



Impact of an Organic Acid and Calcium Chloride Solution on Minimally Processed Breadfruit Quality

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Abstract

Breadfruit (*Artocarpus altilis*) is described as an important food source in the diet of several countries and it is considered a valuable food resource with the potential to positively affect food security in the world. The aim of this study was to evaluate the impact of an organic acid and calcium chloride solution on the physicochemical characteristics of fresh-cut breadfruit. Breadfruit was processed and two groups were separated with and without immersion treatment in a solution containing 2% citric acid, 0.2% ascorbic acid, and 5% calcium chloride. Samples were drained and stored at 6 °C ± 1 °C for 15 days. Chemical composition, physical characteristics, bioactive compounds, antioxidant activity, and microbial behavior were determined every two days. The direct relationship between total phenolic content and the antioxidant capacity of the analyzed samples was observed. Treated samples had higher total phenolic content statistically significant, ($p < 0.05$), when compared with control samples during the storage time 0 = 350.65 ± 1.89; 3 days = 250.61 ± 1.75; 5 days = 240.11 ± 2.03; 7 days = 150.69 ± 2.68; 10 days = 280.40 ± 1.43; 13 days = 240.87 ± 1.46; 15 days = 350.60 ± 1.64 mg GAE.100 g-1 dry sample. And higher antioxidant activity, ($p < 0.05$), compared with control samples, for both DPPH. time 0 = 69.34 ± 0.05; 3 days = 50.69 ± 0.07; 5 days = 46.31 ± 0.09; 7 days = 200.36 ± 0.04; 10 days = 83.05 ± 0.06; 13 days = 59.70 ± 0.07; 15 days = 33.33 ± 0.04 EC50 in µg/mL and ABTS+ radicals, at time 0 = 53.98 ± 0.04; 3 days = 54.57 ± 0.05; 5 days = 92.30 ± 0.20; 7 days = 58.59 ± 0.10; 10 days = 82.54 ± 0.03; 13 days = 78.32 ± 0.09; 15 days = 188.60 ± 0.06 µmol Trolox/g sample. It was concluded that the used solution improved the physicochemical characteristics of the fruit, by increasing its phenolic content and antioxidant activity.

Keywords: Antioxidant Capacity; Lifespan; Bioactive Compounds; Wild Plant

Abbreviations

ABTS: Ability to Sequester Radical 2,2'-Azinobis (3-Ethylbenzothiazoline-6-Sulfonic Acid); AC: Citric Acid; a_w : Water activity; B: Mold; CT: Total Coliforms; CTM: Total Mesophyll Count; DI: Darkening Index; DPPH: Ability to Sequester Radical 2,2-Diphenyl-1-Picrylhydrazyl; EC: Escherichia Coli; L: Yeast; NDF: Neutral Detergent Solution; pH: Hydrogen Potential; PPO: Polyphenol Oxidase; RH: Relative Humidity; SS: Soluble Solids; TEAC: Total Antioxidant Capacity Equivalent to Trolox; WI: Whiteness Index

Introduction

Breadfruit has a long and historic history of cultivation and use. In recent years, there has been a growing interest in promoting breadfruit as a nutritious and income-generating food. From a nutritional point of view, breadfruit is rich in carbohydrates, minerals, vitamins and low in fat. It is a good source of fiber, calcium, copper, iron, magnesium, potassium, thiamine and niacin; some varieties are good sources of antioxidants and carotenoids. According to the National Tropical Botanical Garden, more than 80% of the population that does not have a guarantee of nutritional security lives

in tropical and subtropical regions, being the perfect environment for the cultivation of breadfruit. This fruit could become the next “superfood” capable of alleviating hunger and ensuring nutrition for the population. In 2016, a breadfruit flour received the status of ‘Generally recognized as safe’, thus opening up the possibility of using this flour as an ingredient in different foods [1-3].

The term “minimal processing” refers to the use of one or more methods, techniques, or procedures to transform either plant or animal-derived foods into ready-to-eat products while maintaining the original nutritional and organoleptic qualities. As the world is increasingly interconnected through globalization, it has become a priority to expand the market as much as possible. Consumption of minimally processed fruits and vegetables has become increasingly popular over the last decade due to a greater interest in healthy and nutritious diets and changes in consumer lifestyle. The successful application of minimal processing delays nutrient loss and undesirable changes in texture, color, flavor, and aroma related to ripening or microbial spoilage [4]. In the case of fruits and vegetables, one of the main and persistent problems faced by the food industry for minimal processing is related to perishability, rapid deterioration, and loss of quality attributes. These products have higher perishability and susceptibility to deterioration than raw fruits or vegetables because mechanical injuries to plant tissue promote physiological changes, microbiological spoilage, and a loss of overall quality attributes [4,5].

Many studies suggest that treatment with additives along with other techniques such as modified atmosphere packaging, and dielectric barrier discharge cold plasma treatment, γ -irradiation, non-thermal plasma treatments, edible films, and other emerging technologies can reduce oxidative stress and consequently the restriction on dimming, as well as the increase of the firmness and also the extension of product lifespan [6-10].

In order to study northeastern breadfruit, especially from Pernambuco state orchards, this study had the objective to evaluate physicochemical characteristics of minimally processed breadfruit, with the addition of a conservative solution, aiming the increase its lifespan and, consequently, provide greater profitability to producers.

Materials and Methods

Materials

Twenty kilograms of breadfruit selected according to maturation stage “swollen (between green and ripe)”, skin color dark

green, and size at the average of 1615 g were washed and sanitized in dehydrated sodium dichloroisocyanurate solution for 15 minutes. They were, then, peeled and cut manually with stainless steel knives and immediately placed underwater at $7\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$.

Methods

Sample preparation for analysis

For the analysis, after selection, twelve kilos of peeled breadfruit were cut in half-moon format (15x5x5cm) and washed with water, drained, and divided into two groups. Control (six kilograms were packed in trays of expanded polystyrene, covered with a film of polyvinyl polychloride (20 μm thick, containing 200 g each) and with treatment (six kilograms submitted to immersion in a solution containing 2% citric acid, 0.2% ascorbic acid and 5% calcium chloride for 5 minutes at room temperature ($25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$). They were then, drained and packaged in the same manner as the control sample. Samples were stored for 15 days at $6\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ and relative humidity (RH) of 82-90%.

Physico-chemical parameters

The analyzes were performed in triplicate according to the following procedures:

- **Soluble solids:** Expressed in $^{\circ}\text{Brix}$ and determined at $25\text{ }^{\circ}\text{C}$ using a Reichert digital bench refractometer (Model r2i300, São Paulo, Brazil).
- **pH:** Expressed in pH unit, determined by direct immersion of the electrode of the pH-meter Tec-3 MP (Technal brand, São Paulo, Brazil) in the comminuted and homogenized pulp with value automatic correction by the temperature.
- **Water Activity:** Determined in water activity analyzer (Aqualab brand, Series 4TE, Washington, EUA) at $25\text{ }^{\circ}\text{C}$.
- **Titrateable acidity:** Titrateable acidity was determined by titration with 0.1 N NaOH until pH 8.1 was reached (AOAC Official Method, 920.15) [11], and expressed as mg citric acid.100g⁻¹ on a fresh weight basis.
- **Pulp firmness:** In order to indicate the consistency of the pulp penetration, some tests were performed in 3 points, using a suitable analog penetrometer for fruits (Facchini brand Srl, model FT 327, Italy).

Color measurement

The surface color of breadfruit was determined in the CIE $L^*a^*b^*$ system, using Konica Minolta portable colorimeter (Model CR/400/410 - Sensing, INC, Japan) calibrated with a white porcelain plate. Approximately 2 g of sample was placed in a plastic base of 2 mm of thickness. For each sample, an average value of analy-

sis of the extremities and the center of the sample was collected in triplicate. CIE $L^*a^*b^*$, L^* system represents the brightness ($L^* = 0$ is black and $L^* = 100$ white). The parameters that indicate color direction are: $+a^* =$ red and $-a^* =$ green; $+b^* =$ yellow and $-b^* =$ blue [12].

Whiteness index (WI) and darkening index (DI) were calculated according to the following Equation 1 [13].

$$IB = L^* - 3b^*$$

$$IE = [100(x - 0.31)]/0.172. \text{ Where } x = (a^* + 1.75 L^*) / (5.645 L^* + a^* - 3.012 b^*) \text{ [Equation 1]}$$

Mass Loss Determination

Mass loss during storage of minimally processed (control and treated) breadfruit was carried out using an electronic analytical scale (Mark JK-EAB, model 2204N, China) with an accuracy of ± 0.0001 g. Mass loss was calculated by the following Equation 2.

Mass loss (%) = $(mt - mc) \times 100/mc$. Where mc is the initial weight of minimally processed breadfruit and mt is the final sample weight at time t . [Equation 2]

Chemical composition

Moisture - Determined in infrared humidity meter with coupled scale (Model ID 50, brand Marte®) at 105°C for 30 min by weight difference, before and after drying, up to constant weight and expressed in g.100g⁻¹ of fruit.

Fixed Mineral Residue (Ashes) - Determined by gravimetry in a muffle kiln microprocessor (Model Edgcon 1P, brand EDG, São Paulo) at 550-570°C until constant weight, according to AOAC: Official Method 940.26 [11] and results expressed in g.100g⁻¹ of fruit.

Lipids (Ethereal Extract) - Ethereal extract fraction was determined in an intermittent Soxhlet extractor, using Ethyl Ether P.A. as solvent. After solvent extraction and removal, the number of lipids present in the sample (AOAC Official Method, 920.39 C) [11] was gravimetrically determined. The results were expressed in g.100g⁻¹ of fruit.

Proteins - Determined by Kjeldahl method, based on organic matter destruction followed by distillation, nitrogen being measured by volumetry. Nitrogen present in the resulting acid solution was determined by steam distillation followed by titration with dilute acid. The percentage of sample protein fraction is calculated using factor 6.25, considering for each 100g of protein, on average,

16g of nitrogen, according to AOAC Official Method 960.52 [11]. They were expressed in g.100⁻¹ of fruit.

Total Sugars - It was determined by the Lane-Eynon method. It is based on the ability of glycols, in a strongly hot alkaline medium, to form enediol, a compound with strong reducing power, which in the presence of Cu^{++} oxidizes and reduces copper to Cu^+ , giving rise to a red brick precipitate of Cu_2O , according to methodology recommended by AOAC Official Method 991.43 [11]. The results were expressed in g.100⁻¹ of fruit.

Carbohydrates - Carbohydrate content was calculated by the difference between 100 and the sum of percentages of water, protein, total lipids, and ashes and expressed in g.100⁻¹ of fruit.

Fibers - It was determined by the method proposed by Van Söest [14], using neutral detergent solution (NDF), which solubilizes all plant material, except for the wall structure, which are insoluble fibers (cellulose, hemicellulose, and lignin). This method quantifies the insoluble fraction of fiber. The results were expressed in g.100⁻¹ of fruit.

Total phenolics

Breadfruit Extract - It was obtained using distilled water to result in an aqueous extract. 25 g of breadfruit pulp and 25 ml of distilled water of 1:1 (m: v) were used to prepare the extracts. The mixture was homogenized for 1 hour in Erlenmeyer flasks, using a magnetic stirrer; then it was centrifuged at 6,000 rpm for 10 minutes. The supernatant was stored in amber glass under refrigeration at 8°C until analyses.

Determination of total phenolic - the content of total phenolic compounds was determined according to the Folin-Ciocalteu method [15]. To the extract (100 μ L), 3.9 mL of water was added, followed by the addition of 250 μ L of Folin-Ciocalteu reagent and 750 μ L of sodium carbonate solution. The mixture was maintained in a homogenizer (Certomat® MV, B. Braun Biotech International) and then incubated for 1 h at room temperature ($22 \pm 2^\circ$ C) in the absence of light. The absorbance of the mixture was measured at 765 nm by spectrophotometry (Hitachi U-2800 Spectrophotometer, Japan). Total phenol content was determined from the calibration curve, prepared with the gallic acid standard solution, and expressed as mass of gallic acid equivalents per fresh fruit mass. The results were expressed in mg of total phenolics in gallic acid equivalents/100g of the sample.

Antioxidant capacity evaluation

Ability to sequester radical 1.1-diphenyl-2-picrylhydrazyl DPPH - It was determined according to the method described by Brand-Williams, Cuvelier, and Berset [16], modified by Sanchez-Moreno, Larrauri, Saura-Calixto [17]. In this method, DPPH. reduction by the antioxidant compound results in loss of absorbance and the degree of discoloration of the solution indicates the efficacy of the tested compound. Different concentrations of breadfruit extracts were added to DPPH. solution in methanol (0.1M), in order to reach final concentrations of 7.5; 15 and 30µg of phenolic of the extract. Absorbance at 517 nm was recorded in a spectrophotometer (Shimadzu Europa UV-1650PC) until the reaction reaches the plateau. The ability to sequester DPPH. radical was expressed as a percentage; calculated in relation to the control (without the addition of antioxidant). Using the Equation 3. The absorbance of control to express in EC50 plot the % inhibition x phenolic concentration of extract. Line equation was plotted using line equation EC 50.

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$
 [Equation 3]

Ability to sequester radical ABTS^{•+} - Ability to sequester 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS. +) radical was determined according to the method described by Re., *et al.* [18]. ABTS. + radical was generated from its aqueous solution reaction with (7 mM) with 2.45 mM of potassium persulfate. A radical solution was diluted in ethanol until the absorbance measurement was 0.7 ± 0.005 , at wavelength 734 nm. Several concentrations of breadfruit extracts diluted in ethanol were added to ABTS. + solution, reaching the final concentrations of 0.5; 1 and 2 µg phenolic of the extract. Absorbance was recorded after 6 minutes of reaction in a spectrophotometer (Shimadzu Europa UV-1650PC). Sample antioxidant capacity was calculated in relation to synthetic antioxidant Trolox activity (6-hydroxy-2,5,7,8-tetramethylchromo-2-carboxylic acid) under the same conditions and the results were expressed in an antioxidant activity equivalent to Trolox µmol TEAC.g⁻¹).

Microbiological analysis

Microbiological analyzes were performed according to Wu and Chen method [19], with modifications. From each sample, a 25g portion was used, which was diluted in 225 ml of 0.1% of peptone water in a sterile bag. The diluted sample was manually macerated and homogenized. From each dilution, successive dilutions were prepared, up to 10⁻⁴. From each dilution, 1mL portions were inoc-

ulated for 3 types of Petrifilm™ plates: EC (Official AOAC Method 991.14 and 998.08) [11] to determine coliforms and Escherichia coli, YM for yeasts and molds (Official AOAC Method 997.02) [11], and AC for Total Mesophil Count (AOAC Official Method 990.12) [11]. AC and EC plates were incubated at $35^\circ\text{C} \pm 1^\circ\text{C}/24\text{h}-48\text{h}$ and YM plates at $25^\circ\text{C} \pm 1^\circ\text{C}/$ up to 120 hours. Results were expressed as a log of colony-forming units per gram of sample (log UFC/g).

Statistical analysis

All determinations were performed in triplicate and data were computed and submitted to variance-ANOVA analysis. The averages of obtained values were submitted to Student's t-test and Tukey's test, using the statistical program "Statistica" (version 7, StatSoft, Inc, Tulsa, USA). Significance level of 5% probability ($p < 0.05$) was set.

Results and Discussion

Physicochemical characteristics of minimally processed breadfruit

Factors influencing the quality of minimally processed fruits result in a series of biochemical and physical-chemical modifications, which mainly affect the taste, color, and texture [4]. It is known that pH influences the rate of various chemical, enzymatic and microbiological reactions. Variations of this parameter indicate important changes in fruit quality. The pH found in the studied sample (Figure 1) was higher than that reported by Roopa., *et al.* [20] when evaluating the lifespan of minimally processed breadfruit, packaged under a passive modified atmosphere and stored under refrigeration.

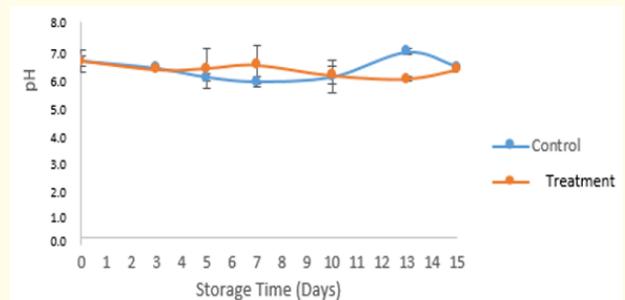


Figure 1: Mean values and standard deviation of the pH of minimally processed breadfruit depending on the treatment and storage times.

The analyzes were performed in triplicate. No difference by student's t-test ($p < 0.05$) between control and treatment.

Water activity average (a_w) showed no difference ($p < 0.05$) between control and treatment during the storage period (Figure 2). The fruit presented high a_w content (0.98). A_w is directly related to fruit moisture and describes the degree to which water is available to act as a solvent, participating in chemical reactions and microorganism development. It also indicates the intensity of the association between water and non-aqueous constituents and influences the speed of various degradation reactions, such as enzyme-catalyzed reactions [21].

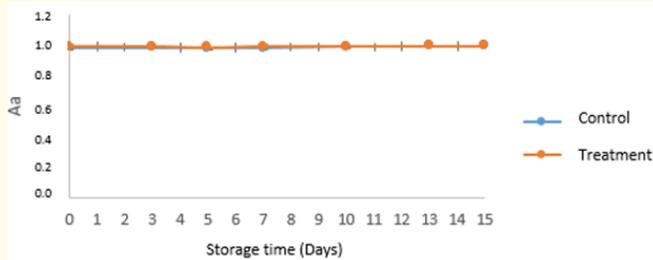


Figure 2: Mean values and standard deviation of the water activity (A_w) of minimally processed breadfruit depending on the treatment and storage time.

The analyzes were performed in triplicate. No difference by student's t-test ($p < 0.05$) between control and treatment.

Titrateable acidity differed ($p > 0.05$) between control and treatment samples on the 3rd and 10th day of storage (Figure 3). At this point, there was an increase in acidity. These values are higher than those found by Moreira, *et al.* [22] to physically and chemically characterize *Apyrena* breadfruit (0.035 mg citric acid.100g⁻¹) and Souza, *et al.* [23] for fresh breadfruit (0.164 mg of citric acid.100g⁻¹).

The analyzes were performed in triplicate. A significant difference by student's t-test ($p > 0.05$) between control and treatment samples on the 3rd and 10th days of storage.

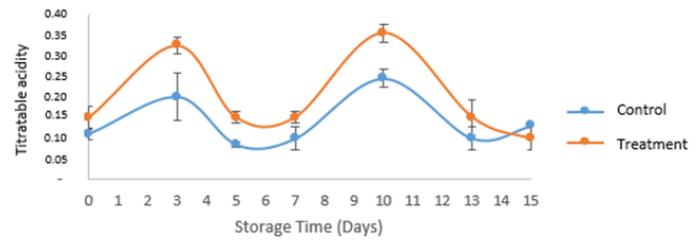


Figure 3: Mean values and standard deviation of titrateable acidity (mg citric acid.100g⁻¹) of minimally processed breadfruit depending on the treatment and storage times.

The sugars represent a major part of the soluble solids (SS) and are shown in figure 4. The SS of breadfruit presented a difference ($p > 0.05$), depending on the treatment and storage time. The control sample presented higher values, except on the 13th day. This is explained by the addition of acids used in the treatment, which can be breathable substrates. The increase of SS value up to the 10th day is justified by fruit natural maturation and the decrease from the 13th day coincides with the increase in the number of microorganisms of total mesophil count (Figure 12), indicating the use of sugars by the microorganisms. Souza, *et al.* [23], when analyzing soluble solid content in fresh breadfruit, found a lower value (5.6 °Brix).

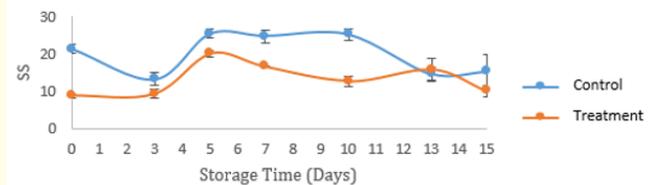


Figure 4: Mean values and standard deviation of Soluble Solids (SS) in °Brix of minimally processed breadfruit as a function of treatment and storage.

The analyzes were performed in triplicate. A significant difference by student's t-test ($p > 0.05$) between control and treatment.

Mechanical lesions of fruit and vegetable minimal processing lead to biochemical deterioration, such as enzymatic browning. This is caused mainly by the action of the enzyme polyphenol oxidase (PPO) on phenolic compounds present in certain fruits. This is considered the lifespan limiting factor of certain minimally processed fruits. Ascorbic acid as a reducing agent is traditionally applied in combination with other organic acids to prevent enzymatic browning [24].

As breadfruit matures, the firmness of the pulp decreases, making it softer. Values varied between the control and treatment samples, during the storage period, according to figure 5. Treated samples remained firmer than the controls. These results are justified by the addition of calcium chloride solution. Calcium salts are traditionally used to maintain fruit firmness [25]. Guo., *et al.* [26], when analyzing soluble solids content and pear firmness during ripening, found that storage time generated a significant decrease in firmness after 4 days. According to Brasil and Siddiqui [27], the loss of pulp firmness is an indicator of ripeness and quality that can determine the shelf life of fruit.

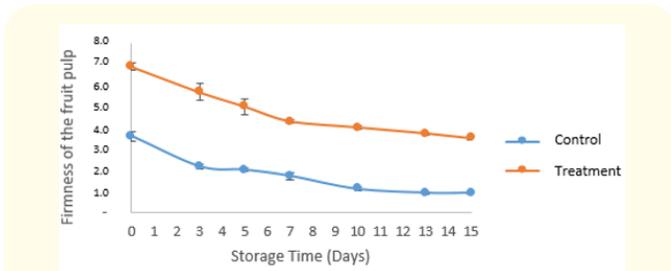


Figure 5: Mean values and standard deviation of firmness of the fruit pulp minimally processed according to the treatment and storage times.

The analyzes were performed in triplicate. A significant difference by student’s t-test ($p > 0.05$) between control and treatment.

Minimal processing exposes fruit tissue in half, causing a substantial mass loss. Mass loss in minimally processed breadfruit presented in figure 6, was lower in the samples with improvement solution; however, these values did not differ ($p < 0.05$). With the storage passing time, there was an increase in mass loss for all analyzed samples. Chen., *et al.* [28], studying the effect of ascorbic

acid and UV-C combination on the quality of minimally processed apples, did not also find significant differences between the control samples and samples enriched with ascorbic acid.

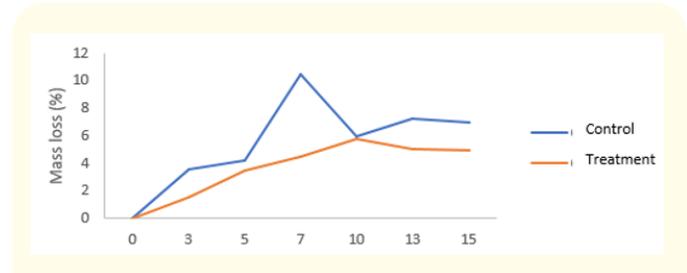


Figure 6: Average values and standard deviation of mass loss of minimally processed breadfruit depending on the treatment and storage times.

The analyzes were performed in triplicate. No difference by student’s t-test ($p < 0.05$) between control and treatment.

As for luminosity (L^*), presented in figure 7, there was a significant difference ($p > 0.05$) between control and treatment samples. Samples with treatment presented a higher index for this parameter, justified by the use of the improvement solution. Reducing agents of this solution are used to avoid browning of processed products, as well as calcium chloride, which action may be due to inhibition of PPO by chloride ions, as well as by diffusion of calcium on tissues, which may have helped to maintain quality, reducing browning. This characteristic was maintained at all times analyzed. These changes in the value of L^* are attributed to enzymatic browning caused by the damage to the fruit tissue and consequent increase of the contact with the PPO enzyme and its substrates.

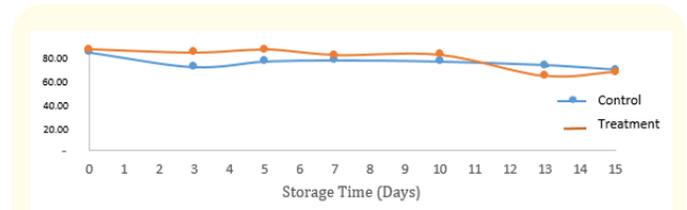


Figure 7: Mean values and standard deviation of L^* of freshly baked breadfruit depending on the treatment and storage times.

The analyzes were performed in triplicate. Significant difference by student's t-test ($p > 0.05$) between control and treatment samples.

As for a^* coordinate, there was a significant difference between control and treatment samples. Values of a^* , shown in figure 8, may be used to indicate fruit browning. The results indicated a tendency to brown staining in control samples, characteristic of PPO enzyme action.

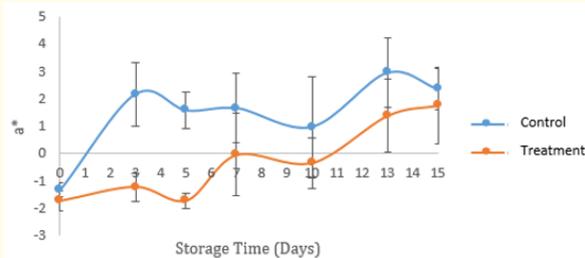


Figure 8: Mean values and standard deviation of a^* of minimally processed breadfruit as a function of treatment and storage times.

The analyzes were performed in triplicate. Significant difference by student's t-test ($p > 0.05$) between control and treatment samples.

Values of coordinate b^* were positive, according to figure 9, which indicates proximity to yellow. This parameter had a significant difference between control and treatment samples, with a higher index in control samples. Degradation reactions may occur during the processing and storage of minimally processed fruits, resulting in browning. These data indicate that the use of acid and calcium chloride improvement solutions showed greater inhibition of PPO enzyme action. Zambrano-Zaragoza, *et al.* [29] emphasize that the use of chemical additives, such as acidulants (citric acid) and reducing agents (ascorbic acid) have been used in minimally processed fruits and vegetables to control browning.

The analyzes were performed in triplicate. Significant difference by student's t-test ($p > 0.05$) between control and treatment samples.

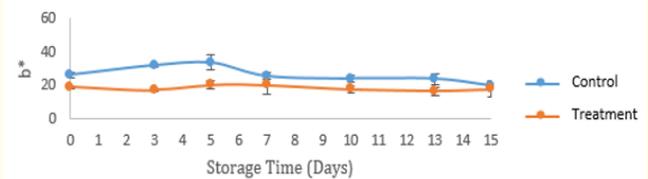


Figure 9: Mean values and standard deviation of b^* of freshly baked breadfruit depending on the treatment and storage times.

Roopa, *et al.* [20], when analyzing the effect of minimum processing and the use of modified atmosphere packaging as a promising way to extend breadfruit lifespan, showed that during the 45 days of storage, there was a significant decrease in the value of L^* , a^* , and b^* over storage time. These results corroborate findings by Chen, *et al.* [28] when analyzing the effect of citric acid and UV-C use on the quality of minimally processed apples.

Related to the whiteness index, shown in figure 10, it was verified that there was a significant difference between control and treatment samples, during storage. A decrease in this parameter is indicative of browning. Samples with the addition of improvement solution showed higher values for this parameter, except at the end of the evaluated period (13th and 15th days).

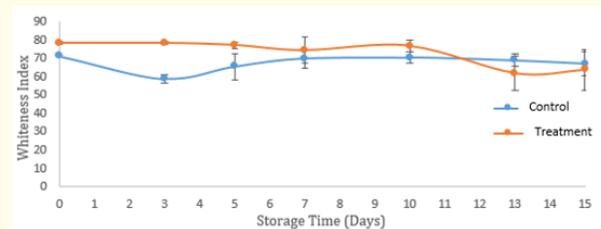


Figure 10: Mean values and standard deviation of the breadfruit whiteness Index minimally processed according to the treatment and storage times.

The analyzes were performed in triplicate. Significant difference by student's t-test ($p > 0.05$) between control and treatment samples.

For darkening index (Figure 11) samples behaved inversely, that is, control samples were darker than those with treatment, justified by the absence of an improvement solution. There is a significant difference between samples over the storage period. This parameter is a factor that limits the lifespan and commercialization of minimally processed fruits.

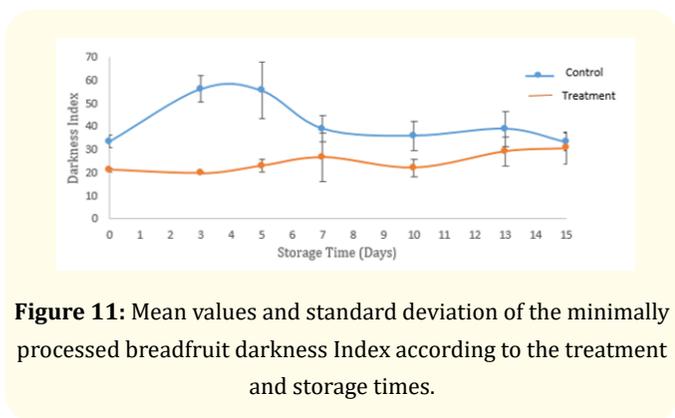


Figure 11: Mean values and standard deviation of the minimally processed breadfruit darkness Index according to the treatment and storage times.

The analyzes were performed in triplicate. Significant difference by student’s t-test ($p > 0.05$) between control and treatment samples.

These data corroborate those found by Chen., *et al.* [28], when they evaluated the effect of the addition of citric acid (AC), UV-C and in combination (AC + UV-C) under browning of minimally processed apples, during storage for 15 days at 5°C. They showed that treatment with citric acid could aggravate the browning of minimally processed apples in a certain period of storage. At the end of the storage, apples treated with citric acid became darker than control samples.

Chemical composition of minimally processed breadfruit

The chemical composition of food manifests its potential in nutrients, being important data for the food industry. In the literature, information on the approximate composition of minimally processed breadfruit is scarce. Values of moisture, ashes, lipids, proteins, total sugars, carbohydrates, and fibers are presented in table 1.

Fraction/Source	data
Humidity	57.52 ± 0.98
Ashes	1.23 ± 0.09
Lipids	0.67 ± 0.04
Proteins	1.64 ± 0.03
Total Sugars	3.23 ± 0.05
Carbohydrates	38.94
Fibers	18.91 ± 0.02

Table 1: Chemical composition (g.100g⁻¹) of minimally processed breadfruit.

The breadfruit used in the present study had a mean moisture content similar to that found by Almeida, Feijó, and Marcellini [30] when characterizing green breadfruit biomass. Moisture is a parameter that must be considered because it facilitates fruit deterioration and microbiological development, is related to its stability, quality, and composition.

Ash content analysis provides us with prior information on nutritional value and depends on the food nature and determination method used. Ash content determined in this experiment (Table 1) was higher than that reported in other studies [1,30]. This variation can occur naturally influenced by climate, period of the year, and cultivar type of soil.

Lipid contents found are higher (Table 1) than those presented by Turi., *et al.* [1] and Almeida, Feijó e Marcellini [30]. This variation can be attributed to fruit varieties. This justifies the differences between different sources consulted. The mean value found for proteins was 1.64 g.100g⁻¹; it is close to those found by Almeida, Feijó e Marcellini [30]. Turi., *et al.* [1] carried out a review of the literature on this fruit and presented mean values of 5.2 g.100g⁻¹ for fresh breadfruit proteins. The mean carbohydrate result in breadfruit studied is within the average of the values presented by the other authors cited. In total sugar analysis and fiber fraction, the values found are close to that reported by Almeida, Feijó, and Marcellini [30] when developing and characterizing green breadfruit biomass.

Total phenolic

All plants produce a remarkable range of secondary metabolites, being phenolic compounds one of the most important metabolites of this group, presenting antioxidant action. The results of total phenol content for aqueous extract of minimally processed breadfruit over a period of 15 days are shown in table 2. The option was water extraction, for most of the phenolic compounds of this fruit presents greater polarity, therefore, they are more water-soluble [30].

Storage time (days)	Control	With treatment
0	310.1 ± 1.05 ^{Ba}	350.65 ± 1.89 ^{aA}
3	280.83 ± 1.05 ^{aC}	250.61 ± 1.75 ^{aBC}
5	200.76 ± 2.30 ^{aD}	240.11 ± 2.03 ^{aC}
7	170.67 ± 1.86 ^{aE}	150.69 ± 2.68 ^{aD}
10	260.52 ± 2.02 ^{aBC}	280.40 ± 1.43 ^{aB}
13	280.99 ± 1.36 ^{aAB}	240.87 ± 1.46 ^{bC}
15	200.04 ± 0.61 ^{bDE}	350.60 ± 1.64 ^{aA}

Table 2: Total phenolic contents in fresh-cut breadfruit (mg GAE.100 g⁻¹ dry sample).

Means followed by the same lowercase letter in the line do not differ significantly from each other by the student's t-test ($p < 0.05$). Averages, in the same column, followed by different letters differ statistically according to Tukey's test ($p < 0.05$).

Total phenolic content of control and treatment samples decreased between 9 and 28% after 3 days of storage. It was verified that there was a significant difference between control and treatment samples, except for the 7th day of storage. The behavior for treated samples did not differ from control on days 0 and 7, presenting the lowest total phenolic content on the 7th day of storage (150.69 ± 2.68 mg of gallic acid equivalent per 100 g dry sample) and the highest value on 0 (zero) storage day (350.65 ± 1.89 mg of gallic acid equivalent per 100 g dry sample). However, it presented a significant difference for the other days. This fact can be explained by the addition of citric and ascorbic acids in improvement solution preparation; thus demonstrating the antioxidant action of these acids. The results show that the fresh-cut breadfruit extract promising antioxidant activities have potential bioactivities due to the high content of phenolic compounds.

Jalal., *et al.* [31] found total phenolic values in breadfruit between 203.17 ± 7.65 until 781 ± 52.97 mg GAE/g dry weight of the sample, using methanol and dichloromethane as solvents. Almeida, Feijó, and Marcellini [30] quantified 615.98 ± 12.44 mg of gallic acid equivalent.100 g⁻¹ of dry sample in green breadfruit aqueous extract.

Variations in the contents of phenolic compounds can be influenced by several factors, such as maturation, species, cultivation practices, geographical origin, growth stage, harvest conditions, and fruit storage process. Methodological peculiarity related to the solvent extractor, whether fresh or dry sample and the phenolic used as a standard for quantification of total phenolic compounds can cooperate for the observed differences.

Evaluation of antioxidant capacity

Antioxidant activity may depend on several factors, such as oxidation conditions and stages, radical formation and stability, as well as a possible location of antioxidants, and stability in different stages of food processing [32].

For the samples without immersion in improvement solution, EC50 values remained above-treated samples, showing lower values of antioxidant activity (Table 3). Almeida, Feijó, and Marcellini [30] when evaluating the antioxidant capacity of green breadfruit in aqueous extract presented an 85% reduction of DPPH. after one hour of contact. Jalal., *et al.* [31], when evaluating pulp, bark, and bark plus breadfruit pulp, using different solvents (55 ± 5.89 µg/mL) and the control group found significant differences ($p < 0.05$) between extraction values by part of the (55 ± 5.89 µg/mL) and control (ascorbic acid, 61 ± 4.51 µg/mL). Methanol extract from the part of pulp inhibited the 50% free radicals of DPPH., being the greater antioxidant activity in the breadfruit pulp.

Means followed by the same lowercase letter in the line do not differ significantly from each other by the student's t-test ($p < 0.05$). Averages, in the same column, followed by different letters differ statistically according to Tukey's test ($p < 0.05$).

The treated sample had the highest antioxidant capacity in the fresh sample on day 0 for the samples with treatment and control.

Storage time (days)	Control	With treatment
0	214.7 ± 0.5 ^{aB}	69.34 ± 0.05 ^{bC}
3	68.97 ± 0.03 ^{aF}	50.69 ± 0.07 ^{bE}
5	52.76 ± 0.09 ^{aG}	46.31 ± 0.09 ^{bF}
7	337.84 ± 0.01 ^{aA}	200.36 ± 0.04 ^{bA}
10	143.80 ± 0.02 ^{aD}	83.05 ± 0.06 ^{bB}
13	180.43 ± 0.03 ^{aC}	59.70 ± 0.07 ^{bD}
15	71.01 ± 0.08 ^{aE}	33.33 ± 0.04 ^{bG}

Table 3: Antioxidant capacity (EC50 in µg/mL) of the minimally processed aqueous fruit extract using the DPPH free radical.

Total Antioxidant Capacity Equivalent to Trolox (TEAC) values found in this study were higher than those obtained by Lan., *et al.* [33] when analyzing breadfruit antioxidant activity, whose value was 7.2 ± 1.6 µmol of Trolox/g of the sample (Table 4).

Means followed by the same lowercase letter in the line do not differ significantly from each other by the student's t-test (p < 0.05). Averages, in the same column, followed by different letters differ statistically according to Tukey's test (p < 0.05).

Storage time (days)	Control	With treatment
0	5.55 ± 0.04 ^{bF}	53.98 ± 0.04 ^{aG}
3	54.47 ± 0.04 ^{aE}	54.57 ± 0.05 ^{aF}
5	79.36 ± 0.04 ^{bB}	92.30 ± 0.20 ^{aB}
7	43.56 ± 0.04 ^{bG}	58.59 ± 0.10 ^{aE}
10	68.82 ± 0.05 ^{bC}	82.54 ± 0.03 ^{aC}
13	68.49 ± 0.13 ^{bD}	78.32 ± 0.09 ^{aD}
15	129.56 ± 0.07 ^{bA}	188.60 ± 0.06 ^{aA}

Table 4: Total Antioxidant Capacity Equivalent to Trolox (µmol Trolox/g sample) by the ABTS method + for minimally processed aqueous fruit extract.

Microbiological analysis

Like other vegetables, minimally processed fruits have a favorable environment for microbial development. The very composition with high water activity, presence of sugars, and, above all, surface increased by cuts favor microbial development. Microbiological counts of minimally processed and stored breadfruit for 15 days are shown in figure 12.

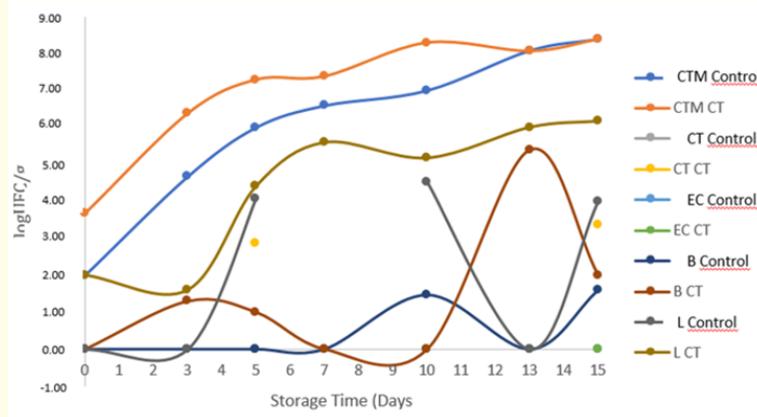


Figure 11: The behavior of the microbiota of breadfruit minimally processed and stored under refrigeration. CTM: Total Mesophyll Count; CT: Total Coliforms; EC: Escherichia Coli; B: Mold and L: Yeast

It is observed that the counts of mesophilic bacteria, coliforms and molds, and yeasts in the control sample and those submitted to treatment increased during storage so that the treatment did

not influence product conservation. Insignificant coliform load in control and treatment samples and the slight change during storage indicate that this type of microorganism was not a problem for

product conservation up to 10 days of storage. Chen, *et al.* [28], when evaluating the effect of citric acid, UV-C, and citric acid together with UV-C, in minimally processed apples also observed this behavior. They reported that the bacterial count of all treatments increased as the storage time increased, with treatment with citric acid and UV-C presented the lowest rate among all.

Product lifespan definition is determined by sensorial characteristics associated with the results of mesophylls and fungi total count. Roopa, *et al.* [20], when evaluating minimally processed breadfruit lifespan stored under refrigeration, concluded that the treatment with a conservative solution helped to guarantee product microbiological safety, with no incidence of coliforms or pathogens, having control samples displayed coliforms throughout the storage range.

Fungi behavior (Molds and Yeasts) was determinant for the establishment of product lifespan. Up to the 5th day, it was verified that the microbial load of these microorganisms was below acceptable levels for industrialized plant products, that is, 4logUFC/g [34]. Microbiological parameters are not a good indicator to determine the lifespan of this type of minimally processed product, since this will be consumed after thermal treatment.

Conclusion

This study indicated that immersion in organic acid and calcium chloride solution for 5 minutes was useful to ensure better preservation of physicochemical characteristics of the breadfruit and microbiological quality, with the maintenance of its sensorial aspects.

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