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Maintaining the firmness of minimally processed papaya using pectin methylesterase and calcium lactate

Manutenção da firmeza do mamão minimamente processado utilizando pectina metilesterase e lactato de cálcio

Mantener la firmeza de la papaya mínimamente procesada con pectina metilesterasa y lactato de calcio

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Abstract

The objective of this work was to evaluate the effect of vacuum infusion of pectin methylesterase (PME) and calcium lactate ($C_6H_{10}CaO_6$) in maintaining the firmness of minimally processed papaya, in order to maintain the quality and cellular integrity of the fruit. After minimal processing, the treatments used were: fruit without infusion (control), with H_2O infusion, with PME infusion, with $C_6H_{10}CaO_6$ infusion and with PME+$C_6H_{10}CaO_6$ infusion. At zero times, four and eight days of storage, analyzes of total galacturonic acid, methanol, cell integrity, vitamin C, pH, acidity, soluble solids, damage, freshness and contamination were performed. Papaya treated with the PME+calcium combination showed an increase in firmness (5.8 N) on the eighth day of storage, differing from the control treatment (1.3 N), reporting the least leakage of electrolytes. On the fourth day, the fruit treated with PME+$C_6H_{10}CaO_6$ showed the highest content of galacturonic acid and on the eighth day the highest content of methanol, indicating an effective action of the enzyme PME and calcium in this period. The PME+$C_6H_{10}CaO_6$ treatment was effective in maintaining and improving the quality of papaya while preserving freshness, soluble solids content, acidity and pH throughout storage.

Keywords: Vacuum infusion; Cell integrity; Pectin demethylation.

Resumo

O objetivo deste trabalho foi avaliar o efeito da infusão a vácuo de pectina metilesterase (PME) e lactato de cálcio ($C_6H_{10}CaO_6$) na manutenção da firmeza do mamão minimamente processado, visando manter a qualidade e a integridade celular do fruto. Após o processamento mínimo, os tratamentos empregados foram: fruto sem infusão (controle); com infusão de H_2O; com infusão de PME; com infusão de $C_6H_{10}CaO_6$ e com infusão de PME+$C_6H_{10}CaO_6$. Nos tempos zero, quatro e oito dias de armazenamento foram realizadas análises de ácido galacturônico total, metanol, integridade celular, vitamina C, pH, acidez, sólidos solúveis, danos, frescor e contaminação. O mamão tratado com a combinação PME+cálcio apresentou um aumento da firmeza (5,8 N) no oitavo dia de armazenamento, diferindo em relação ao tratamento controle (1,3 N), reportando o menor vazamento de eletrólitos. No quarto dia, o fruto tratado com PME+$C_6H_{10}CaO_6$ apresentou o maior teor de ácido galacturônico e no oitavo dia o maior teor de metanol, indicando uma ação efetiva da enzima PME e do cálcio nesse período. O tratamento PME+$C_6H_{10}CaO_6$ foi eficaz em manter e melhorar a qualidade do mamão preservando o frescor, o conteúdo de sólidos solúveis, a acidez e o pH ao longo do armazenamento.

Palavras-chave: Infusão a vácuo; Integridade celular; Desmetilação da pectina.

Resumen

El objetivo de este trabajo fue evaluar el efecto de la infusión al vacío de pectina metilesterasa (PME) y lactato de calcio ($C_6H_{10}CaO_6$) en el mantenimiento de la firmeza de la papaya mínimamente procesada, con el fin de mantener la calidad e integridad celular del fruto. Después de un procesamiento mínimo, los tratamientos utilizados fueron: fruto sin infusión (control); con infusión de H_2O; infundido con PME; con infusión de $C_6H_{10}CaO_6$ y con infusión de PME+$C_6H_{10}CaO_6$. En tiempos cero, cuatro y ocho días de almacenamiento, se realizaron análisis de ácido galacturónico total, metanol, integridad celular, vitamina C, pH, acidez, sólidos solubles, daño, frescura y contaminación. La papaya tratada con la combinación PME+calcio mostró un aumento de firmeza (5,8 N) en el octavo día de almacenamiento, diferenciándose del tratamiento control (1,3 N), reportando la menor fuga de electrolitos. Al cuarto día, la fruta tratada con PME+$C_6H_{10}CaO_6$ tuvo el mayor contenido de ácido galacturónico y al octavo día el mayor contenido de metanol, lo que indica una acción efectiva de la enzima PME y calcio en este período. El tratamiento PME+$C_6H_{10}CaO_6$ fue eficaz para mantener y mejorar la calidad de la papaya al tiempo que conservaba la frescura, el contenido de sólidos solubles, la acidez y el pH durante el almacenamiento.

Palabras clave: Infusión al vacío; Integridad celular; Desmetilación de pectina.

1. Introduction

Papaya (*Carica papaya* L.) is one of the main fruit trees present in tropical and subtropical regions of the planet, with varieties classified into two groups, *Solo* and *Formosa* (Dantas et al., 2013). The fruits of the *Formosa* group are well accepted by consumers due to the quality of their pulp, however they are not convenient for individual use, as they are large and need preparation before consumption. In this way, minimal processing becomes a marketing alternative, providing a more practical consumption, adding value to the final product (Teixeira et al., 2001).

When a fruit is harvested, it continues to breathe and undergo a series of endogenous transformations, which will reflect changes in its characteristics (Batista, 2015), mainly changes in texture and firmness. The ripening process is accompanied by a loss of firmness, caused by changes in the polysaccharides that make up the cell wall, such as cellulose, hemicellulose and pectin. These changes result from the performance of endogenous enzymes

in the fruit (Zhang et al., 2019).

The decrease in pulp firmness during ripening is attributed to the action of polygalacturonase (PG; EC 3.2.1.15) and pectin methylesterase (PME; EC 3.1.1.11) (Kohli et al., 2015). PME catalyzes the demethylation of pectin, damaging the horizontal calcium links of the acid polysaccharide chain, leading to cell separation, resulting in a greater number of carboxylic acid groups, which facilitates the action of polygalacturonase. PG acts by hydrolyzing pectin acid, causing pectin degradation, cell wall dissolution and fruit softening (Zhang et al., 2019).

The demethylation of pectin caused by the action of PME can improve the texture of fruits and vegetables, since the carboxylic groups resulting from this process when interacting with divalent ions such as exogenous Ca^{+2}, form a network maintaining the stability of pectin and the cell wall structure (Guillemin et al., 2008; Kohli et al., 2015). In the cell wall of plants, calcium suppresses the decline in quality, preserves integrity and reduces membrane permeability during storage (Aguayo et al., 2015).

Calcium applications have been used for producing beneficial effects on the texture of fresh fruits (Yang et al., 2017). The process of de-esterification of pectin by the PME and subsequent association with calcium forms the complex known as “egg box”, which acts as a cement, providing firmness to vegetables (Aghdam et al., 2012). The technique that has been used for the application of calcium and exogenous PME to fruits is vacuum impregnation, which consists of exchanging native gases and liquids with an impregnation solution under the action of the hydrodynamic mechanism (Derossi et al., 2013).

Recently, studies have been carried out applying calcium chloride to pre-harvest tangerines (Vasconcelos et al., 2020) and to post-harvest apricots (Liu et al., 2017) and using the vacuum infusion of PME+calcium in plant matrices such as apples (Guillemin et al., 2008), mangoes (Batista, 2015), papayas (Yang et al., 2017) strawberries (Carnelossi et al., 2018) and peppers (Paixão et al., 2020).

Thus, the combined use of the enzyme pectin methylesterase with calcium becomes important to avoid post-harvest losses related to decreased firmness of MP fruits, being an alternative for maintaining the sensory characteristics, prolonging the shelf life of these products. The objective of this work was to evaluate the effect of the infusion of pectin methylesterase (PME) and calcium in maintaining the firmness of the minimally processed papaya in order to maintain the quality and cellular integrity, as well as to increase the useful life of the fruit.

2. Methodology

Minimal processing

Papaya of the Formosa variety were selected according to their degree of ripeness (½ ripe fruit, 25 to 50% of the yellowish peel surface), according to the classification by Ceagesp (2015).

After reception, the fruits were washed in running water and sanitized in an aqueous chlorine solution at a concentration of 200 mg L^{-1} at a temperature of 5±1°C for 10 minutes. Then, peel removal and manual cutting into cubes was promoted. The cubes were sanitized in an aqueous chlorine solution at 5±1°C at a concentration of 5 mg L^{-1} for 3 minutes.

Vacuum infusion and treatments

The vacuum infusion was performed using 85 g of minimally processed papaya arranged in 250 mL beakers with 125 mL of aqueous solution. A pressure of 33 KPa or 250 mmHg was applied for 10 minutes using a desiccator with a pressure gauge attached to a vacuum pump. When the pressure was reached, the system was turned off.

The minimally processed papayas were divided into 5 treatments: control (without infusion); infusion with water only; infusion with aqueous solution containing commercial PME (10 IU); infusion with aqueous solution containing commercial PME and Ca^{+2} (calcium lactate); and infusion with aqueous solution containing Ca^{+2} (calcium lactate) (Batista, 2015; Carnelossi et al., 2018).

The enzyme used in the experiments was pectin methylesterase (NovoShape, Novozymes, Denmark) diluted to achieve the activity recommended by the manufacturer, 10 PEU/mL, that is, 1 mL PME/kg of fruit. The concentration of calcium lactate ($C_6H_{10}CaO_6$), at 1% was determined based on the experimental results obtained by (Batista, 2015; Carnelossi et al., 2018).

After infusion, the minimally processed papayas (85 g) were drained and then packed in polypropylene packages and stored in a vertical display with air circulation (Springer) at a temperature of 5°C range 1 for 8 days, under relative humidity of 78-82%. Samples were collected at times 0, 4 and 8 days for biochemical and physical-chemical analysis, previously crushed and homogenized with the aid of the Sorvall Ominimixer equipment and stored at -18°C until the time of analysis.

Physical-chemical and biochemical analyzes

Determination of firmness

The determination of firmness was performed on the papaya MP with the aid of the Brookfield CT3 Texture Analyzer equipment using the TexturePro CT V1.2 Build 9 program with application of the TPA test. The test was carried out with an aluminum probe of 5.0 mm in diameter at a speed of 0.5 mm/s. The strain distance used was 3.0 mm and the firing load 0.06 N. Triplicate readings were performed and the result was expressed in Newton (N).

Cell integrity

The analysis of cell integrity was determined using the methodology of Villalta and Sargent (2004). For this, a 2 g sample of the papaya MP cut in cubes of 5mm-2mm was used. After weighing, the sample was washed with distilled water on filter paper, placed in centrifuge tubes (50 mL) and added with 35 mL of 0.6 M isotonic mannitol solution at 23°C for 4 hours. After this time, the conductivity was determined and the samples were frozen (24 h at -20°C). After 24 h, the samples were thawed and heated in boiling water for 30 minutes. After cooling, the conductivity was again determined, with the electrolytic flow expressed as a percentage of the total tissue electrolytes.

Determination of methanol

The analysis of methanol determination was performed according to the methodology described by Wood and Siddiqui (1971), with adaptations. For analysis, 250 mg of the sample were suspended in 2 mL of distilled water and taken to an ultrasonic bath for 10 minutes. Then 0.8 mL of 2M NaOH were added for deesterification and the sample was incubated at 20°C with occasional shaking. Then, neutralization was carried out with the addition of 0.8 mL of 2M HCl, equilibrated at a temperature of 25°C in a water bath for 15 minutes. Soon after, the sample was centrifuged at 8000 rpm for 10 minutes at 25°C.

The quantification of methanol was determined using a 1 mL aliquot of the sample preparation collected and placed in a test tube with 1 mL of 1.0 N H_2SO_4. The test tubes were cooled in an ice water bath and adding 0.2 mL of 0.2% potassium permanganate. It was mixed and kept in an ice bath for 15 minutes. Subsequently, 0.2 mL of 0.5 M sodium arsenate solution in 0.12N H_2SO_4 was added, followed by the addition of 0.6 mL of distilled water, remaining for 1 hour at room temperature. Soon after, 2 mL of the 0.02 M acetylacetone solution dissolved in a solution containing 2M ammonium acetate and 0.05M acetic acid were

added, the tubes were shaken, capped with "marbles" and heated in a water bath the temperature of 58-60°C for 15 minutes. After heating, the sample was centrifuged at 10,000 rpm for 10 minutes, for deposition of the precipitate, followed by the absorbance reading in a spectrophotometer at 412 nm.

Determination of total galacturonic acid

The contents of galacturonic acid were determined according to the methodology of Ahmed and Labavitch (1977), with adaptations. For sample preparation, 100 mg was weighed in a 20 mL beaker containing a magnetic stir bar. 2 mL of refrigerated sulfuric acid and 0.5 mL of cold distilled water were added, promoting gentle agitation for 5 minutes. Another 0.5 mL of cold distilled water was added, followed by another 10 minutes of stirring. 17 mL of cold distilled water was added to complete the volume of the beaker.

The total galacturonic acid was determined by collecting a 0.5 mL aliquot of the sample contained in the beaker, placing it in test tubes previously cooled in an ice water bath. Afterwards, 3.6 mL of the 0.0125 M sodium tetraborate solution in refrigerated sulfuric acid was added and the mixture was promoted. The tubes were heated in a boiling water bath for 5 minutes and cooled under running water. After being cooled, 60 µL of the 0.15% m-hydroxydiphenyl solution in 0.5% NaOH was added. A rapid homogenization was carried out and the absorbance was read on a spectrophotometer at 520 nm.

pH, soluble solids and titratable acidity

The pH of the samples was determined in a digital pH meter model DLA-PH, previously calibrated and the soluble solids were quantified by adding drops of the sample previously homogenized on the prism of the digital refractometer (Hanna Instruments). Titratable acidity was determined using the 1% phenolphthalein indicator and 0.1 M sodium hydroxide solution, the acidity being expressed as a percentage of citric acid (IAL, 2008).

Vitamin C

To quantify the vitamin C content, 5 g of the sample were weighed in a beaker and homogenized with an extraction solution, 2% oxalic acid. Subsequently, the sample was filtered with the aid of filter paper into a 50 mL volumetric flask and the flask volume was completed with extraction solution. Then, 7 mL of the flask solution were collected, transferred to a 50 mL conical flask and titrated with the 2,6-dichlorophenolindophenol solution (DCPIP), until the formation of a persistent pink color. The results were expressed in

mg of ascorbic acid/100g of fresh matter (AOAC, 1992).

Damage, freshness and contamination

The damage analysis consisted of counting the number of minimally processed papaya cubes that presented some type of post-harvest mechanical damage, the result being expressed as a percentage of the total papaya cubes present in the packaging. The freshness of the fruit was assessed using the following scale: 9 = excellent (completely fresh appearance, high gloss); 7 = good (still looks fresh, still shiny); 5 = fair (does not have a fresh appearance, low gloss, liquidity limit); 3 = poor (dull, usability limit); 1 = extremely poor (withered appearance), the result being expressed as the average of repetitions per treatment. The contamination consisted of counting the papaya cubes that had an incidence of post-harvest rot and/or the presence of visible fungi, the results being expressed as a percentage (Jacomino et al., 2011).

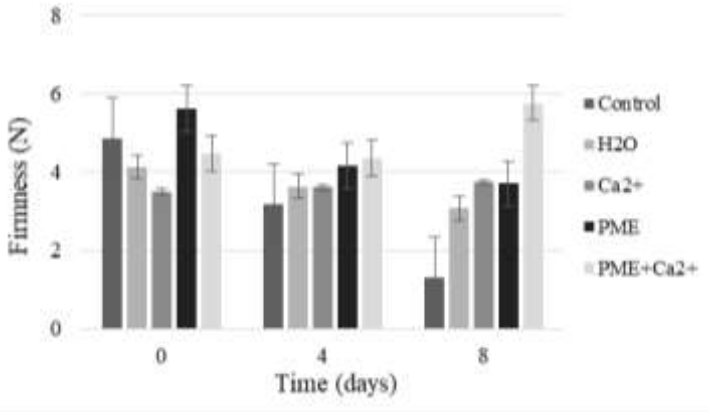
Statistical analysis

A completely randomized design with a 5 x 3 factorial scheme (5 treatments and 3 times) was used, with three replications per treatment. The data collected were submitted to analysis of variance (ANOVA) and the means compared by the Tukey test ($p<0.05$), expressed as mean ± standard deviation, with the aid of the Sisvar 5.7 computer program (Ferreira, 2011).

3. Results and Discussion

After the vacuum infusion process, all treatments showed a decrease in firmness when compared to the control sample, with the exception of minimally processed papaya treated only with PME (Figure 1). The decrease in firmness, in this first moment, can be explained by the pressure difference to which the papaya cubes are submitted when the vacuum is applied, this change in pressure may have weakened the cellular structures, resulting in the loss of firmness soon after impregnation vacuum (Guillemin et al., 2008; Yang et al., 2017).

Figure 1. Firmness of minimally processed papaya after infusion (day 0) and during storage (4 and 8 days).



Treatments: Control, without infusion; H20, water infusion; Ca2+, infusion with calcium lactate; PME, infusion with the enzyme pectin methylesterase; and PME+Ca2+, infusion with the enzyme pectin methylesterase and calcium lactate. Three repetitions were performed. The bars indicate the standard error of the mean. Source: Author's own compilation (2020).

On the other hand, the momentary increase in firmness in treatment only with PME may have happened due to the action of the exogenous PME associated with the endogenous calcium present in the fruit matrix. However, as endogenous calcium ions are insufficient to bind to all free carboxyl groups released by the action of the PME, after the momentary increase in firmness, papaya subsequently softens (Lara et al., 2004).

It was found that papaya treated with the PME+calcium combination showed an increase in firmness on the eighth day of storage compared to the other treatments, differing significantly ($p<0.05$) from the control treatment (Figure 1). This result demonstrates that the PME+$C_6H_{10}CaO_6$ treatment was effective in maintaining and increasing papaya firmness during storage.

The increase in firmness occurs once the enzyme PME breaks the chains of galacturonic acid, the methanol clusters of the pectin are hydrolyzed and then the calcium is bound with the groups of carboxylic acids, maintaining the structure of the wall, making it the firmer (Guillemin et al., 2008; Batista, 2015).

The control sample showed a gradual decrease in firmness, differing significantly ($p<0.05$) over time. This behavior may be associated with the action of the polygalacturonase enzyme, which can be elevated in freshly cut papayas, when compared to intact fruit, due to the increase in ethylene production and damage caused by cuts, as observed by Karakurt and Huber (2003).

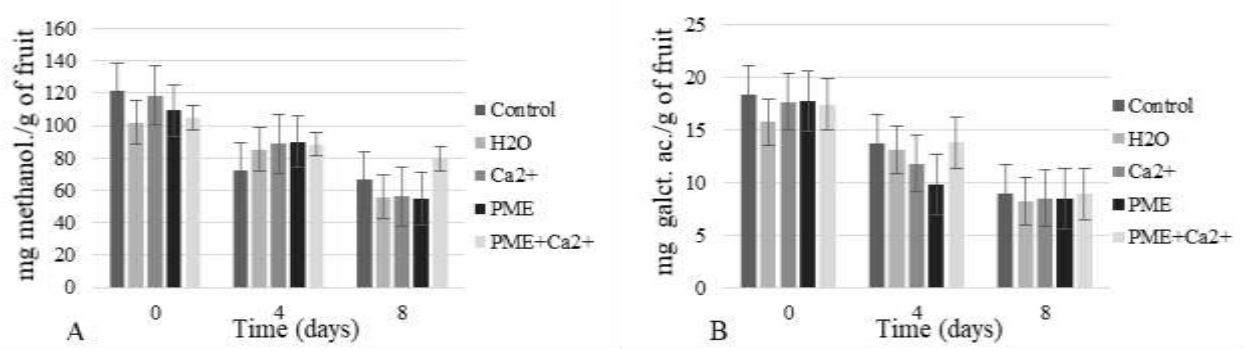
The treatment with $C_6H_{10}CaO_6$ maintained the firmness of the minimally processed papaya achieved after vacuum impregnation during storage; however the combined treatment with the PME enzyme proved to be more efficient because in addition to maintaining, it increased firmness over the 8 days. According to Aghadam et al. (2012), calcium lactate can inhibit endogenous PME and/or promote greater "egg box" conformation, maintaining the firmness of the plant matrix, which may have occurred in the present study.

Similar results were found in the study by Yang et al. (2017) using vacuum impregnation of calcium lactate and pectin methylesterase in fresh papayas cut from the cultivar “Sekaki” at 5 KPa. It was observed that the hardness of all papayas treated with vacuum impregnation dropped shortly after the process and that the samples subjected to treatment with the enzyme and Ca^{2+} had high hardness during storage.

The content of methanol and galacturonic acid present in the samples decreased as a function of time in all treatments applied (Figure 2). It is possible to observe that there was a higher quantification of methanol in mg/g of the fruit (Figure 2 A) when compared to the content of galacturonic acid (Figure 2 B). As these compounds were directly quantified in the fresh sample, there was interference from other constituents that are naturally present in the fruit composition.

On the fourth day of storage, papayas treated with PME+$C_6H_{10}CaO_6$ had the highest content of galacturonic acid and on the eighth day of storage the highest content of methanol (Figure 2), indicating an effective action of PME and calcium in this period, since the enzyme acts in the breakdown of the galacturonic acid chain, releasing methanol, facilitating the association of calcium and the formation of the “egg box” complex (Aghdam et al., 2012).

Figure 2. A, methanol content; and B, galacturonic acid content in papaya minimally processed after infusion (day 0) and during storage (4 and 8 days).



Treatments: Control, without infusion; H20, water infusion; Ca2+, infusion with calcium lactate; PME, infusion with the enzyme pectin methylesterase; and PME+Ca2+, infusion with the enzyme pectin methylesterase and calcium lactate. Three repetitions were performed. The bars indicate the standard error of the mean. Source: Author's own compilation (2020).

The leakage of electrolytes from the minimally processed papaya mesocarp increased significantly ($p<0.05$) as a function of time for all treatments applied, with the exception of the PME+$C_6H_{10}CaO_6$ treatment, for which there was also an increase, but without significant difference (Table 1). Ion leakage is directly related to the integrity of cell membranes because high electrolyte leakage rates indicate changes in membrane permeability (Villalta & Sargent, 2004).

Table 1. Leakage of electrolytes (%) in minimally processed papaya after infusion (day 0) and during storage (4 and 8 days).

Time (days)	Treatments				
	Control	H_2O	$C_6H_{10}CaO_6$	PME	PME+$C_6H_{10}CaO_6$
0	27.89±1.28^{bA}	31.24±3.07^{bA}	32.95±3.34^{bA}	28.81±2.03^{bA}	30.57±1.77^{aA}
4	34.55±0.88^{abA}	38.99±2.93^{abA}	39.38±2.34^{abA}	37.19±2.65^{abA}	36.71±1.89^{aA}
8	48.11±0.73^{aA}	48.84±2.47^{aA}	52.28±0.94^{aA}	46.93±1.63^{aA}	40.47±2.35^{aA}

The means followed by the same letter, lower case in the column and upper case in the line, do not differ by the Tukey test at the 5% level of significance. Treatments: Control, without infusion; H_20, water infusion; $C_6H_{10}CaO_6$, infusion with calcium lactate; PME, infusion with the enzyme pectin methylesterase; and PME+$C_6H_{10}CaO_6$, infusion with the enzyme pectin methylesterase and calcium lactate. Three repetitions were performed. Source: Author's own compilation (2020).

Although there was no significant difference between treatments, on the eighth day it was possible to observe that the PME+$C_6H_{10}CaO_6$ treatment had the lowest percentage of electrolyte leakage, indicating that the use of these substances can help to maintain the

structural integrity of papaya tissues. The calcium and pectin complex, formed from the action of the PME, acts as cement, providing firmness to the plant tissue, contributing to the maintenance of cellular integrity, promoting the delay of maturation and senescence of the fruit (Aghdam et al., 2012).

The rapid softening of freshly cut papaya can be a limiting factor in its useful life, as the rupture of the cell wall and the consequent loss of integrity are inevitable, since cutting the fruit releases pectinolytic enzymes that act in the degradation of pectin and wall dissolution (Toivonen & Brummell, 2008; Yang et al., 2017). Therefore, the application of PME+ calcium is an alternative to maintain and improve the firmness of minimally processed papaya, delaying the rupture of the cellular structure, the leakage of electrolytes and the loss of integrity.

The levels of vitamin C present in the minimally processed papaya cubes increased on the fourth day when compared to day 0, in all treatments (Table 2). This increase may be associated with ascorbic acid synthesis under the conditions in which the fruit was (stored at 5°C in polypropylene packaging) or the loss of water by the vegetable tissue (Dea et al., 2010). When some citrus fruits or vegetables are stored, they may show retention or increase in the vitamin C content (Batista, 2015).

Between the fourth and the eighth day, in turn, the levels of vitamin C decreased in all treatments, differing significantly ($p<0.05$) in the treatments with H_2O infusion and with $C_6H_{10}CaO_6$ infusion (Table 2). Vitamin C is an antioxidant component present in the plant matrix, which can act as a reducing and chelating agent in the elimination of free radicals, decomposing during storage to prevent oxidation, leading to a decrease in its content (Zhang et al., 2019). Therefore, the decrease in vitamin C content observed in papaya MP between the fourth and eighth day may be associated with its performance as an antioxidant in response to oxidative reactions that occur due to ripening, preventing oxidation of the fruit.

Table 2. Vitamin C (mg/100g), titratable acidity (%), pH, soluble solids (°BRIX), freshness and damage (%) in minimally processed papaya after infusion (day 0) and during storage (4 and 8 days).

Variables	Time (days)	Treatments				
		Control	H_2O	$C_6H_{10}CaO_6$	PME	PME+$C_6H_{10}CaO_6$
Vitamin C (mg/100g)	0	53.99±3.86^{aA}	45.42±2.92^{abA}	43.81±2.86^{abA}	43.89±2.21^{aA}	40.06±1.41^{aA}
	4	56.50±1.45^{aA}	55.39±0.92^{aA}	53.99±1.78^{aA}	44.03±2.34^{aA}	53.41±2.89^{aA}
	8	51.05±2.11^{aA}	40.70±2.97^{bA}	39.61±1.35^{bA}	39.54±0.30^{aA}	42.12±1.93^{aA}
Titratable acidity (% citric acid)	0	0.10±0.01^{aAB}	0.06±0.00^{aB}	0.09±0.01^{aAB}	0.11±0.01^{aA}	0.08±0.01^{aAB}
	4	0.09±0.01^{aA}	0.08±0.00^{aA}	0.07±0.00^{abA}	0.08±0.01^{abA}	0.07±0.00^{aA}
	8	0.08±0.01^{aA}	0.06±0.01^{aA}	0.05±0.01^{bA}	0.08±0.01^{bA}	0.06±0.01^{aA}
pH	0	5.7±0.1^{aAB}	5.9±0.0^{aA}	5.7±0.2^{aAB}	5.3±0.3^{bB}	5.8±0.1^{aA}
	4	5.9±0.0^{aA}	5.8±0.1^{aA}	5.9±0.0^{aA}	5.7±0.3^{abA}	5.8±0.1^{aA}
	8	5.6±0.1^{aA}	5.7±0.0^{aA}	5.8±0.2^{aA}	5.6±0.1^{aA}	5.8±0.2^{aA}
Soluble solids (°BRIX)	0	10.8±0.4^{aA}	8.4±0.4^{aB}	8.8±0.1^{aB}	9.7±0.1^{aAB}	8.9±0.0^{aB}
	4	10.2±0.1^{aA}	9.3±0.2^{aA}	8.8±0.3^{aA}	8.8±0.5^{aA}	8.7±0.1^{aA}
	8	9.8±0.4^{aA}	8.9±0.4^{aA}	9.1±0.3^{aA}	8.8±0.4^{aA}	8.8±0.7^{aA}
Freshness	0	9.0±0.0^{aA}	9.0±0,0^{aA}	9.0±0.0^{aA}	9.0±0.0^{aA}	9.0±0.0^{aA}
	4	7.0±0.5^{abA}	7.0±0.0^{abA}	7.2±0.6^{aA}	6.3±0.6^{bA}	7.8±0.3^{aA}
	8	6.3±0.3^{bA}	5.5±0.3^{bA}	6.8±0.4^{aA}	6.2±0.3^{bA}	7.0±0.2^{aA}
Damage (%)	0	0.0±0.0^{bA}	0.0±0.0^{aA}	0.0±0.0^{aA}	0.0±0.0^{bA}	0.0±0.0^{aA}
	4	6.7±5.8^{abA}	13.3±5.8^{aA}	0.0±0.0^{aA}	30.0±10.0^{aA}	0.0±0.0^{aA}
	8	26.7±5.8^{aAB}	40.0±10.0^{bA}	6.7±5.8^{aB}	20.0±10.0^{abAB}	6.7±5.8^{aB}

The means followed by the same letter, lower case in the column and upper case in the line, do not differ by the Tukey test at the 5% level of significance. Treatments: Control, without infusion; H_20, water infusion; $C_6H_{10}CaO_6$, infusion with calcium lactate; PME, infusion with the enzyme pectin methylesterase and PME+$C_6H_{10}CaO_6$, infusion with the enzyme pectin methylesterase and calcium lactate. Three repetitions were performed. Source: Author's own compilation (2020).

Titratable acidity had a significant reduction ($p<0.05$) in treatments with $C_6H_{10}CaO_6$ infusion and with PME infusion between the day of vacuum impregnation (day 0) and the eighth day (Table 2). The control and infusion samples PME+$C_6H_{10}CaO_6$ did not differ in acidity content over time, demonstrating that the combination of the enzyme PME with calcium was effective in maintaining acidity during storage, without changing its content when compared to the control sample.

Although the control, water and PME+$C_6H_{10}CaO_6$ treatments did not differ significantly ($p<0.05$) as a function of time, all treatments had reduced acidity during storage, which may be related to the fact that organic acids are the first compounds consumed during breathing (Chitarra & Chitarra, 2005).

After vacuum impregnation, it was possible to observe that papaya treated only with PME had the highest percentage of acidity in citric acid (Table 2). This behavior can be explained by an increase in the concentration of acids resulting from the performance of exogenous PME and other pectic enzymes (Pinto et al., 2011).

As for pH, it was possible to observe that there were no significant differences ($p<0.05$) as a function of time, with the exception of the PME treatment for which the values differed between day zero and day eight. At zero time, this treatment reported the lowest pH value, which can be explained by the same reason that led to an increase in the acidity of this treatment at zero time, that is, after vacuum impregnation there is a greater performance of pectic enzymes and consequent release of organic acids, making the pH more acidic. It was also possible to observe a reduction in the levels of soluble solids for all treatments (Table 2), and the samples with H_2O, $C_6H_{10}CaO_6$, PME+$C_6H_{10}CaO_6$ infusion differed significantly ($p<0.05$) from the control sample.

There were no significant differences ($p<0.05$) in the content of soluble solids as a function of storage time, but it was observed that in the treatment with water there was an increase in the content on the fourth day. An increase in the content of soluble solids was also found in the study by Paixão et al. (2020) to evaluate the postharvest behavior of green peppers after application of PME and calcium, with an increase in the content of soluble solids from the sixth to the ninth day of storage from 3.1 to 5.0ºBrix, in peppers treated with H_2O infusion.

For the PME+$C_6H_{10}CaO_6$ treatment, it was observed that the soluble solids content practically did not vary over time (Table 2), demonstrating that this treatment, besides improving and maintaining the firmness of the plant tissue, does not interfere negatively in the quality parameters of the plant fruit. The content of soluble solids is an important parameter of fruit quality during post-harvest storage, being directly related to ripening, contributing to the flavor, especially sweetness (Zhang et al., 2019).

As for freshness, at time zero, all treatments received a score of 9 (Table 2), indicating that samples had a fresh appearance and high gloss, that is, they had the same degree of freshness. On the fourth day, only papaya treated with the PME enzyme differed significantly ($p<0.05$) when compared to day zero, presenting an appearance with low freshness and low

brightness. The other treatments had a freshness rating ≥ 7, classified as “good”, keeping them fresh and shiny.

Papayas treated with PME+$C_6H_{10}CaO_6$ and only with $C_6H_{10}CaO_6$ did not differ ($p<0.05$) in freshness over time. On the last day, the combined treatment of PME+calcium presented the highest score for assessing freshness.

The minimal processing of papaya is convenient, but the accelerated catabolism of cell wall components and the loss of fluid, result in decreased firmness and loss of freshness (Karakurt & Huber, 2003; Yang et al., 2017). The reduction of freshness is associated with factors such as water loss, mechanical damage and contamination (Batista, 2015). Therefore, the vacuum impregnation of PME+Ca^{2+} proves to be an effective alternative to prevent deterioration, maintaining freshness and improving firmness during storage.

As for the damage, on the fourth day the papaya treated with PME showed the highest percentage of damage to the structure of the plant tissue (Table 2). This behavior is explained because the PME acts on the fraction of the pectin that makes up the cell wall, catalyzing its demethylation, leading to cell separation and decreased firmness, increasing the percentage of damage to the minimally processed product (Zhang et al., 2019).

The treatments with $C_6H_{10}CaO_6$ and PME+ $C_6H_{10}CaO_6$ had similar behavior, both reduced the appearance of damage during storage. This can be explained by the fact that Ca^{2+} is able to inhibit the action of endogenous PME and thereby maintain the structure of the cell wall, but it cannot maintain or increase the firmness of the fruit (Yamamoto et al., 2011; Batista, 2015). On the other hand, when vacuum impregnation of PME+$C_6H_{10}CaO_6$ occurs, the formation of calcium pectate occurs, which improves and maintains the firmness of the fruit, thus preventing the appearance of damage to its structure (Carnelossi et al., 2018).

During the storage period, no incidence of contamination was observed in papaya, that is, there was no occurrence of rot and/or the growth of visible fungi.

4. Final Considerations

The use of vacuum infusion of PME and calcium lactate in minimally processed papaya is an alternative to preserve the firmness of the fruit of the Formosa variety. In addition to increasing and maintaining papaya firmness until the eighth day, the combination PME+calcium lactate promoted the least leakage of electrolytes in the papaya's vegetable tissues, demonstrating that there was preservation of the cellular structure.

The vacuum impregnation process of PME+Ca^{2+}, did not influence the quality parameters of the product, since the levels of vitamin C, soluble solids, pH and acidity were maintained after processing and throughout storage.

There was also an increase in the useful life of the minimally processed papaya, by maintaining freshness, reducing damage and the absence of contamination in the product.

The use of vacuum infusion of PME and calcium lactate preserved the quality and firmness of minimally processed papaya, it is suggested that future work be carried out in order to explore the impact of this technique on the acceptability of minimally processed papaya.

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