SCIENTIA PLENA
www.scientiaplena.org.br
VOL. 17, NUM. 5 2021
doi: 10.14808/sci.plena.2021.051501

Minimally processed cassava leaves: effect of packaging on the microbiological and physical-chemical standards

Folhas de mandioca minimamente processadas: efeito da embalagem sobre o padrão microbiológico e físico-químico

J. F. C. Moraes[1]; A. P. R. Campos[1]; A. L. Araújo[1]; A. S. Lopes[2]; R. S. Pena[2*]

[1]*Graduate Program in Food Science and Technology, Federal University of Pará, 66075-110, Belém-Pará, Brazil*
[2]*Institute of Technology/Faculty of Food Engineering, Federal University of Pará, 66075-110, Belém-Pará, Brazil*

**rspena@ufpa.br*
(Recebido em 11 de outubro de 2020; aceito em 24 de maio de 2021)

The study presents itself as an alternative for the minimal processing of crushed cassava leaves, a product widely used in the Northern Brazilian cuisine. A Box-Behnken design was used to define the concentration of the sodium hypochlorite solution (NaClO) and the immersion time (t) capable of guaranteeing acceptable levels for thermotolerant coliforms and molds and yeasts in the leaves. The leaves sanitized in this condition were crushed, packed in polyethylene packaging under standard atmosphere (PE-WV) and under vacuum (PE-V); also packed in polyamide packaging under vacuum (PA-V), and stored at 7ºC for 30 days. The following properties were monitored: fresh weight loss, water activity, pH, titratable acidity, soluble solids, instrumental color, chlorophyll *a* and *b*, and total phenolic contents. In addition, there were assessed *Salmonella* spp, coliform at 45ºC, molds and yeasts and psychrotrophic bacteria. The 20 min immersion time and 250 mg/L NaClO solution were defined as the optimal conditions for the sanitization of the leaves and, after that, the leaves were rinsed with water. The behavior of the physical-chemical and microbiological properties indicated that the sanitized and crushed cassava leaves will be suitable for consumption for 24 h when packed in PE-WV; for 7 days when packed in PE-V and for 14 days when packed in PA-V, at 7°C storage.
Keywords: *Manihot esculenta* Crantz, sanitization, minimal processing.

O estudo apresenta uma alternativa para o processamento mínimo das folhas da mandioca trituradas; um produto amplamente utilizado na culinária da região Norte. Um planejamento Box-Behnken foi utilizado para definir a concentração da solução de hipoclorito de sódio (NaClO) e o tempo de imersão (t) capazes de garantir níveis aceitáveis para coliformes termotolerantes e bolores e leveduras, nas folhas. As folhas sanitizadas nesta condição foram trituradas; acondicionadas em embalagens de polietileno, em atmosfera padrão (PE-WV) e sob vácuo (PE-V), e em embalagem de poliamida sob vácuo (PA-V); e armazenadas a 7°C, por 30 dias. Foram acompanhadas as propriedades: perda de massa fresca, atividade de água, pH, acidez, sólidos solúveis, cor instrumental, clorofila a e b, e compostos fenólicos; além da avaliação de *Salmonella* spp, coliformes a 45°C, bolores e leveduras e bactérias psicrotróficas. Foi definida como condição ótima para a sanitização, a imersão das folhas por 20 min, em uma solução a 250 mg/L de NaClO, seguida de lavagem com água. O comportamento das propriedades físico-químicas e microbiológicas indicaram que as folhas da mandioca sanitizadas e trituradas estarão adequadas para o consumo por 24 h, se acondicionadas em PE-WV, por até 7 dias em PE-V e por 14 dias em PA-V, se armazenadas a 7°C.
Palavras-chave: *Manihot esculenta* Crantz, sanitização, processamento mínimo.

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a tropical root crop, which has an abundant composition of starch. This species stands out for presenting a world production of 296.8 million tons and a cultivated area of 26.6 million hectares [1]. In Brazil, a cassava production of 20.2 million tons is estimated, as the Northern region accounting for 36.1% of this production [2].

In Brazil, cassava roots can be found in the minimally processed or pre-cooked and in frozen forms [3], as the main use of this root destined for the production of flour and tucupi, in the Northern region [4]. Cassava leaves, in turn, are used as an ingredient in typical dishes from the Amazonian culture [5], as maniçoba: a product obtained from the crushed cassava leaves that are boiled with water for several days to eliminate cyanide [6]. Cassava leaves are commonly commercialized in

open markets, in the state of Pará, *in natura* or in crushed form, with or without cooking. These leaves are sold in bulk or in polyethylene packaging at room temperature. In countries like Congo, Indonesia, Malaysia and Tanzania, cassava leaves are consumed as vegetables [7].

The composition of raw materials from plant origin has promoted an increase on demand for this type of product, as well as encouraging studies, due to the beneficial actions of these products to health [8]. The consumer market, in turn, has shown changes in the form of consumption of these products and, in this context, minimally processed products have been highlighted, due to the nutritional advantages and practicality of use [9].

The minimal processing has become an important tool for the food industry, especially for vegetables. However, the use of pre-treatments, such as vegetable cutting, can promote the release of cellular material at the site of lesion and, thus, favor the growth of microorganisms and the increase in senescence rates, reducing product's shelf life [10].

The minimum processing is composed by steps, such as: rinsing, classification, sanitization, cutting, centrifuging, packaging and refrigeration [11]. The sanitization of minimally processed products is important to control the deteriorating processes from microbiological origin, once this step is intended to eliminate microorganisms and reduce deterioration to safer levels [12, 13].

On the basis of the above reasoning, the objective of this work was to evaluate the microbiological and physical-chemical profile of cassava leaves, submitted to minimal processing conditions; aiming to present a safer preservation alternative for these leaves, as well as adding value to the cassava production chain.

2. MATERIAL AND METHODS

2.1 Raw material

The cassava leaves used in this research were collected from the municipality of Salvaterra (Pará, Brazil) (00º 45' 12" S, 48º 31' 00" W, 5 m altitude), in a farm at the banks of PA-154 highway. The leaves were harvested in June 2019, with a cultivar cycle of eight months. The leaves were transported, at room temperature (≈ 28°C), to the Federal University of Pará (Belém, Pará, Brazil), 96 km away from the collection place, in polyethylene packages with 10 kg capacity, dimensions of 60 cm x 40 cm (length x width) and film thickness of 0.10 mm. The leaves were separated from the stems, using stainless steel scissors, in less than 24 hours after harvesting (time related to processing). Then, the leaves were subjected to different conditions of minimum processing.

2.2 Definition of sanitization conditions of the leaves

Before performing the experimental design tests, the cassava leaves were submitted to a selection stage of the intact leaves, followed by rinsing with running water. Then, the sanitization was performed under different experimental design conditions. At this research stage, an experimental design was used to define the concentration of sodium hypochlorite and the immersion time to be used in the minimum processing of the leaves. For this purpose, the Box-Behnken factorial design, the response surface methodology (RSM) and the desirability function were used. The effect of the input variables (independent variables): concentration of sodium hypochlorite (C) in the sanitizing solution (100–300 mg/L), and immersion time (t) (10-30 min), were assessed on the responses (dependent variables): coliform at 45ºC and molds and yeasts. The matrix of the experimental design is presented in Table 1 (first to third columns). After the contact time, the leaves were rinsed with distilled water to remove chlorine in excess and then dried superficially with the aid of a manual polypropylene centrifuge (Alves Plastic, NCM:39241000, Gaspar Mirim, Brazil) with capacity to 4.5 L, for leaves and vegetables. After these steps, the leaves underwent microbiological analyses.

Table 1: Matrix of the Box-Behnken factorial design and the results for coliform at 45ºC and molds and yeasts for the sanitization process of the cassava leaves.

Run order	Original variables (coded variables)		Responses	
	C (X1)	t (X2)	Coliform at 45ºC (MPN/g)	Molds and yeasts (CFU/g)
1	100 (-1)	10 (-1)	< 3.0	$1.18x10^1$
2	100 (-1)	20 (0)	< 3.0	$1.18x10^1$
3	100 (-1)	30 (+1)	< 3.0	$0.45x10^1$
4	200 (0)	10 (-1)	< 3.0	$0.09x10^1$
5	200 (0)	20 (0)	< 3.0	$1.82\ x10^1$
6	200 (0)	30 (+1)	< 3.0	$0.36x10^1$
7	300 (+1)	10 (-1)	< 3.0	$0.45x10^1$
8	300 (+1)	20 (0)	< 3.0	ND
9	300 (+1)	30 (+1)	< 3.0	$0.54x10^1$
10	200 (0)	20 (0)	< 3.0	$0.27x10^1$
11	200 (0)	20 (0)	< 3.0	$0.27x10^1$

C: concentration of sodium hypochlorite (mg/L); t: immersion time (min); X_1: C; X_2: t; MPN: most probable number; CFU: colony forming unit; ND: non-detected.

2.3 Minimum processing of the leaves

After defining the sanitization condition, the tests were performed to define the minimum processing condition for the cassava leaves. For this end, the following steps were performed: selection of the intact leaves; rinsing with running water; sanitization with sodium hypochlorite, in the optimal condition; rinsing with distilled water to remove chlorine in excess; and surface drying by centrifugation. Then, the leaves were crushed in a benchtop crusher (Mondial, Power Mixer 500W, China). The study was carried out with the crushed leaves, because this is the main way of marketing the product.

To evaluate the packaging condition, the crushed leaves were divided into three groups. In the first group, the leaves were packed in polyethylene packaging with vacuum (PE-V), in the second group the leaves were packed in polyamide packaging with vacuum (PA-V) and in the third group, used as control, the leaves were packed in polyethylene packaging without vacuum (PE-WV). After packaging, all samples were stored in the BOD Refrigerated Incubator (Quimis, Q315M16, Brazil) (7°C ± 1°C), for 30 days. This temperature was chosen based on the study from Zhan et al. (2012) [14], who observed a higher retention of the green color in the minimally processed broccoli, when stored at 7°C; besides, it is a condition with lower energy costs. The green color is remarkable characteristic of cassava leaves, which interferes with the consumer's choice for the product. For the samples that were packed under vacuum, a vacuum sealer (Selovac, Jumbo Mini, São Paulo, Brazil) was used at a pressure of 1.3 kPa, for 3 seconds. The storage control was performed at 0, 1, 7, 14, 21 and 30 days, when the samples were taken to undergo microbiological and physical-chemical analyses. The processing steps are presented in the flowchart of Figure 1.

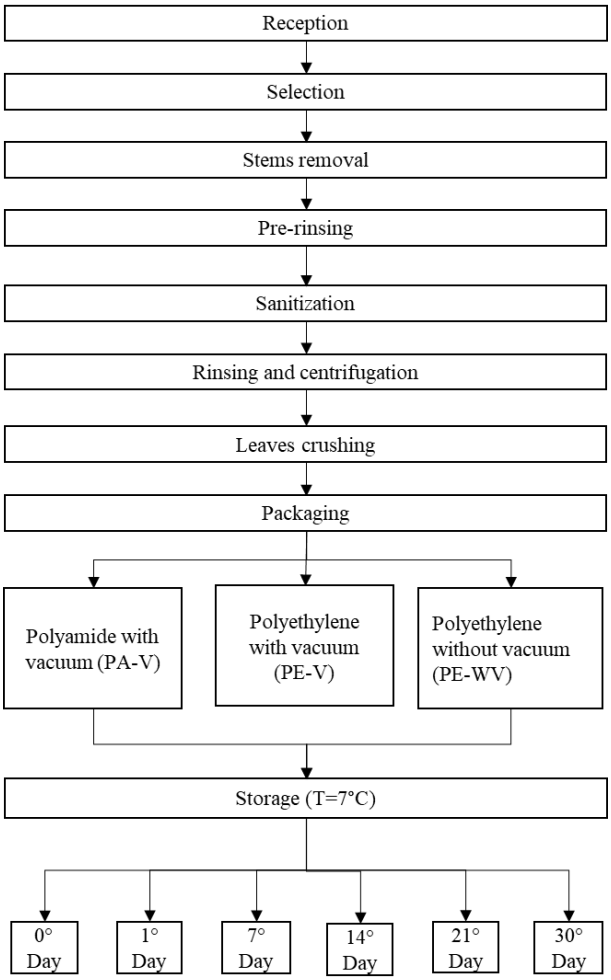


Figure 1: Steps flowchart of the minimum processing of cassava leaves.

2.4 Analytical determinations

2.4.1 Microbiological analyses

In the microbiological determinations, the methodology described by Downes and Ito (2001) [15] was used for *Salmonella* spp. The most probable number (MPN) method APHA 9:2015 was used for coliform at 45ºC; the plating method APHA 21:2015, for molds and yeasts; and the plating method APHA 13.61:2015, for psychrotrophic bacteria in food; all according to official methods from American Public Health Association – APHA [16]. All analyzes were performed in duplicate.

2.4.2 Physical-chemical analyses

In the storage control, additionally to microbiological analyses: *Salmonella* spp absence or presence in 25g of sample, coliform at 45ºC (MPN/g), molds and yeasts (CFU/g) and psychrotrophic bacteria (CFU/g), the samples were submitted to fresh weight loss (%) analyses by gravimetry [17]; water activity (a_w), in digital thermohygrometer (Aqualab 4TE, Decagon Devices Inc, USA); pH, on a benchtop pHmeter (KASVI, k39-2014B, China); titratable acidity (TA) (mEq NaOH 0.01N/ 100g), titration with sodium hydroxide [18]; and soluble solids (SS) (°BRIX), in a digital benchtop refractometer (Quimis, Q76780, São Paulo, Brazil) [18].

Chemical compounds of interest have also been quantified. *Chlorophyll a* and *chlorophyll b* were determined by reading the extracts at 470 nm, 661.6 nm and 664.8 nm, in spectrophotometer (BEL Photonics, BEL Engineering, Monza, Italy), and the results were expressed as µg/g fresh leaf

[19]. *Total phenolic contents* were determined by the Folin-Ciocalteau method [20], and the quantification was made with the aid of an analytical curve of gallic acid, in the concentration range of 0.02 to 0.06 μg of gallic acid/μL (absorbance between 0.160 and 0.895). The extracts were obtained according to Boeing et al. (2014) [21] and the results were expressed as mg gallic acid equivalent/g fresh leaf.

The samples were also subjected to the instrumental color analysis, in a digital colorimeter (Chroma Meter CR-300, Konica Minolta, Japan), based on the CIELAB color space. The following calibration was used: light source D65, 0° vision geometry (specular component included), and values for white X = 0.3174 and gray Y = 0.3349. The parameters a*, b*, L*, c* and h° were determined by direct reading on the equipment. The determination of the color difference (ΔE) was calculated by the Equation 1 [22]. All analyzes were performed in duplicate.

$$\Delta E = \left[\left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2 + \left(\Delta L^*\right)^2\right]^{1/2} \quad (1)$$

where, ΔL* = difference in lightness/darkness, indicating the difference between lighter (+) and darker (–), Δa* = difference between red (+) and green (–), Δb* = difference between yellow (+) and blue (–), and ΔE = total color difference.

2.5 Statistical Analysis

The results of the experimental design were submitted to analysis of variance (ANOVA) to estimate the statistical parameters, the model's lack-of-fit and the coefficient of determination (R^2). The fitting tests and prediction of the polynomial model (Equation 2) were performed at the significance level of 5%.

$$Y = \beta_0 + \beta_1 C + \beta_{11} C^2 + \beta_2 t + \beta_{22} t^2 + \beta_{12} Ct + \beta_{112} C^2 t + \beta_{122} Ct^2 \quad (2)$$

where, Y is the dependent variable (coliform at 45ºC and molds and yeasts); C and t represent the independent variables of concentration of hypochlorite and immersion time, respectively; β_0 represents the constant term; β_1 and β_2 are the linear coefficients; β_{11} and β_{22} are the quadratic coefficients; β_{12} is the linear interaction coefficient, and β_{112} and β_{122} are the quadratic interaction coefficients.

To define the optimum condition, for the sanitization of the leaves, the response surface methodology and the desirability function were used. Desirability values range from 0 to 1, where 0 represents a completely undesirable value and 1 the most desirable value [23]. To obtain the graph of the desirability function, rates of change in desirability (s and t) equal to 2 and a grid factor of 4 were used. To evaluate the behavior of the microbiological and physical-chemical properties analyzed during the storage of the minimally processed cassava leaves, the results were subjected to analysis of variance (ANOVA) and Tukey's test, with 5% significance, for the comparison of means. All statistical analyzes were performed using the Statistica 7.0 program.

3. RESULTS AND DISCUSSION

3.1 Optimization of the sanitization

The results obtained for coliform at 45ºC and molds and yeasts, for Box-Behnken design, are shown in Table 1 (fourth and fifth columns). The National Health Surveillance Agency – ANVISA establishes the maximum limit for coliform at 45°C of $5x10^2$ MPN/g for related products [24] and does not recommend a standard for molds and yeasts. As all the sanitization conditions ensured very low counts for coliform at 45°C (< 3.0 MPN/g), the molds and yeasts were used to evaluate the efficiency of the leaf sanitization process. The results of ANOVA applied to this response are shown in Table 2, considering only the significant effects ($p \leq 0.05$).

Table 2: Estimate effect, pure error, t test and level of statistical significance (p), for the factors of the model fitted to the molds and yeasts

Factor	Effect estimate	Pure error	*t*	*p*
Mean	5.03	0.16	30.89	0.001
C	-6.06	0.43	-14.14	0.005
C^2	-4.00	0.32	-12.59	0.006
Ct	4.09	0.53	7.79	0.016
Ct^2	-4.32	0.45	-9.50	0.011
C^2t	2.95	0.45	6.50	0.023

C: concentration of sodium hypochlorite (mg/L); t: immersion time (min); *t*: t test; *p*: level of probability.

The concentration of hypochlorite (C) had a negative and desirable effect, once the increase in C promoted a reduction in the molds and yeasts (MY) in the leaves. The immersion time (t), in turn, had no significant effect when assessed individually; only when present in the interactions with C. The ordering of effects from the studied variables on the responses, for the process of sanitization of cassava leaves, can be better visualized in the Pareto chart (Figure 2), as it can be visualized that the concentration of sodium hypochlorite (C and C^2) was the variable that presented the greatest isolated effect on the reduction of molds and yeasts.

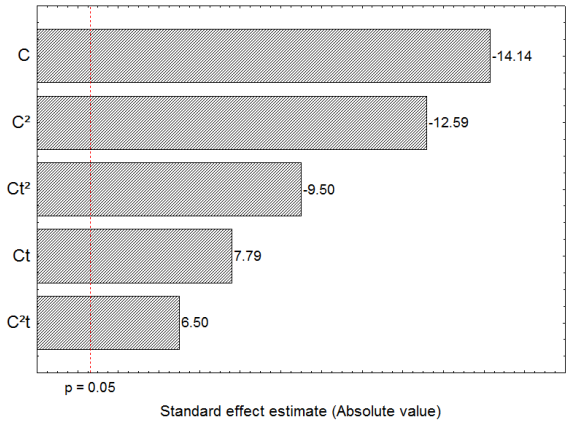


Figure 2: Pareto chart for molds and yeasts.

Table 3 shows the results of ANOVA, for the regression of the second order model, with the F value (F_{value}) and F table (F_{table}), along with the coefficient of determination (R^2), for the molds and yeasts (MY) response; considering only the significant effects. The results indicate that the fitting of the model to the response was significant, within 95% confidence level. The value of F_{value} was higher than the value of F_{table} ($F_{value}/F_{table} > 8$), confirming that the fitted model (Equation 3) is predictive [25]. The R^2 value, in turn, indicates that the model explains 98% of the total variation of the observed data, for the variable molds and yeasts. In addition, a value of $F_{table}/F_{value} > 5$ (for the lack-of-fit) ensures the absence of lack-of-fit.

Table 3: Analysis of variance (ANOVA) for the regression of the model fitted to the response of molds and yeasts.

Source of variation	SS	DF	QM	F_{value}	F_{table}	R^2
Regression	151.97	5	30.39	44.67	5.05	0.98
Residue	3.40	5	0.68			
Lack-of-fit	2.85	3	0.95	3.45	19.16	
Pure error	0.55	2	0.28			
Total	155.37	10				

SS: sum of squares; DF: degree of freedom; QM: quadratic mean.

$$MY = 23.42 - 0.112C + 1.92 \times 10^{-4} C^2 - 4.73 \times 10^{-3} Ct + 6.29 \times 10^{-5} Ct^2 + 8.73 \times 10^{-6} tC^2 \quad (3)$$

The response surface and the contour curves generated by the Equation 3, for the MY response, are shown in Figure 3. Figure 3a shows that the increase in C promoted a reduction in MY, which effects were more representative for values of C between 200 and 300 mg/L and in intermediate t (20 min); conditions in which sanitization was most effective. Figure 3b also shows that, for t over 20 min it was possible to obtain the same levels of destruction for MY (2 CFU/g), using lower C (between 200 and 250 mg/L). A similar effect was observed for treatments with higher C values (between 250 and 300 mg/L), which allowed the use lower t (between 14 and 20 min). These results are important, for practical purposes, as it allows to reduce costs for the sanitization of the cassava leaves, due to the reduction in the demand for sodium hypochlorite or the use of shorter processing times.

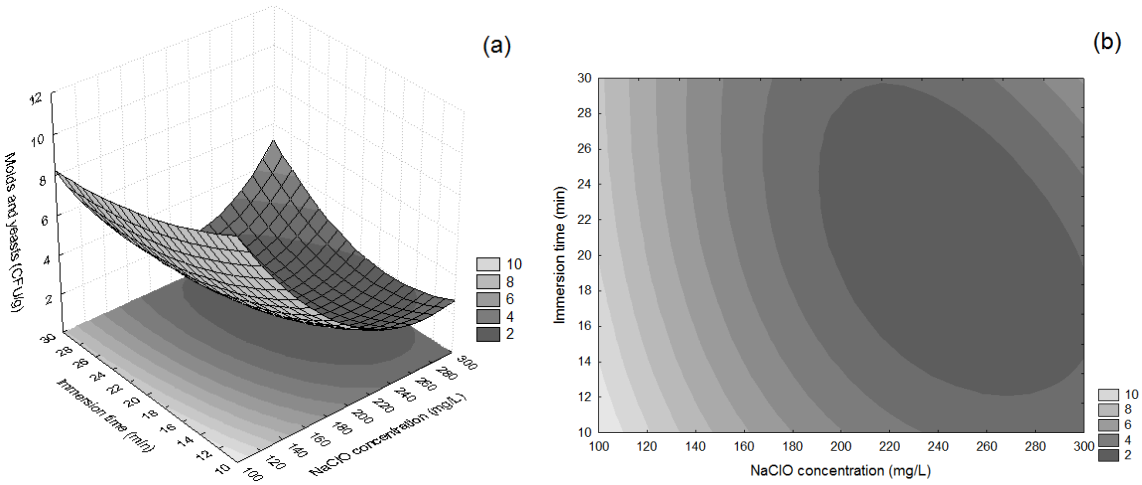


Figure 3: Response surface (a) and contour curves (b) for molds and yeasts.

Figure 4 presents the graphs with the profiles for the concentration of sodium hypochlorite (C) and the immersion time (t), as well as for the function of global desirability, in the experimental domain of the cassava leaves sanitization process. The values attributed to the desirability function, for the optimization of the sanitization process were: 0 for 11.82 CFU/g (undesirable), 0.5 for 5.91 CFU/g (moderately desirable) and 1 for 0.0 CFU/g (desirable); maximum, medium and minimum values for the MY response.

The global desirability varies from 0 to 1 and, the closer the value approaches 1, the better the simultaneous optimization of the studied variables [26]. Thus, the observed value for the global desirability (0.98) allows to affirm that the individual optimum for C and t, relative to the MY, are close to each other, which allows the selection of an experimental condition that meets both variables. Therefore, the optimal conditions defined by the desirability function, for the sanitization process of the cassava leaves, are: an aqueous hypochlorite solution concentration of 250 mg/L, and an immersion time of leaves in the solution of 20 min. In this condition, the count of molds and yeasts was estimated at 1.05 CFU/g.

Berbari et al. (2001) [27] evaluated the efficiency of chlorine, in three different concentrations (70, 100 and 130 mg/L), for an immersion time of 15 min, on the sanitization of lettuce. The minimally processed products presented a count of molds and yeasts of $5x10^5$, $6x10^3$ and $3x10^3$ CFU/g, respectively, after nine days of storage at 2°C. These levels of contamination were higher than those observed for cassava leaves, in the experimental domain (Table 1), most likely to the lowest concentrations used by the cited authors.

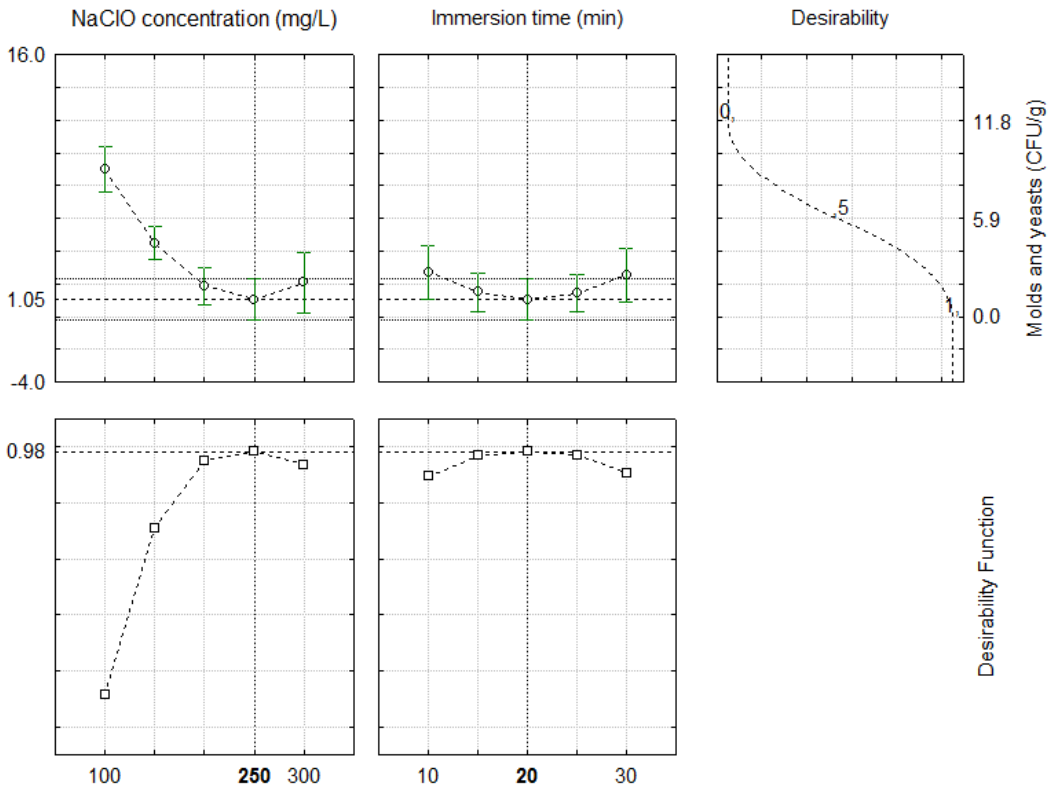


Figure 4: Desirability function graph for molds and yeasts.

3.2 Storage of the minimally processed leaves

3.2.1 Fresh weight loss and water activity

The results of fresh weight loss of the cassava leaves, in different packaging, are shown in Figure 5. The fresh weight loss of the cassava leaves increased with the storage time, but this increase was statistically greater ($p \leq 0.05$) for leaves packed in polyethylene (PE) packaging, regardless the application of vacuum (PE-V) or the non-application of vacuum (PE-WV). The observed increase was more representative, after 21 days of storage, when the fresh weight loss of the PE-WV and PE-V samples continued to increase, while the values observed for the leaves packed in a polyamide packaging with vacuum (PA-V) remained statistically unchanged ($p > 0.05$). In 30 days of storage, the fresh weight loss of the leaves packed in PE-V and PE-WV reached an approximately value of 4.5%, while for the leaves packed in PA-V, the weight loss remained less than 2%.

According to Saltveit (2002) [28], the physical injuries caused by freshly crushed vegetables promote an immediate physical and physiological response in the plant tissue, generating the accumulation of water on the surface and the exposure of the tissue to the spread of contaminants (physical changes) and subsequent changes, such as the diffusion of gases and change in the appearance of the surface (physiological changes). The accumulation of water on the surface favors the loss of water and, consequently, the fresh weight loss of the vegetable. In summary, the results indicate that when the control variable is fresh weight loss, the crushed cassava leaves can be packed in PE packaging without vacuum, for up to 21 days, if stored at 7°C. However, if the storage time (at 7°C) is desired, up to 30 days, it is recommended the leaves to be packed in PA packaging, under vacuum, for a more effective control of the variable.

The water activities (a_w) of the minimally processed products did not present discrepant values (data not shown) between the types of packaging used (polyethylene and polyamide), the storage conditions (with and without vacuum) and between the storage days. The a_w values close to 0.99 are the favorable conditions for the development of microorganisms in the cassava leaves, if it is not rinsed, sanitized, conditioned and stored under appropriate conditions.

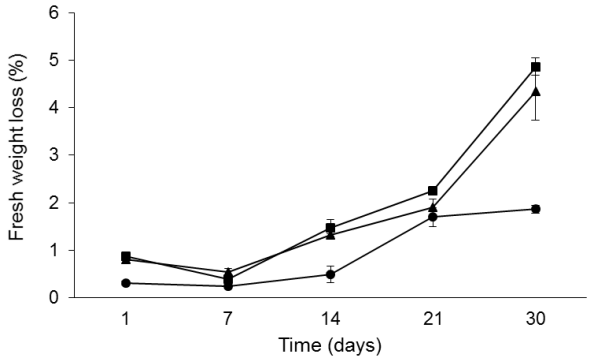


Figure 5: Fresh weight loss (%) of the minimally processed cassava leaves, stored at 7°C; in different packaging conditions. (■) Polyethylene without vacuum, (▲) Polyethylene with vacuum and (●) Polyamide with vacuum.

3.2.2 pH, titratable acidity and soluble solids

Figure 6 shows the behavior of pH, titratable acidity (TA) and soluble solids (SS), in minimally processed cassava leaves, during storage at 7°C. A pH reduction in the leaves was observed in the first day of storage, which was more significant ($p \leq 0.05$) for PE-WV, followed by PE-V and PA-V. On the seventh day, the pH of the leaves increased to values statistically equal to the initial ones, and remained practically unchanged until the end of storage. Rinaldi et al. (2005) [29] evaluated the storage of minimally processed cabbage, at 5°C and 10°C, and observed the increase in the pH of the vegetable, during storage. The increase in pH is attributed to the need to neutralize the acidification of the medium caused by carbon dioxide (CO_2), a product of the post-harvest respiration reaction [30, 31]. Thus, the slight pH variation can be attributed to the low respiration rate of cassava leaves at the storage temperature of 7°C.

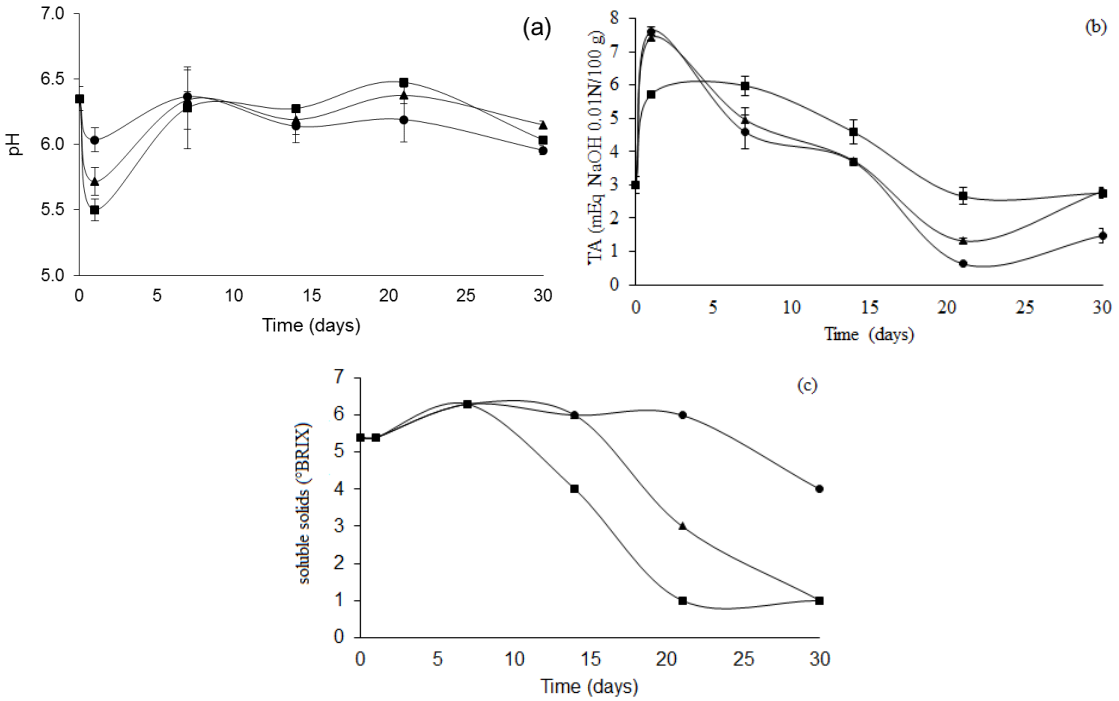


Figure 6: Values of pH (a), titratable acidity (TA) (b) and soluble solids (c), for the minimally processed cassava leaves, during the storage at 7 °C. (■) Polyethylene without vacuum, (▲) Polyethylene with vacuum and (●) Polyamide with vacuum.

Titratable acidity experienced a significant increase ($p \leq 0.05$) on the first day of storage, for all packaging conditions, and then there was a decrease up to the initial value, for PE-WV, and for values lower than the initial ones, for PE-V and PA-V; on the 21st day of storage. The abrupt increase in TA in the first 24 hours of storage can be related to the high consumption of oxygen (O_2), due to the respiration process of the plant, which accumulates CO_2 and water. The generated CO_2 can dissolve in water to form carbonic acid (H_2CO_3), a weak acid, which tends to ionize and form hydrogen ions, bicarbonate (HCO_3^{1-}) and carbonate (CO_3^{2-}), promoting a pH stabilization (Figure 6a) and increasing acidity (Figure 6b) [32].

The reduction in TA that occurred from the first day of storage, for leaves packed under vacuum (PE-V and PA-V) and from the seventh day for leaves packed in standard atmosphere (PE-WV), can be attributed to the consumption of organic acids, as substrate by microorganisms, due to the limitation of the leaf respiration process and the decrease in energy reserves; once plant tissues do not act as carbon storage sites [29, 33]. A lower concentration of O_2 and, consequently, a greater limitation to the breathing process has made the reduction in TA to occur in a shorter time and with greater intensity, for the leaves packed with vacuum (PE-V and PA-V). When packaging materials with low O_2 permeability are used, such as polyamide, the effect mentioned is more significant [34], which was observed for the leaves packed in PA-V (Figure 6b).

Up to the seventh day of storage, there was an increase of 0.5 °Brix, in the soluble solids (SS) content, for cassava leaves, in all packaging conditions (Figure 6c). This behavior can be attributed to traces of sugars arising from cell wall degradation or to soluble sugars derived from the degradation of vegetable storage carbohydrates [35]. There was an abrupt decrease in SS from the seventh day in the leaves packed in standard atmosphere (PE-WV), from 6 °Brix to 1 °Brix; on the 21st day. For leaves packed with vacuum, SS decreased from the 14th day for PE-V and only from the 21st day for PA-V; reaching 1 °Brix and 4 °Brix, respectively, on the 30th day. Therefore, the use of polyamide packaging under vacuum for the packing of crushed cassava leaves was the condition that proved to be the most efficient in controlling SS for storage at 7°C.

As the storage time progressed, the plant's respiration rates are minimized, promoting the change from the aerobic route to the anaerobic route, to supply the energy demand. In vegetables, alcoholic fermentation is the main process, which is preceded by lactic fermentation, which promotes the accumulation of lactic acid and induces acidification of cytosol, a signal for the activation of alcoholic fermentation, due to the hypoxia conditions created in the environment [36-38]. In the anaerobic route, the Krebs cycle will change to the glycolytic pathway, in which the pyruvic acid is decarboxylated to form acetaldehyde and, consequently, CO_2 and ethanol [30]. The production of lactic acid, ethanol, among other intermediates, promote changes in pH, TA and SS during storage.

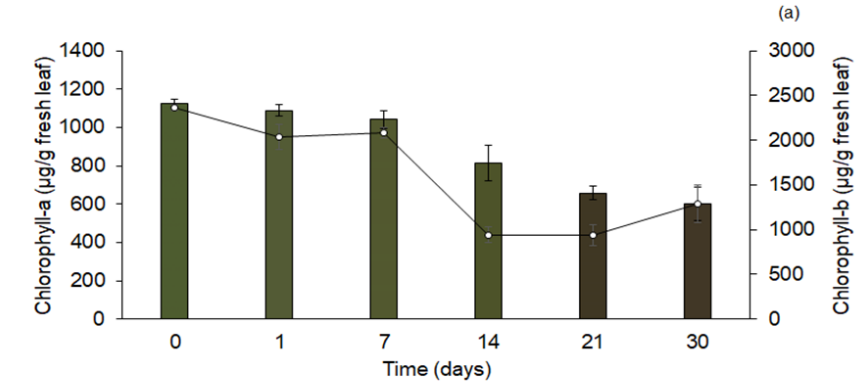
The changes observed during storage can also be attributed to the increasing in the concentration of CO_2, inside the packaging. Temperatures between 6°C and 10°C, in the presence of CO_2, can induce microbiological changes, such as the development of mesophilic microorganisms, when compared to standard atmospheric conditions [39]. The growth of microorganisms from the lactic acid flora can also occur, promoting a reduction in TA (Figure 6b) and SS (Figure 6c) [40], or the growth of psychrotrophic microorganisms, such as *Pseudomona*s spp, which are the greatest vegetables deteriorating [41].

3.2.3 Color, chlorophyll a and b

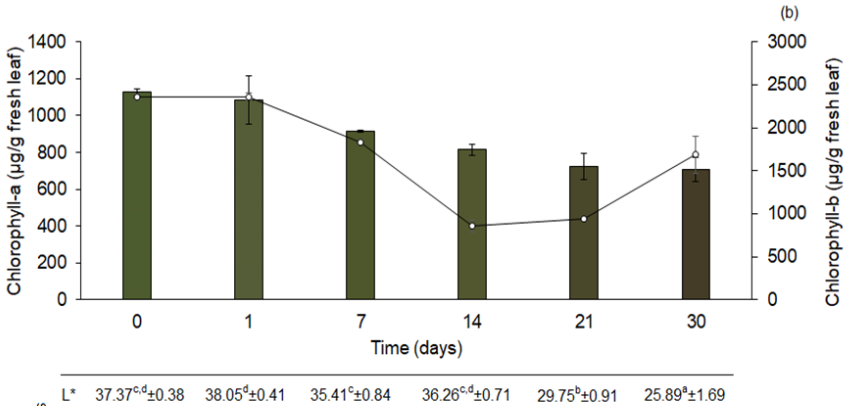
Figure 7 shows the behaviors of the instrumental color parameters, as well as the chlorophyll *a* and chlorophyll *b*, in minimally processed cassava leaves, during storage at 7°C. The color parameters values indicate the presence of the colors green (-a*), yellow (+b*) and dark ($L^* < 40$), in the cassava leaves. The losses in green and yellow colorings, as well as the darkening of the leaves packed in PE-WV and PE-V were more significant after the 14th day of storage. These results indicated that the use of vacuum was not favorable in retaining the color, when the polyethylene packages were used.

For the leaves packed in PA-V, in turn, the colors green and yellow, as well as the lightness, were maintained during the 30 days of storage. This behavior is attributed to the greater efficiency

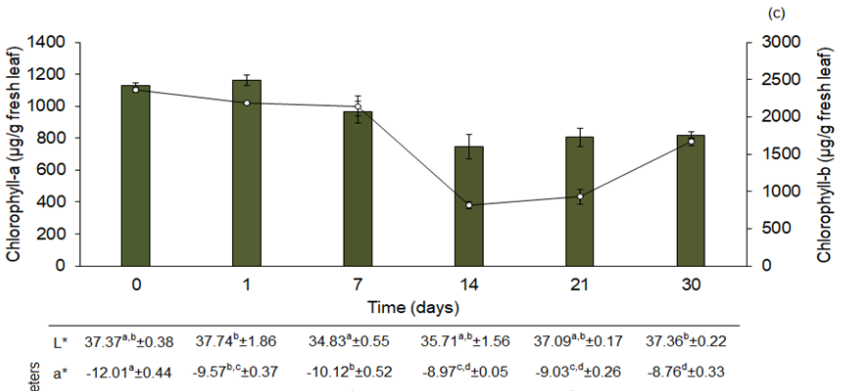
of the polyamide packaging in maintaining the vacuum [42]. The results are confirmed by the behavior observed for the Chroma (c*), the Hue angle (h°) and the total color difference (ΔE).



Color Parameters	0	1	7	14	21	30
L*	37.37^{b}±0.38	36.90^{b}±1.49	36.52^{b}±4.88	33.77^{b}±1.97	24.53^{a}±0.30	23.14^{a}±1.14
a*	-12.01^{a}±0.44	-7.76^{b}±0.74	-8.54^{b}±1.55	-7.27^{b}±0.41	1.57^{c}±0.13	1.71^{c}±0.40
b*	25.23^{d}±0.29	19.54^{b}±1.16	22.95^{c}±0.69	22.95^{c}±1.07	13.74^{a}±0.62	13.18^{a}±0.54
c*	28.15^{d}±0.61	21.05^{b}±1.15	25.71^{c,d}±1.85	24.08^{c}±0.99	13.82^{a}±0.62	13.28^{a}±0.55
h°	115.46^{b}±0.92	111.79^{b}±3.18	106.86^{b}±8.58	107.61^{b}±1.39	83.47^{a}±0.49	82.86^{a}±1.47
ΔE		7.35^{a}±0.68	5.91^{a}±2.04	6.57^{a}±1.37	22.00^{b}±0.22	23.17^{b}±0.82



Color Parametres	0	1	7	14	21	30
L*	37.37^{c,d}±0.38	38.05^{d}±0.41	35.41^{c}±0.84	36.26^{c,d}±0.71	29.75^{b}±0.91	25.89^{a}±1.69
a*	-12.01^{a}±0.44	-9.09^{b}±0.90	-9.47^{b}±0.35	-7.28^{c}±0.71	-2.63^{d}±0.84	0.60^{e}±0.12
b*	25.23^{d}±0.29	20.31^{b}±1.30	23.18^{c,d}±0.31	22.09^{b,c}±0.87	15.88^{a}±1.18	13.88^{a}±1.39
c*	28.15^{d}±0.61	22.25^{b}±1.55	25.04^{c}±0.42	23.03^{b,c}±0.39	16.10^{a}±1.31	13.93^{a}±1.36
h°	115.46^{d}±0.92	114.07^{d}±0.81	112.21^{c,d}±0.49	108.43^{c}±1.82	99.27^{b}±2.14	91.19^{a}±4.08
ΔE		5.79^{a}±1.52	3.89^{a}±0.34	5.88^{a}±0.65	15.34^{b}±0.78	20.52^{c}±1.71



Color Parameters	0	1	7	14	21	30
L*	37.37^{a,b}±0.38	37.74^{b}±1.86	34.83^{a}±0.55	35.71^{a,b}±1.56	37.09^{a,b}±0.17	37.36^{b}±0.22
a*	-12.01^{a}±0.44	-9.57^{b,c}±0.37	-10.12^{b}±0.52	-8.97^{c,d}±0.05	-9.03^{c,d}±0.26	-8.76^{d}±0.33
b*	25.23^{c}±0.29	23.38^{a}±0.75	23.28^{a,b}±1.17	22.26^{a}±0.37	23.94^{b,c}±0.27	22.07^{a}±0.52
c*	28.15^{c}±0.61	24.36^{a,b}±0.66	25.29^{a,b}±1.33	24.00^{a,b}±0.34	25.59^{b}±0.30	23.75^{a}±0.56
h°	115.46^{d}±0.92	113.15^{b,c}±1.38	113.70^{c,d}±0.80	111.95^{a,b,c}±0.37	110.65^{a}±0.50	111.65^{a,b}±0.59
ΔE		4.16^{a,b}±0.28	3.80^{a,b}±1.07	4.74^{b}±0.67	3.28^{a}±0.28	4.55^{a,b}±0.54

Figure 7: Color parameters, and profile of chlorophyll a (bars) and chlorophyll b (markers), for the minimally processed cassava leaves, during storage at 7°C. (a) PE-WV, (b) PE-V and (c) PA-V. Means followed by the same letter do not differ statistically from each other on the same line, by the Tukey's test, at 5% probability.

The darkening of the leaves can be attributed to injuries suffered during the long period of cold storage. Tomás-Barberán et al. (1997) [43] and Mai and Glomb (2013) [44] observed that chlorogenic acid was one of the phenolic compounds responsible for the darkening of lettuce leaves. Zhan et al. (2012) [14] evaluated the effect of exposure to light (24 µmol/m.s) and temperature (4°C and 7°C) during the storage of minimally processed broccoli, and observed that these conditions were effective in maintaining the color, texture, odor and acceptability of the product.

Regarding the behavior of chlorophyll during the storage of minimally processed cassava leaves, the same degradation standard was observed for chlorophyll *a* and chlorophyll *b*; which its reduction was more significant after the seventh day of storage. The beginning of the chlorophyll degradation can be attributed to the removal of the phytol group and the formation of chlorophyll, by the action of chlorophyllase. The reaction is favored by the enzyme release present in the chloroplast, during the leaf crushing process [45]. The increase in the concentration of chlorophylls *a* and *b*, observed from the 21st day of storage, can be attributed to the increase in the leaf's fresh weight loss (Figure 5), which promoted the concentration of pigments.

3.2.4 Total phenolic contents

Figure 8 shows the behavior of the total phenolic contents, in minimally processed cassava leaves, during storage. The initial content of total phenolics, in the leaves, was close to the values observed by Suresh et al. (2011) [46], in extracts obtained with methanol (64 mg/g), acidified methanol (136 mg/g) and acetone (164 mg/g), from the cassava leaves stems.

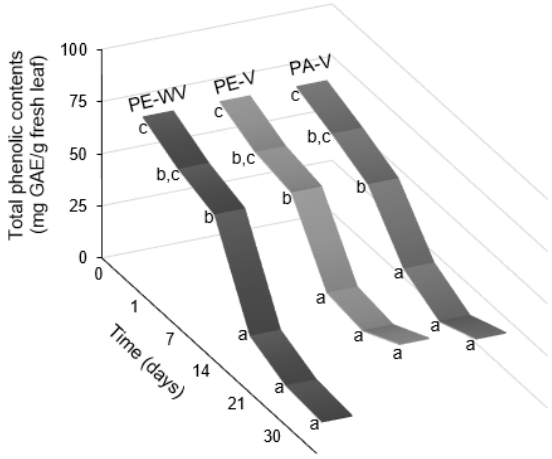


Figure 8: Total phenolic contents in minimally processed cassava leaves, during storage at 7°C. Values with the same letters, in the same range, do not differ, at 5% probability, by the Tukey's test.

The degradation of total phenolic contents of the leaves was observed during the entire storage, which was more significant from the seventh day of storage, regardless of the packaging and the atmosphere used. After 30 days of storage, the maximum levels of degradation observed were 93%, 49% and 62%, for leaves packed in PE-WV, PE-V and PA-V. The results show that packaging under vacuum was much more efficient in retaining the phenolic compounds from the leaves, and that packaging in PE-V was more efficient than in PA-V. This behavior can be attributed to the fact that the leaves packed in polyethylene packaging have presented a fresh weight loss twice higher than the leaves packed in polyamide packaging (Figure 5), which promoted the highest concentration of compounds in the first one.

Vallejo et al. (2003) [47] studied the freshly harvested broccoli stored for 7 days at 1°C to simulate the maximum time spent on transportation and distribution of the product and for another 3 days at 15°C, in order to simulate the time spent in the food market. Furthermore, the authors found that after 10 days, a considerable loss in total phenolic contents occurred, around 44-51, 59-

62 and 73-74% on a wet basis for synaptic acid derivatives, total flavonoids and derivatives of caffeoylquinic acid.

3.2.5 Microbiological analysis

After the sanitization process (at the optimized condition of 250 mg/L for 20 minutes), the cassava leaves underwent analyzes of *Salmonella* spp., coliform at 45ºC, molds and yeasts and psychrotrophic bacteria. As the results did not reveal the presence of *Salmonella*, in 25 g of fresh leaf, this analysis was not performed during leaf storage. The other microorganisms, in turn, were analyzed during the 30 days of storage, and the results are shown in Table 4.

Table 4: Molds and yeasts, coliform at 45°C and psychrotrophic bacteria count, on the minimally processed cassava leaves, during storage at 7°C.

Time (days)	Coliform at 45°C (MPN/g)			Molds and yeasts (CFU/g)			Psychrotrophic bacteria (CFU/g)		
	PE-WV	PE-V	PA-V	PE-WV	PE-V	PA-V	PE-WV	PE-V	PA-V
0	<3.0	<3.0	<3.0	$6.82x10^2$	$6.82x10^2$	$6.82x10^2$	$6.82x10^2$	$6.82x10^2$	$6.82x10^2$
1	<3.0	<3.0	<3.0	$7.73x10^4$	$4.86x10^4$	$1.91x10^3$	$7.73x10^4$	$4.86x10^4$	$1.91x10^3$
7	3.6	3.6	3.6	$1.58x10^6$	$8.18x10^5$	$2.52x10^4$	$1.58x10^6$	$8.18x10^5$	$2.52x10^4$
14	<3.0	<3.0	<3.0	$1.20x10^7$	$7.32x10^6$	$2.73x10^5$	$1.20x10^7$	$7.32x10^6$	$2.73x10^5$
21	<3.0	<3.0	<3.0	$3.14x10^8$	$1.10x10^8$	$1.03x10^7$	$3.14x10^8$	$1.10x10^8$	$1.03x10^7$
30	9.2	<3.0	<3.0	$4.00x10^8$	$1.82x10^8$	$9.55x10^7$	$4.00x10^8$	$1.82x10^8$	$9.55x10^7$

PE-WV: Polyethylene without vacuum, PE-V: Polyethylene with vacuum, PA-V: Polyamide with vacuum.

In general, as the storage time advanced, an increase in the count of the evaluated microorganisms was observed in the minimally processed cassava leaves; regardless of the packaging condition. However, a greater increase was observed for leaves packed in polyethylene, with the highest counts observed for packaging without vacuum (PE-WV). A better microbiological control was observed for the leaves packed in PA-V.

Schuh et al. (2020) [48] assessed products based on minimally processed vegetables (sprouts, cabbage, collards, lettuce, tropical and Italian salads), sold in three different supermarkets in the municipality of Concórdia, in the Santa Catarina state (Brazil), which showed values of coliform at 45°C below 3.0 MPN/g. Santos et al. (2019) [9], in turn, analyzed minimally processed vegetable salad, which showed a count of coliform at 45°C also below 3.0 MPN/g.

During the 30 days of storage, there was no increase in the count of coliform at 45°C, in the minimally processed cassava leaves, except on the 30^{th} day, for PE-WV. However, the observed values did not exceed the limit established by Brazilian legislation (10^2 MPN/g) [24]. These results confirm the efficiency of the sanitization condition used, in the minimal processing of cassava leaves, as well as the storage temperature.

The initial contamination of molds and yeasts in the leaves of minimally processed cassava was $6x10^2$ CFU/g. After 30 days of storage, high levels of contamination were observed for these microorganisms, in PE-WV ($4.00x10^8$ CFU/g), PE-V ($1.82x10^8$ CFU/g) and PA-V ($9.55x10^7$ CFU/g). Pereira et al. (2011) [49] evaluated the behavior of yeasts in 84 samples of minimally processed vegetables, sanitized with sodium hypochlorite, in concentrations of 50 to 400 mg/L, and observed that the yeasts also presented resistance against the action of the sanitizer, even at the highest concentrations.

Regarding the psychrotrophic bacteria, the count reached values in the order of magnitude of 10^8 CFU/g fresh leaf, at 30 days of storage. The highest counts (> 10^6 CFU/g) were observed from the seventh day for PE-WV, 14^{th} day for PE-V and 21^{st} day for PA-V. However, Brazilian legislation does not define psychrotrophic bacteria as possible contaminants for minimally processed vegetables. Hébraud and Potier (1999) [50] emphasize that the presence of psychrotrophic bacteria in refrigerated foods is relevant, once the presence of these microorganisms is a cause of deterioration and food intoxication.

According to Francis et al. (1999) [51], temperature is the variable with the greatest influence on the growth of microorganisms in minimally processed vegetables. These authors observed that the psychrotrophic bacteria *Aeromonas hydrophila* grew in minimally processed products, stored under refrigeration (2°C-5°C). Szabo et al. (2000) [52] evaluated 120 commercial samples of minimally processed lettuce and identified the presence of the following psychrotrophic microorganisms: *Yersinia enterocolitica*, *Aeromonas hydrophila* or *Aeromonas caviae* and *Listeria monocytogenes*.

Santos et al. (2019) [9] found a count of psychrotrophic bacteria greater than $3x10^2$ CFU/g in a commercial vegetable salad. Fantuzzi et al. (2004) [11] observed a count of 10^4 CFU/g, for psychrotrophic bacteria, in minimally processed cabbage, sanitized with sodium hypochlorite at 200 mg/L, for 10 min. Garg et al. (1990) [53] and Fan and Song (2008) [54] found counts between 10^3 and 10^5 CFU/g, for psychrotrophic bacteria, in products such as lettuce, cabbage salad and cauliflower. In a study with chopped American lettuce, Barriga et al. (1991) [55], observed an increase in the count of psychrotrophic bacteria from 10^4 to 10^7 CFU/g, during storage in a modified atmosphere, for 12 days.

In refrigerated conditions, the growth of bacteria, fungi and yeasts can be observed. Most of the bacteria have suppressed growth, except psychrotrophic bacteria, which can grow at refrigerated temperatures [56, 57]. *Pseudomonas* spp gender is one of the main microorganisms responsible for food losses under refrigeration storages; in different parts of the world. *Pseudomonas marginalis* bacteria is an important pathogen that causes soft rot in the postharvest of a wide variety of vegetables [58].

Additional studies are necessary, aiming to establish reference values, for psychrotrophic bacteria and for molds and yeasts, as these microorganisms can be indicators of the good state of conservation in a food. The evaluation of psychrotrophic bacteria in minimally processed vegetables is very important, because at ideal refrigeration temperatures for vegetable products (0°C to 7°C), there may be a great development of these microorganisms.

4. CONCLUSION

The minimal processing of crushed cassava leaves was studied for the first time. The optimum condition defined for the sanitization of cassava leaves was immersion time of 20 min, in an aqueous solution with 250 mg/L sodium hypochlorite. In turn, the behavior of the physical-chemical (fresh weight loss, a_w, pH, TA, SS, color parameters, chlorophyll *a* and *b*, and total phenolic contents) and microbiological (*Salmonella* spp, coliform at 45ºC, molds and yeasts and psychrotrophic bacteria) properties during the storage at 7ºC showed that, the crushed cassava leaves will be suitable for consumption for only 24 hours when packed in polyethylene under a standard atmosphere (PE-WV); for up to 7 days when packed in polyethylene with vacuum (PE-V); and for up to 14 days when packed in polyamide with vacuum (PA-V). Although the behavior of the physical-chemical and microbiological properties evaluated have shown that the PA-V packaging was the most efficient for the conservation of minimally processed cassava leaves; the PE-V packaging presents itself as a good alternative; for lower packaging costs.

5. ACKNOWLEDGEMENTS

The authors acknowledge Coordenação de Pessoal de Nível Superior (CAPES, Brazil) for the scholarship of J. F. C. Moraes (99992418249), CNPq (Conselho Nacional de Desenvolvimento Cientifico e Tecnológico, Brazil, Project 428403/2016-6) and PROPESP (UFPA, Brazil).

6. REFERENCES

1. Wang S, He H, Yu J, Chen S, Li X, Bo F, Guo Q, Xu Z, Jin M. Cellulase-added cassava ethanol process boosts ethanol titer and reduces glycerol production. Ind Crops Prod. 2020 Mar;148:112304, doi: 10.1016/j.indcrop.2020.112304.

2. IBGE – Instituto Brasileiro de Geografia e Estatística. Levantamento sistemático da produção agrícola [Internet]. 2019 Jan [cited 2020 Jul 01]. Available from: https://biblioteca.ibge.gov.br/visualizacao/periodicos/2415/epag_2019_jan.pdf
3. Aguiar EB, Valle, TL, Lorenzi JO, Kanthack RAD, Miranda Filho H, Granja NP. Efeito da densidade populacional e época de colheita na produção de raízes de mandioca de mesa. Rev Bragantia. 2011 Dez;70(3):561-9, doi: 10.1590/S0006-87052011005000010.
4. Chisté RC, Cohen KO. Estudo do processo de fabricação da farinha de mandioca – Documentos 267. Belém (PA): Embrapa Amazônia Oriental; 2006. 75 p.
5. Modesto Junior EM, Chisté RC, Pena RS. Oven drying and hot water cooking processes decrease HCN contents of cassava leaves. Food Res Int. 2019 Jan;119:517-23, doi: 10.1016/j.foodres.2019.01.029.
6. Câmara FS, Madruga MS. Cyanic acid, phytic acid, total tannin and aflotoxin contentes of a Brazilian multimistura preparation. Rev Nutr. 2001 Jan/Apr;14(1):33-6, doi: 10.1590/S1415-52732001000100005.
7. Latif S, Muller J. Potential of cassava leaves in human nutrition: A review. Trends Food Sci Technol. 2015 Apr;44(2):147-58, doi: 10.1016/j.tifs.2015.04.006.
8. Khanam UKS, Oba S, Yanase E, Murakami, Y. Phenolic acids, flavonoids and total antioxidant capacity of selected leafy vegetables. J Funct Foods. 2012 Jul;4(4):979-87, doi: 10.1016/j.jff.2012.07.006.
9. Santos RB, Silva JM, Silva CS, Nascimento MS, Santos TMC, Costa JHQ. Qualidade microbiológica de alimentos in natura minimamente processados. Global Sci Technol. 2019 Jan/Abr;12(1):43-52.
10. Ali A, Yeoh WK, Forney C, Siddiqui MW. Advances in postharvest technologies to extend the storage life of minimally processed fruits and vegetables. Crit Rev Food Sci Nutr. 2018 Oct;58(15):2632-49, doi: 10.1080/10408398.2017.1339180.
11. Fantuzzi E, Puschmann R, Vanetti MCD. Microbiota contaminante em repolho minimamente processado. Food Sci. Technol. 2004 Jan;24(2):207-11, doi: 10.1590/S0101-20612004000200008.
12. Brackett RE. Microbiological consequences of minimally processed fruits and vegetables. J Food Qual. 1987 Jun;10(3):195-206, doi: 10.1111/j.1745-4557.1987.tb00858.x.
13. Vilarin SP, Teixeira TMR, Lima CMG, Pagnossa JP, Figueiredo RM, Medeiros UBC, Santana RF. Effect of sanitization on minimally processed cabbage (*Brassica oleracea* L.). Res Soc Dev. 2020 Apr;9(6):1-9, doi: 10.33448/rsd-v9i6.3467.
14. Zhan L, Hub J, Li Y, Pang L. Combination of light exposure and low temperature in preserving quality and extending shelf-life of fresh-cut broccoli (*Brassica oleracea* L.). Postharvest Biol Technol. 2012 Jan;72:76-81, doi: 10.1016/j.postharvbio.2012.05.001.
15. Downes FP, Ito K. Compendium of methods for the microbiological examinations of foods. 4th ed. Washington (DC): APHA; 2001. 687 p.
16. Silva N. Manual de métodos de análise microbiológica de alimentos e água. São Paulo: Blucher; 2017. 535 p.
17. Simões NA, Coelho DG, Fonseca KS, Barros Júnior AP, Ferreira-Silva SL, Freire CS, Silva EF. Effect of multilayer nylon packages on the oxidative damage of minimally processed yam. Braz J Food Technol. 2019 May;22:1-15, doi: 10.1590/1981-6723.09017.
18. AOAC. Official methods of analysis of the Association of Official Analytical Chemists. Maryland (US): AOAC International; 2016. 3172 p.
19. Lichtenthaler HK, Buschmann C. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. Curr Protoc Food Anal Chem. 2001 Nov;1(1):1-8, doi:10.1002/0471142913.faf0403s01.
20. Waterhouse AL. Determination of total phenolics. Curr Protoc Food Anal Chem. 2002 Feb;6(1):1-8, doi:10.1002/0471142913.fai0101s06.
21. Boeing JS, Barizão EO, Silva BC, Montanher PF, Almeida VC, Visentainer JV. Evaluation of solvent effect on the extraction of phenolic compounds and antioxidant capacities from the berries: Application of principal component analysis. Chem Cent J. 2014 Aug;8(1):48, doi: 10.1186/s13065-014-0048-1
22. Pathare PB, Opara UL, Al-Said FAJ. Colour measurement and analysis in fresh and processed foods: A review. Food Bioprocess Technol. 2013 May;6(1):36-60, doi: 10.1007/s11947-012-0867-9.
23. Derringer G, Suich R. Simultaneous optimization of several response variables. J Qual Technol. 1980 Oct;12(4):214-9, doi: 10.1080/00224065.1980.11980968.
24. Brasil, Ministério da Saúde, Agência Nacional de Vigilância Sanitária – Anvisa. Resolução RDC nº 12, de 2 de janeiro de 2001. Diário Oficial [da] República Federativa do Brasil. 10 jan 2001;7-E(Seção 1):45-53.
25. Bruns RE, Scarmínio IS, Barros Neto B. Como fazer experimentos: Pesquisa e desenvolvimento na ciência e na indústria. Campinas (SP): Editora da Unicamp; 2001. 401 p.
26. Akhnazarova S, Kafarov V. Experiment optimization in chemistry and chemical engineering. California (US): Mir Publishers; 1982. 312 p.

27. Berbari SAG, Paschoalino JE, Silveira NFA. Efeito do cloro na água de lavagem para desinfecção de alface minimamente processada. Food Sci Technol. 2001 Mai;21(2):197-201, doi: 10.1590/S0101-20612001000200014.
28. Saltveit ME. Fresh-cut vegetables. In: Bartz JA, Brecht JK, editors. Postharvest physiology and pathology of vegetables. 2nd ed. New York (US): Marcel Dekker; 2002. p. 691-712.
29. Rinaldi MM, Benedetti BC, Calore L. Efeito da embalagem e temperatura de armazenamento em repolho minimamente processado. Food Sci. Technol. 2005 May;25(3):480-6, doi: 10.1590/S0101-20612005000300015.
30. Kader AA. Biochemical and physiological basis for effects of controlled and modified atmospheres on fruits and vegetables. Food Technol. 1986 Jun;40(5):99-104.
31. Moreno LB, Scherwinski R, Silva JMT, Scalon SPQ, Carnevalli TO. Conservação de repolho minimamente processado sob efeito de diferentes embalagens, tempo de estocagem e temperatura. Rev Agric Neotrop. 2016 Apr/Jun;3(2):68-74, doi: 10.32404/rean.v3i2.1139.
32. Newton DE. Food chemistry. New York (US): Facts on File. An imprint of Infobase Publishing; 2007. Chapter 2, Food additives; p. 17-48.
33. Roura SI, Davidovich LA, Del Valle CE. Quality loss in minimally processed swiss chard related to amount of damaged area. Lebensm-Wiss u-Technol. 2000 Feb;33(1):53-9, doi: 10.1006/fstl.1999.0615.
34. Ngadi MO, Latheef MB, Kassama L. Emerging technologies for microbial control in food processing. In: Boye JI, Arcand Y, editors. Green technologies in food production and processing. New York (US): Springer; 2012. p. 363-411.
35. Maness N, Perkins-Veazie P. Soluble and storage carbohydrates. In: Bartz JA, Brecht JK, editors. Postharvest physiology and pathology of vegetables. 2nd ed. New York (US): Marcel Dekker; 2002. p. 361-82.
36. Porte A, Maia LH. Alterações fisiológicas, bioquímicas e microbiológicas de alimentos minimamente processados. Bol CEPPA. 2001 Jan/Jun;19(1):105-18, doi: 10.5380/cep.v19i1.1227.
37. Damodaran S, Parkin KL, Fennema OR. Química de alimentos de Fennema. 4 ed. Porto Alegre (RS): Artmed; 2010. 900 p.
38. Nelson DL, Cox MM. Princípios de bioquímica de Lehninger. 5 ed. Porto Alegre (RS): Artmed; 2011. 1304 p.
39. Varoquaux P, Wiley RC. Cambios biológicos y bioquímicos en frutas y hortalizas refrigeradas minimamente processadas. In: Wiley RC, editor. Frutas y hortalizas minimamente procesadas y refrigeradas. Zaragoza (ES): Acribia; 1997. p. 221-262.
40. Hintlian, C.B.; Hotchkiss, J.H. The safety of modified atmosphere packaging: A review. Food Technol. 1986;40(12):70-6.
41. Wiley CR. Alteración microbiológicas y microorganismos patógenos de frutas y hortalizas refrigeradas mínimamente procesadas. In: Wiley RC, editor. Frutas y hortalizas minimamente procesadas y refrigeradas. Zaragoza: Acribia (ES); 1997. p. 263-304.
42. Tyuftin AA, Kerry JP. Review of surface treatment methods for polyamide films for potential application as smart packaging materials: Surface structure, antimicrobial and spectral properties. Food Packag Shelf Life. 2020 Jun;24:100475, doi: 10.1016/j.fpsl.2020.100475.
43. Tomás-Barberán FA, Gil MI, Castaner M, Artés F, Saltveit ME. Effect of selected browning inhibitors on phenolic metabolism in stem tissue of harvested lettuce. J Agric Food Chem. 1997 Mar;45(3):583-9, doi: doi.org/10.1021/jf960478f.
44. Mai F, Glomb MA. Isolation of phenolic compounds from iceberg lettuce and impact on enzymatic browning. J Agric Food Chem. 2013 Mar;61(11):2868-74, doi: 10.1021/jf305182u.
45. Streit NM, Canterle LP, Canto MW, Hecktheuer LHH. As clorofilas. Cienc Rural. 2005 Mai/Jun;35(3):748-55, doi: 10.1590/S0103-84782005000300043.
46. Suresh R, Saravanakumar M, Suganyadevi P. Anthocyanins from indian cassava (*Manihot esculenta* Crantz) and its antioxidant properties. Int J Pharm Pharm Res. 2011 Jun;2(7):1819-28, doi: 10.13040/IJPSR.0975-8232.2(7).1819-28.
47. Vallejo F, Tomás-Barberán F, García-Viguera C. Healthpromoting compounds in broccoli as influenced by refrigerated transport and retail sale period. J Agric Food Chem. 2003 May;51(10):3029-34, doi: 10.1021/jf021065j.
48. Schuh V, Schuh J, Fronza N, Foralosso FB, Verruck S, Vargas Junior A, Silveira SM. Evaluation of the microbiological quality of minimally processed vegetables. Food Sci Technol. 2020 Apr/Jun; 40(2):290-5, doi: 10.1590/fst.38118.
49. Pereira APM, Werle CH, Gonçalves TMV, Hoffmann FL. Identificação e avaliação da resistência antimicrobiana de leveduras em vegetais minimamente processados. Rev Inst Adolfo Lutz. 2011 Jun; 70(2):139-43.

50. Hébraud M, Potier P. Cold shock response and low temperature adaptation in psychrotrophic bacteria. J Mol Microbiol Biotechnol. 1999 Dec;1(2):211-19.
51. Francis GA, Thomas C, O'beirne D. The microbiological safety of minimally processed vegetables. Int J Food Sci Technol. 1999 Dec;34:1-22, doi: 10.1046/j.1365-2621.1999.00253.x.
52. Szabo EA, Scurrah KJ, Burrows JM. Survey for psychrotrophic bacterial pathogens in minimally processed lettuce. Lett Appl Microbiol. 2000 Feb;30:456-60, 10.1046/j.1472-765x.2000.00747.x.
53. Garg N, Churey JJ, Splittstoesser DF. Effect of processing conditions on the microflora of fresh-cut vegetables. J Food Prot. 1990 Dec;53(80):701-3, doi: 10.4315/0362-028X-53.8.701.
54. Fan L, Song J. Microbial quality assessment methods for fresh-cut fruits and vegetables. Stewart Postharvest Rev. 2008 Jun;3(10):1-9, doi: 10.2212/spr.2008.3.10.
55. Barriga MI, Trachy G, Willemot C, Simard RE. Microbial changes in shredded iceberg lettuce stored under controlled atmospheres. J Food Sci. 1991 Nov;56(6):1586-8, doi: 10.1111/j.1365-2621.1991.tb08646.x.
56. Jonghe VD, Coorevits A, Hoorde KV, Messens W, Landschoot AV, Vos PD, Heyndrickx M. Influence of storage conditions on the growth of pseudomonas species in refrigerated raw milk. Appl Environ Microbiol. 2011 Jan;72(2):460-70, doi: 10.1128/AEM.00521-10.
57. Wei O, Wang X, Sun DW, Pu H. Rapid detection and control of psychrotrophic microorganisms in cold storage foods: A review. Trends Food Sci. Technol. 2019 Feb;86:453-64, doi: 10.1016/j.tifs.2019.02.009.
58. Achbani EH, Sadik S, El Kahkahi R, Benbouazza1 A, Mazouz H. First report on *Pseudomonas marginalis* bacterium causing soft rot of onion in Morocco. Atlas J Biol. 2014 Oct;3(2):218-23, doi: 10.5147/ajb.2014.0136.