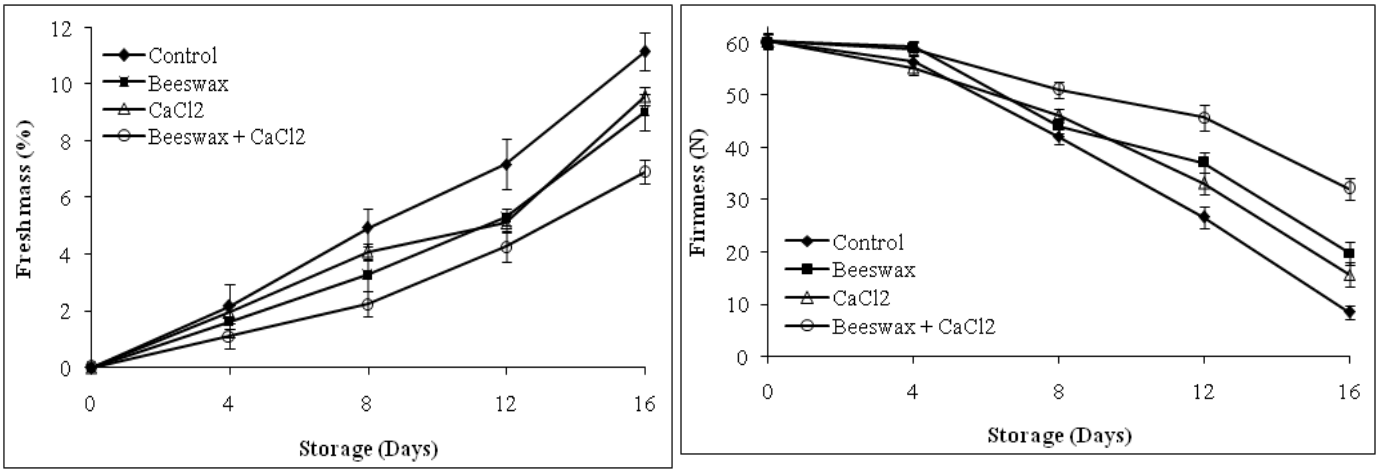
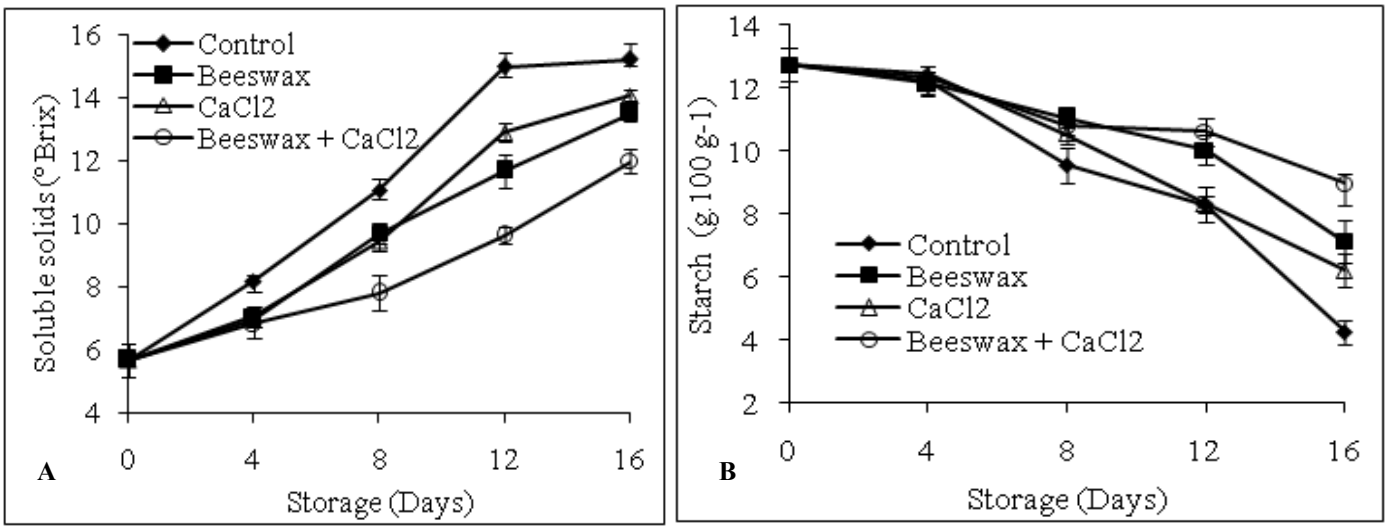



**Figure 1** Fresh mass (A) and reduced firmness (B) in oursop treated with beeswax and calcium chloride with subsequent storage at 13°C for 16 days.

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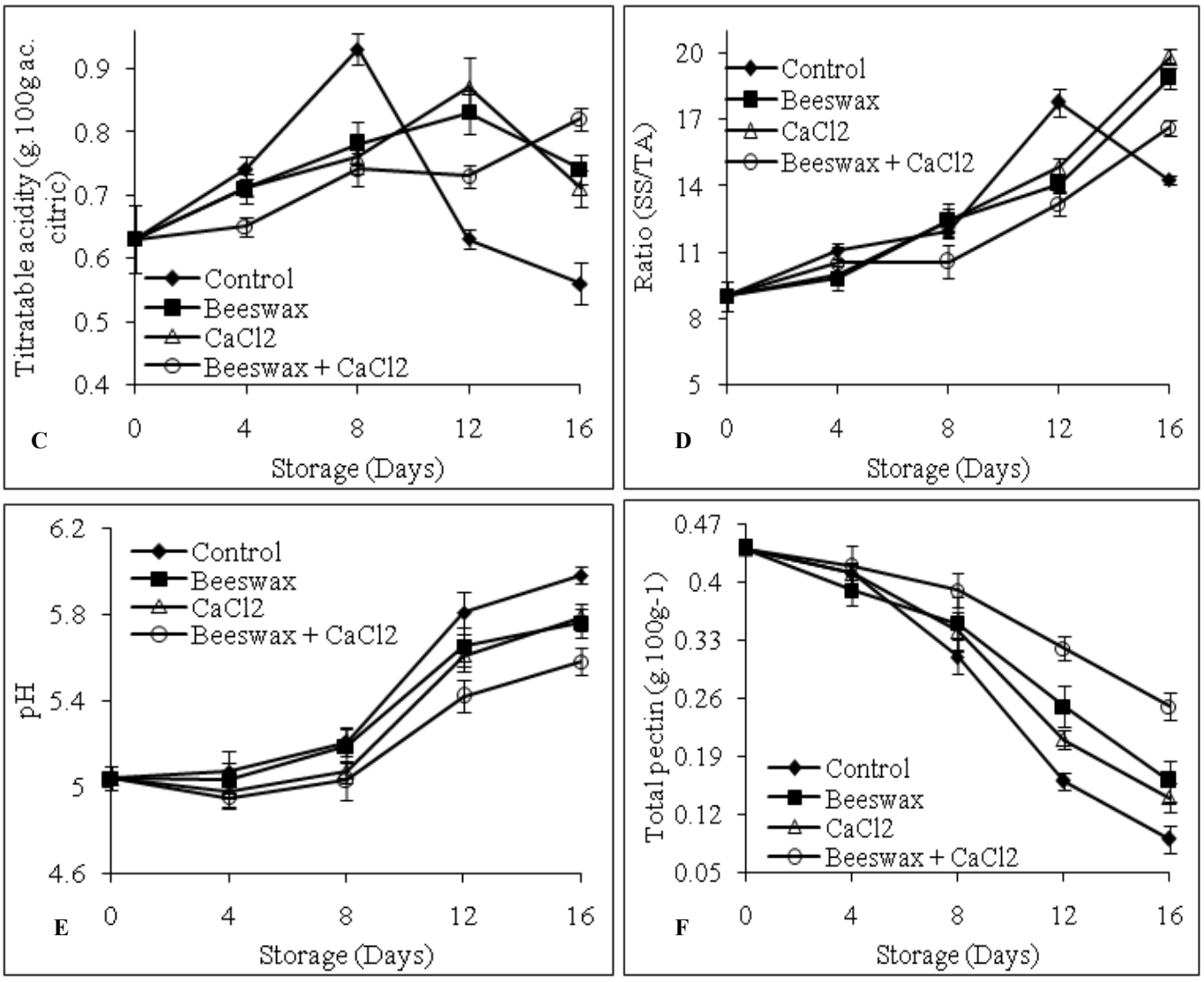


**Figure 2** Soluble solids (A), starch (B), titratable acidity (C), pH (D), ratio SS / AT (E) and total pectin (F) in soursop treated with beeswax and calcium chloride with subsequent storage at 13°C for 16 days.

## Discussion

The lower loss of fresh mass and firmness observed in fruits treated with beeswax + $CaCl_2$ (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 $CaCl_2$, 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 $CaCl_2$ 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 $CaCl_2$ (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 $CaCl_2$ reflects the delay in their ripening through the lower accumulation of SS (Figure 2A) and starch degradation (Figure 2B). These results corroborate the findings ofEshetu et al,[11]in a study with mangos and Sanches et al,[18]in pitangas where concentrations of 2 and 1% of beeswax and $CaCl_2$, 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).

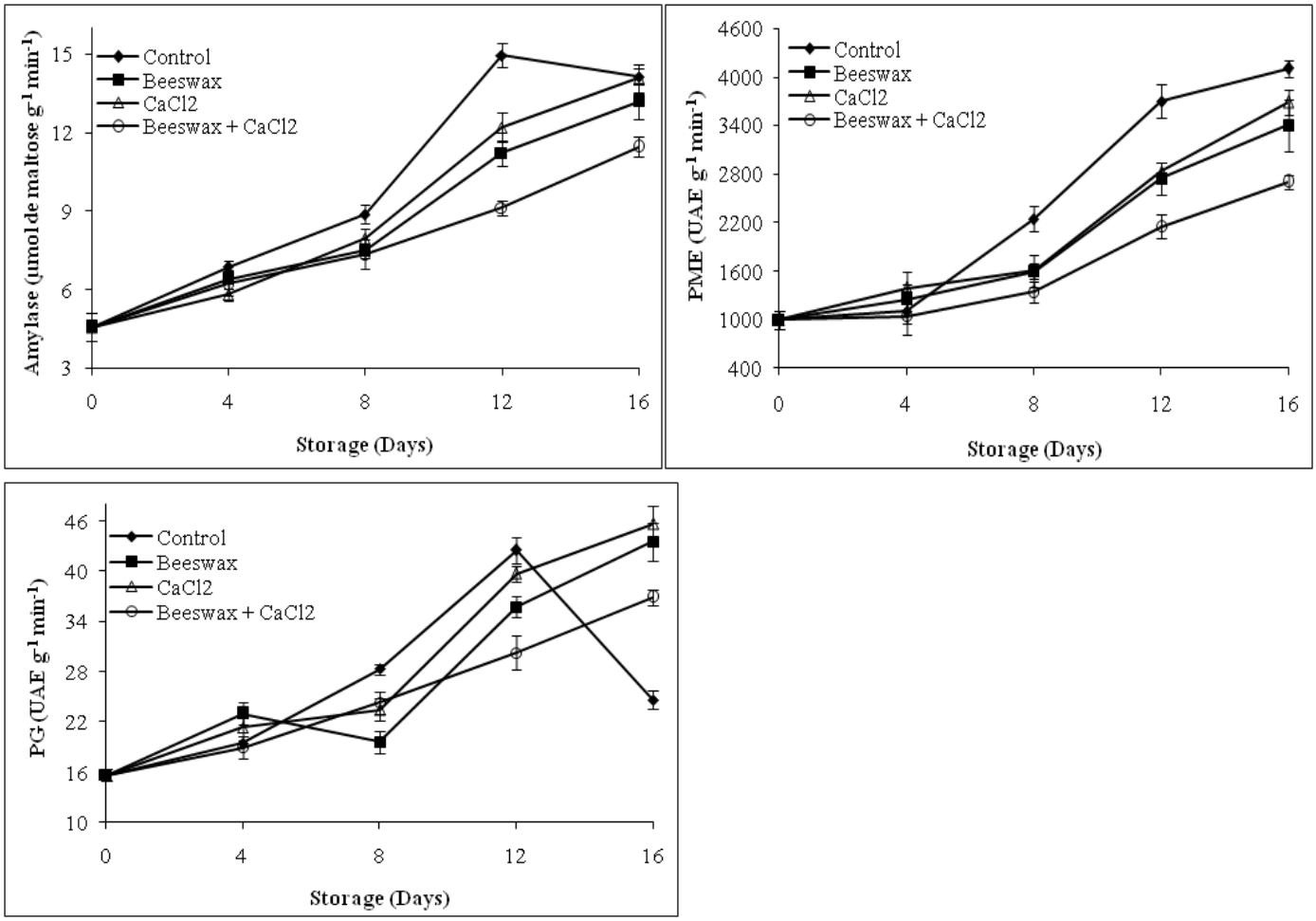


**Figure 3** Activity of amylase (A), pectinamethylesterase (B) and polygalacturonase (C) in soursop treated with beeswax and calcium chloride with subsequent storage at 13°C for 16 days.

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 23ºC, 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 + CaCl2 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.

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## Conflicts of interest

The authors declare that there was no conflict of interest.

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