Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.1590/fst.30817

Optimization of parameters technological to process tucupi and study of product stability

Ana Paula Rocha CAMPOS[1], Rafaella de Andrade MATTIETTO[2], Ana Vânia CARVALHO[2]*

Abstract

Tucupi is a product obtained from the fermentation and cooking of cassava root wastewater which is widely used in the popular cuisine of the North region of Brazil. This study employed an experimental design to select tucupi processing (fermentation and cooking times) as a function of residual cyanidric acid content and assess the stability of the product obtained. Although the design did not generate a predictive mathematical model, the trend plots and physicochemical and sensory tests indicated 24 h of fermentation and 40 min of cooking as process parameters. The tucupi prepared had pH 3.71, titratable acidity of 0.7 g lactic acid/100 mL, 93.91% moisture, 0.5% ashes, 0.52% proteins, 0.24% lipids, 4.83% carbohydrates, total and free cyanide of 6.97 and 1.31 mg HCN/L, respectively. No *Salmonella* spp., coagulase-positive *Staphylococcus*, *Bacillus cereus*, or thermotolerant coliforms were observed, whereas counts of mesophilic aerobic bacteria ($1.3x10^2$ CFU/g) and molds and yeasts ($1.3x10^2$ CFU/g) were low. Over 50 days of follow-up (stored at 10°C), the microbial load remained stable and no significant difference was found in physicochemical characteristics, however, the sensory analysis indicated a decrease in quality at 49 days of storage.

Keywords: *Manihot esculeta*; cyanogenic compound; experimental design; sensory.

Practical Application: Select a proper technological process to preserve tucupi while ensuring safe cyanidric acid and microbiological levels.

1 Introduction

Cassava (*Manihot esculeta* Crantz) is the most important tuberous root in the world, being a source of nutrition for over 500 million people (Ghimire et al., 2015; Uchechukwu-Agua et al., 2015). This crop tolerates dry soils poor in nutrients and is important for food safety and income generation in developing countries, where it is mainly grown by small farmers (Bellotti et al., 2012). However, the presence of toxic cyanogenic glycosides is a limiting factor for the dietary value of cassava (Nambisan, 2011). According to Amorim et al. (2006), cassava is considered the most important cyanogenic species in Brazil, with naturally occurring linamarin and lotaustralin glycosides. The content of those compounds in cassava, in the range of 75 to 1000 mg HCN/kg, depends on plant variety and age, soil conditions, fertilizer use, and climate (Nambisan, 2011; Ngiki et al., 2014).

During root processing, the main component of cassava flour processing effluent is *manipueira*, as cassava wastewater is called in Brazil, from which tucupi is obtained through a fermentation process. According to the Agriculture and Livestock Defense Agency of the State of Pará (ADEPARÁ), tucupi is a product and/or byproduct obtained from cassava root and its varieties using an appropriate technological process (Pará, 2008). Traditionally, tucupi is widely employed in several dishes of Amazonian cuisine, such as duck in tucupi sauce and *tacacá*.

Optimization techniques are often used to solve problems such as minimizing costs and operational time in order to ensure optimal processing and resource application (Pholdee & Bureerat, 2014). Experimental design is considered the most appropriate statistical method to investigate variables influencing processes. It is a structured, organized method used to determine different process input and output factors, involving the definition of the set of experiments, in which all relevant factors are systematically varied (Haridy et al., 2011).

According to Azeredo (2012), the stability of a food product may be preserved for a certain period of time by controlling intrinsic and extrinsic factors that compromise product quality and lead to undesirable sensory and nutritional alterations. The intrinsic factors include, but are not limited to, water activity, pH, chemical composition, and intrinsic microbiological load. Among extrinsic factors, temperature, relative air humidity, exposure to light, and oxygen availability stand out.

Little information is available on tucupi in the technical and scientific literature, which requires optimization studies for a proper technological process aiming at tucupi preservation and safety. Such studies must investigate the influence of fermentation and thermal treatment on residual cyanidric acid contents since this product is obtained from the processing of cassava, which has high levels of that acid.

This research aimed to study tucupi processing parameters while assessing the effect of combining different fermentation and cooking times on the physicochemical processing and cyanide content, besides the stability of the final product.

Received 18 Sept., 2017
Accepted 18 July, 2018
[1]*Programa de Pós-graduação em Ciência e Tecnologia de Alimentos, Instituto de Tecnologia, Universidade Federal do Pará – UFPA, Belém, PA, Brasil*
[2]*Laboratório de Agroindústria, Embrapa Amazônia Oriental, Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA, Belém, PA, Brasil*
*Corresponding author: ana-vania.carvalho@embrapa.br

2 Material and methods

2.1 Material

The cassava wastewater used in the experimental design assays was directly collected at a processing plant and came from the first pressing of cassava roots. Was used 0.5% garlic, 0.5% salt, 0.8% chicory and 0.8% french basil to cook the fermented cassava wastewater which are seasonings usually added to the product and is regulated by current legislation (Pará, 2008).

2.2 Experimental design

A 2^2 central composite rotatable design was carried out with three central points and four axial points for a total of 11 assays (Barros et al., 1995) with the independent variables X_1 = fermentation time (h) and X_2 = cooking time (min) and dependent variables Y_1 = total cyanide content and Y_2 = free cyanide content. After each fermentation time, the liquid was cooked in stainless steel recipients on an industrial stove, under the conditions determined by the experimental design.

After the tucupi obtention process was selected, the final product was prepared to asses its stability.

2.3 Analyse of total and free cyanide

The total (linamarin + acetone cyanohydrin + HCN) and free (HCN) cyanide analyses were carried out according to the enzymatic methodology described and adapted by Essers et al. (1993) with reading in a spectrophotometer (Thermo Scientific, Evolution 300, England) at 605 nm.

2.4 Characterization of the final product

The product obtained according to the parameters assessed in the experimental design was analyzed for its physicochemical, microbiological, sensory, and instrumental color characteristics. Analyses of pH, total titratable acidity, total soluble solids, moisture, ashes, proteins, lipids, and carbohydrates were performed according to the Association of Official Analytical Chemists (1997) and the energy value was calculated according to the United States Department of Agriculture (1963).

The microbiological analyses of total and thermotolerant coliforms, *Salmonella* spp., coagulase-positive *Staphylococcus*, *Bacillus cereus*, mesophilic aerobic bacteria, and molds and yeasts were performed according to the official methods of the American Public Health Association (Vanderzant & Splittstoesser, 1992).

Instrumental color was determined using the CIELAB system for included specular reflectance using a UV-visible colorimeter (HunterLab, ColorQuest XE, USA), with D65 illuminant and 10° observer angle. The parameters determined was the L* (lightness), a* and b* (color coordinates).

The sensory analysis used an acceptance test with a nine-point hedonic scale and assessed color, aroma, flavor, and overall impression (Stone & Sidel, 2004). Was served 20 mL of the sample, in plastic cups coded with three random digits, at 70 °C (± 3 °C). The tucupi is a hot consumed product, therefore, a temperature similar to that used in broth and soup sensory analysis was used. The session was held with 100 untrained tasters of both sexes between 18 and 64 years old who declared to be an usual tucupi consumer. The sensory analysis was approved by the Ethics Committee (CAAE 50781615.7.0000.0018).

2.5 Product stability assay

For the stability assay, the tucupi was placed in 1 L PET containers and stored in a B.O.D. chamber at 10 °C. The stability study was carried out in this temperature, because in commercial establishments tucupi is kept under refrigeration (± 10 °C) during the period of commercialization. The product was analyzed every five days over 50 days of storage regarding physicochemical (pH, titratable acidity, and soluble solids) and microbiological (mesophilic aerobic bacteria, molds and yeasts, and total and thermotolerant coliforms) aspects. The sensory analysis was performed only after the microbiological analysis in order to evaluate whether the tucupi was harmless and ensure the safety of the tasters. The same conditions of the sensory analysis described in item 2.4 were followed, in which the acceptance test with a structured nine-point hedonic scale assessed with 30 fixed tasters, which developed sensorial memory in relation to the product over time, liked or disliked the sample concerning color, aroma, flavor, and overall impression (Stone & Sidel, 2004) along storage. The loss of quality in the tucupi was determined by adopting the value of 5 (neither liked nor disliked) on the nine-point hedonic scale as the cut-off score (Stone & Sidel, 2004). An acceptance index ≥70% was considered good (Dutcosky, 2013).

2.5 Statistical analysis

The statistical analysis was performed using the software Statistica version 7.0 (Statsoft, 2004). The physicochemical and sensory results were assessed using analysis of variance (ANOVA) and Tukey's multiple comparison test ($p \leq 0.05$). The results obtained by the experimental design were analyzed using ANOVA to estimate the statistical parameters and assess whether or not the mathematical model was predictive.

3 Results and discussion

3.1 Tucupi processing

Table 1 shows the results obtained in the assessment of the influence of fermentation and cooking time during tucupi processing on the reduction of total and free cyanide contents in the final product according to the experimental design matrix.

The results that were obtained were submitted to statistical treatments to assess the main and interaction effects to determine the model's regression coefficients and ANOVA for the responses of total and free cyanide (Table 2).

The significant variables, with 95% confidence interval, for total cyanide (Y_1) were cooking time (linear and quadratic) and fermentation time (linear and quadratic) (Figure 1C). The analysis of variance was performed based on those significant variables, which indicated the model's correlation coefficient (R^2) of 0.6256, which is unsatisfactory to generate a predictive mathematical model since only 62% of the variations could be explained by the model.

Table 1. Total and free cyanide contents in the assays carried out.

Assay	Fermentation time in hours (X_1)	Cooking time in minutes (X_2)	Total cyanide (mg HCN/L)	Free cyanide (mg HCN/L)
1	12 (-1)	10 (-1)	8.68 ± 0.31	4.76 ± 0.68
2	12 (-1)	50 (1)	5.37 ± 0.03	4.00 ± 0.35
3	48 (1)	10 (-1)	7.49 ± 0.66	4.64 ± 0.60
4	48 (1)	50 (1)	4.59 ± 0.06	2.41 ± 0.10
5	30 (0)	30 (0)	9.83 ± 0.19	5.36 ± 0.52
6	30 (0)	30 (0)	10.02 ± 0.04	5.38 ± 0.15
7	30 (0)	30 (0)	10.05 ± 0.14	5.24 ± 0.22
8	30 (0)	2 (-1.41)	43.08 ± 0.48	10.03 ± 0.29
9	30 (0)	58 (1.41)	5.12 ± 0.15	3.04 ± 0.15
10	5 (-1.41)	30 (0)	7.92 ± 0.15	4.67 ± 0.69
11	55 (1.41)	30 (0)	6.07 ± 0.24	4.15 ± 0.28

Results are means ± standard deviation.

Table 2. Analysis of variance for the total and free cyanide content response.

Total cyanide						
Factor	SS	df	MS	F_{CAL}	F_{TAB}	R^2
Regression	744.94	4	186.236	2.51	4.53	0.6256
Residue	445.70	6	74.2835			
Lack of fit	445.673	4	111.4182	7931.72	19.25	
Pure error	0.028	2	0.0140			
Total	1190.645	10				
Free cyanide						
Factor	SS	df	MS	F_{CAL}	F_{TAB}	R^2
Regression	17.83	5	3.565203	0.927294	5.05	0.4811
Residue	19.22	5	3.844737			
Lack of fit	19.21219	3	6.404063	1114.16	19.16	
Pure error	0.01150	2	0.005748			
Total	37.04970	10				

SS = sum of squares; df = degree of freedom; MS = mean square; F_{CAL} = F calculated ($MS_{regression}/MS_{residue}$); F_{TAB}= F tabulated.

The $F_{calculated}/F_{tabulated}$ ratio found (0.5541) was well below the expected ratio. According to Box et al. (1978), this ratio must be over 3 for predictive purposes. Confirming the model's lack-of-fit, the value of $F_{calculated}$ (7,931.72) for lack-of-fit was well above $F_{tabulated}$ (19.25), resulting in a ratio of 412, which is equally not ideal to validate the model.

For free cyanide content (Y_2), cooking time (linear and quadratic), fermentation time (linear and quadratic), and the interaction between cooking and fermentation times were significant at 95% confidence (Figure 1D). ANOVA also indicated a low R^2 value, with only 48% of the variability of the response being explained by the model.

The $F_{calculated}/F_{tabulated}$ ratio was also low at 0.1836. Matching the lack-of-fit of the model, the value of $F_{calculated}$ of 1,114.16 was higher than the value of $F_{tabulated}$ of 19.16, yielding a ratio of 58.15, hence the model is not useful or valid for predictive purposes.

It is concluded that no predictive model could be generated for the two parameters studied (reduction in total and free cyanide contents) within the ranges investigated. However, the process trend can be observed in the contour plots obtained and pareto charts of standardized effects (Figure 1).

It can be seen that, when fermentation times are applied with short cooking times (under 10 min), cyanide contents did not decrease (darker area of the plot). However, when cooking time is longer, total and free cyanide contents significantly decreased to safe levels, particularly between 30 and 60 min, regardless of the fermentation time. Thus, Figures 1C and 1D indicated that the cooking time variable had greater influence on the process since longer cooking times lead to greater reductions in cyanide irrespective of the fermentation time.

This way, two processing conditions were selected to be tested so that the influence of the variables studied could be observed on the physicochemical and sensory characteristics of the tucupi obtained. Therefore, since fermentation time had less influence on reducing cyanide, the times of 12 and 24 h, which are commonly used in processing plants, were chosen. The cooking time selected was 40 min, which is within the optimal range (30 to 60 min) for cyanide reduction to safe levels for consumption (Figure 1).

The physicochemical and sensory characteristics of the products derived from the two conditions are shown in Table 3.

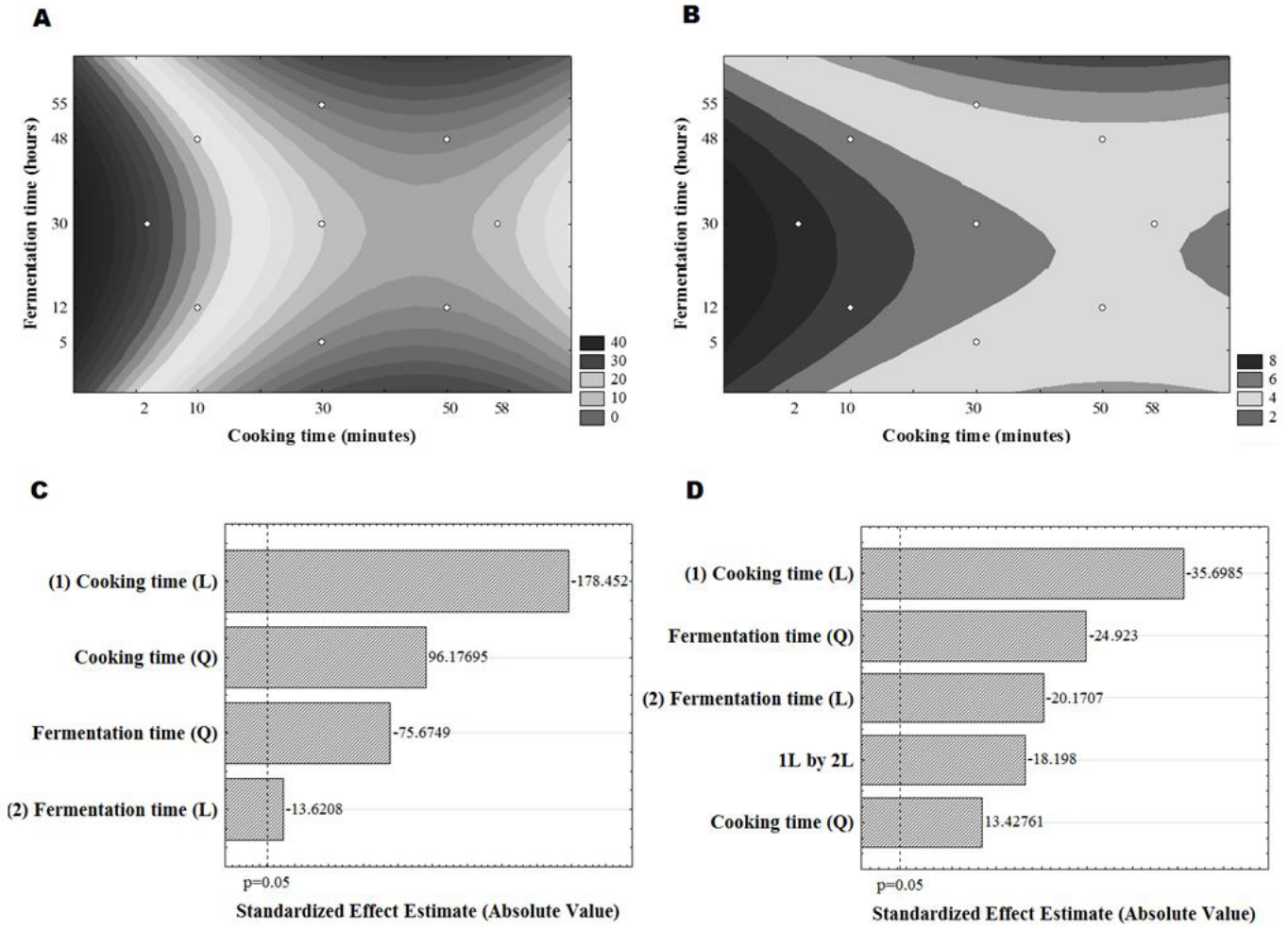

Figure 1. A) Contour plot for the total cyanide content response, B) Contour plot for the free cyanide content response, C) Pareto charts of standardized effects for the total cyanide content response and D) Pareto charts of standardized effects for the free cyanide content response.

Table 3. Physicochemical and sensory characteristics of the tucupi processing experiments with 12 and 24 h of fermentation and 40 min of cooking.

	Characteristics	Fermentation time (hours)	
		12	24
Physicochemical	pH	4.34 ± 0.01[a]	3.97 ± 0.01[b]
	Total titratable acidity (g lactic acid/100 mL)	0.34 ± 0.06[b]	0.70 ± 0.06[a]
	Total cyanide (mg HCN/L)	4.89 ± 0.10[a]	4.30 ± 0.06[b]
	Free cyanide (mg HCN/L)	2.90 ± 0.03[a]	2.13 ± 0.08[b]
Sensory	Color	7.88 ± 1.05[a]	8.06 ± 0.96[a]
	Flavor	6.48 ± 1.86[b]	7.38 ± 1.31[a]
	Aroma	7.37 ± 1.30[a]	7.56 ± 1.10[a]
	Overall impression	6.90 ± 1.53[b]	7.49 ± 1.03[a]

Results are means ± standard deviation. Means followed by the same letter on the same row do not differ statistically at 5% probability.

It was found that titratable acidity increased as a function of the longer fermentation time and, consequently, lower pH values as a consequence of the release of organic acids by fermentation, which causes the characteristic acidification of the product.

The tucupi fermented for 24 h had higher means for all sensory attributes assessed, with a significant difference for the attributes flavor and overall impression compared with tucupi fermented for 12 h. Thus, based on the contour plots and on the physicochemical and sensory characteristics of the tucupi products prepared according to the two complementary experiments carried out, the times of 24 h of fermentation and 40 min of cooking were chosen for the production of the final tucupi.

3.2 Characterization of the final product

The results of the physicochemical characterization of the tucupi processed in the selected conditions are presented in Table 4.

The physicochemical characteristics are in accordance with the Normative Instruction that established the Identity and Quality Standard of Tucupi (Pará, 2008), which sets pH from 3.5 to 4.3 and total titratable acidity between 0.1 and 0.8 g lactic acid/100 mL. However, tucupi is a little-studied regional product, therefore literature references on its physicochemical characterization are rare.

According to the characterization performed, the tucupi had high moisture and low contents of proteins, lipids, and carbohydrates. The values observed for ashes and proteins are in the range found by Chisté et al. (2007), from 0.18 to 1.08% ashes and 0.33 to 0.66% proteins. Those authors found slightly higher moisture values (94.64 to 97.46%) than in the present study. The tucupi prepared had energy value of 23.56 kcal/100 g, being considered a low-calorie food according to the Brazilian legislation (Brasil, 2012). This value is in the same range observed in the label of tucupi products marketed in the city of Belém, Pará, Brazil (on average, 22 kcal/100 g).

The instrumental color analysis of the tucupi showed values of -1.85 and 37.35 for a* and b*, respectively, indicating yellowish color, and 52.02 for L*, indicating the bright yellow color characteristic of the product, which complies with the legislation (Pará, 2008).

The microbiological characteristics of the tucupi meet the requirements of Normative Instruction no. 001/2008 (Pará, 2008), which establishes an absence of *Salmonella* spp., coagulase-positive *Staphylococcus*, and *Bacillus cereus* and MPN<3/mL for total and thermotolerant coliforms. Moreover, the tucupi produced in the selected conditions had low counts of mesophilic aerobic bacteria and molds and yeasts, which indicate the product's hygienic quality. Thus, the tucupi prepared is good for human consumption, with processing under satisfactory hygienic-sanitary conditions that ensure a safe food product.

Table 4. Physicochemical and microbiological characterization of the tucupi processed in the selected conditions.

Analyses		Results
Moisture (% d.b.)		93.91 ± 0.09
Ashes (% d.b.)		0.50 ± 0.01
Proteins (% d.b.)		0.52 ± 0.01
Lipids (% d.b.)		0.24 ± 0.01
Carbohydrates (% d.b.)		4.83 ± 0.11
pH		3.71 ± 0.00
Total titratable acidity (g lactic acid/100 mL)		0.65 ± 0.00
Total soluble solids (°Brix)		6.50 ± 0.06
Energy value (kcal/100 g)		23.56 ± 0.01
Total cyanide (mg HCN/L)		6.97 ± 0.10
Free cyanide (mg HCN/L)		1.31 ± 0.05
Color	L*	52.02 ± 0.09
	a*	-1.85 ± 0.04
	b*	37.35 ± 0.27
Total and thermotolerant coliforms (MPN/mL)		<3
Salmonella spp.		Absence in 25 g
Coagulase-positive *Staphylococcus*		Absent
Bacillus cereus		Absent
Mesophilic aerobic bacteria (CFU/g)		1.3×10^2 (est.)
Molds and yeasts (CFU/g)		1.3×10^2 (est.)

Results are means ± standard deviation.

The DL_{50} threshold set for cassava linamarin is still debatable (Cereda & Lopes, 2003). However, the lethal dose is estimated at approximately 10 mg/kg weight (Cagnon et al., 2002). This way, the total and free cyanide contents found are considered safe for consumption.

3.3 Product stability assay

Table 5 presents the results of the physicochemical analyses performed during refrigerated storage of the tucupi at 10 °C for 50 days.

Means followed by the same letter on the same column do not differ statistically at 5% probability.

Statistical differences, with variation among the times of measurement, were found over storage for pH, soluble solids, and titratable acidity. However, by the end of 50 days, although the values observed are statistically different, such differences are subtle and have no technological importance that affects product stability. Throughout the storage period, the values of pH and titratable acidity remained in accordance with the current legislation by Pará (2008), which establishes pH between 3.5 and 4.3 and total titratable acidity between 0.1 and 0.8 lactic acid/100 mL.

The values observed for soluble solids remained virtually stable during storage, indicating that the tucupi showed no signs of fermentation over the 50 days since the soluble solids contents did not decrease. Overall, it can be said that the product processed under the selected conditions showed physicochemical stability over the follow-up period.

No significant differences were found over the storage period for total cyanide and free cyanide, with averages from 6.61 mg HCN/L and 1.21 mg HCN/L, respectively. Therefore, it is considered that the total and free cyanide levels of the tucupi were safe for consumption. Higher values of 37.1 mg HCN/L for total cyanide and 8.9 mg HCN/L for free cyanide were reported by Chisté & Cohen (2011) for tucupi produced with 72 h of fermentation and 10 min of cooking.

The counts of total and thermotolerant coliforms, mesophilic aerobic bacteria, and molds and yeasts obtained for the tucupi over the 50 days of storage are presented in Table 6.

The Agriculture and Livestock Defense Agency of the State of Pará (Pará, 2008) establishes the maximum limit for thermotolerant coliforms at MPN<3/mL. Therefore, the values of total and thermotolerant coliforms found in the present study meet the current legislation.

Although the legislation does not set standards for mesophilic bacteria or molds and yeasts, counts were performed of those microorganisms since they may indicate the quality of a food

product and its shelf life. In the beginning of the assay, the population levels had estimated values with low counts both for mesophilic bacteria and molds and yeasts. On the fifteenth day of storage, the microbial counts increased at a logarithmic rate, with the final results being expressed as 10^3 CFU/g, which remained constant until the end of storage for both microorganisms.

It is noteworthy that, although the product remained microbiologically stable over 50 days of refrigerated storage, the product's stability assay was interrupted due to the sensory quality depreciation of tucupi evidenced by the acceptance tests (Table 7).

Means followed by the same letter on the same column do not differ statistically at 5% probability.

Table 5. Physicochemical characterization of tucupi over 50 days of storage under refrigeration.

Time (days)	pH	Total titratable acidity (g lactic acid/100 mL)	Soluble solids (°Brix)	Total cyanide (HCN/100 mL)	Free cyanide (HCN/100 mL)
0	3.71 ± 0.00^{cd}	0.65 ± 0.00^{abc}	6.5 ± 0.06^{c}	6.97 ± 0.10^{a}	1.31 ± 0.05^{a}
5	3.51 ± 0.01^{g}	0.64 ± 0.01^{bcd}	6.6 ± 0.06^{c}	6.99 ± 0.39^{a}	1.21 ± 0.01^{a}
10	3.73 ± 0.01^{bc}	0.65 ± 0.00^{abc}	6.3 ± 0.04^{ef}	6.44 ± 0.40^{a}	1.18 ± 0.02^{a}
15	3.79 ± 0.01^{a}	0.65 ± 0.00^{ab}	6.3 ± 0.05^{f}	6.58 ± 0.27^{a}	1.24 ± 0.03^{a}
20	3.58 ± 0.01^{f}	0.63 ± 0.01^{d}	6.9 ± 0.05^{a}	6.65 ± 0.72^{a}	1.19 ± 0.01^{a}
25	3.75 ± 0.01^{b}	0.64 ± 0.01^{cd}	6.5 ± 0.05^{cd}	6.43 ± 0.64^{a}	1.23 ± 0.06^{a}
30	3.68 ± 0.00^{d}	0.66 ± 0.01^{a}	6.9 ± 0.05^{a}	6.48 ± 0.11^{a}	1.20 ± 0.01^{a}
35	3.62 ± 0.01^{e}	0.64 ± 0.01^{bcd}	6.7 ± 0.06^{b}	6.57 ± 0.27^{a}	1.16 ± 0.02^{a}
40	3.58 ± 0.01^{f}	0.64 ± 0.01^{bcd}	6.5 ± 0.06^{c}	6.35 ± 0.28^{a}	1.25 ± 0.06^{a}
45	3.57 ± 0.01^{f}	0.64 ± 0.00^{bcd}	6.5 ± 0.04^{c}	6.69 ± 0.81^{a}	1.17 ± 0.06^{a}
50	3.74 ± 0.00^{bc}	0.63 ± 0.00^{d}	6.4 ± 0.05^{de}	6.59 ± 0.80^{a}	1.19 ± 0.01^{a}

Results are means ± standard deviation. Means in the same column with the same letter are not statistically different at 95% significance by the Tukey test.

Table 6. Counts of total and thermotolerant coliforms, mesophilic aerobic bacteria, and molds and yeasts over tucupi storage.

Time (days)	Total and thermotolerant coliforms (MPN/mL)	Mesophilic aerobic bacteria (CFU/g)	Molds and yeasts (CFU/g)
0	<3	1.3×10^2 (est.)	1.3×10^2 (est.)
5	<3	6.7×10^2	1.4×10^2
10	<3	4×10^2	7.1×10^2
15	<3	1.8×10^3	1.2×10^3
20	<3	4.3×10^3	1.6×10^3
25	<3	3.3×10^3	1.8×10^3
30	<3	2.3×10^3	1.6×10^3
35	<3	1.4×10^3	2.0×10^3
40	<3	1.0×10^3	2.0×10^3
45	<3	1.6×10^3	2.3×10^3
50	<3	1.1×10^3	2.2×10^3

Mean values of two replicates in two randomly selected samples.

Table 7. Mean results of sensory parameters attributed to tucupi and percentage acceptance indices over the refrigerated storage period.

Time (days)	Color	Flavor	Aroma	Overall impression
0	7.96 ± 0.99 (88.44%)^{a}	7.55 ± 1.30 (83.89%)^{ab}	8.07 ± 1.11 (89.67%)^{a}	7.77 ± 1.04 (86.33%)^{a}
3	8.00 ± 0.69 (88.89%)^{a}	7.63 ± 0.89 (84.81%)^{ab}	7.90 ± 0.88 (87.78%)^{ab}	7.70 ± 0.88 (85.56%)^{ab}
10	8.03 ± 0.71 (89.26%)^{a}	8.13 ± 0.86 (90.37%)^{a}	7.83 ± 1.34 (87.04%)^{ab}	8.07 ± 0.74 (89.63%)^{a}
15	8.13 ± 0.90 (90.37%)^{a}	7.63 ± 1.00 (84.81%)^{ab}	7.83 ± 0.83 (87.04%)^{ab}	7.57 ± 1.00 (84.07%)^{ab}
20	8.17 ± 0.79 (90.74%)^{a}	7.23 ± 1.33 (80.37%)^{abc}	7.47 ± 1.43 (82.96%)^{ab}	7.37 ± 1.30 (81.85%)^{abc}
24	8.13 ± 0.77 (90.37%)^{a}	7.50 ± 1.16 (83.33%)^{abc}	7.70 ± 1.12 (85.56%)^{ab}	7.50 ± 1.13 (83.33%)^{abc}
30	7.83 ± 0.95 (87.04%)^{a}	7.20 ± 1.51 (80.00%)^{abc}	7.63 ± 1.07 (84.81%)^{ab}	7.03 ± 1.42 (78.15%)^{abc}
35	7.73 ± 0.83 (85.93%)^{a}	6.63 ± 1.65 (73.70%)^{bcd}	7.37 ± 1.25 (81.85%)^{ab}	6.53 ± 1.63 (72.59%)^{bcd}
44	8.10 ± 0.76 (90.00%)^{a}	6.30 ± 1.23 (70.00%)^{cde}	7.33 ± 1.29 (81.48%)^{ab}	6.37 ± 1.40 (70.74%)^{cd}
49	7.57 ± 1.10 (84.07%)^{a}	5.90 ± 1.93 (66.67%)^{de}	7.00 ± 1.50 (77.78%)^{b}	6.27 ± 15.89 (70.37%)^{cd}
56	6.60 ± 1.71 (75.56%)^{b}	5.27 ± 2.42 (65.19%)^{e}	5.84 ± 2.01 (67.78%)^{c}	5.30 ± 2.36 (63.70%)^{d}

Results are means ± standard deviation. Means in the same column with the same letter are not statistically different at 95% significance by the Tukey test.

Can be observed that the tasters did not notice differences in color among the samples up until 49 days of storage. However, at 56 days, the mean score attributed by the tasters significantly dropped. The scores of flavor, aroma, and overall impression significantly varied over the storage period, with decreasing taster acceptance.

A similar behavior is observed for all four attributes assessed, i.e., a decrease in the acceptance index of tucupi by the tasters over the storage period. Reductions by 14.57%, 22.30%, 24.41%, and 26.21% were observed between the initial and final times of the storage period for the attributes color, flavor, aroma, and overall impression, respectively.

The sensory tests were a decisive factor in determining of the maximum acceptance time for the tucupi. As previously mentioned, the sensory stability of the product was determined by adopting the value of 5 (neither liked nor disliked) on the nine-point hedonic scale as a cut-off score. Thus, up until 49 days of storage, the tucupi was proper for consumption from a sensory standpoint, which is a good result since the storage time of tucupi marketed under refrigeration is usually 30 days according to the manufacturers.

4 Conclusions

Although the research understands that cassava can be considered a raw material with significant physicochemical variability, it is believed that the parameters defined at 24 h of fermentation and 40 min of cooking are able to fit most processing plants to obtain safe tucupi. During the stability assay of the selected product, its physicochemical and microbiological characteristics remained stable, thus ensuring safe levels for human consumption. The sensory analysis showed the maximum period for good acceptance of the tucupi obtained in this study was 49 days.

Acknowledgements

The authors are grateful to the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq - Brazil) for the financial support (process number 407764/2013-5) and to Propesp/UFPA for the financial support in translating the paper.

References

Amorim, S. L., Medeiros, R. M. T., & Riet-Correa, F. (2006). Intoxications by cyanogenic plants in Brazil. *Ciência Animal*, 16(1), 17-26.

Association of Official Analytical Chemists – AOAC. (1997). *Official methods of analysis* (16. ed.). Gaitherburg: AOAC.

Azeredo, H. M. C. (2012). *Fundamentos de estabilidade de alimentos* (2. ed.). Brasília: Embrapa.

Barros, B. No., Scarminio, I. S., & Bruns, R. E. (1995). *Planejamento e otimização de experimentos* (2. ed.). Campinas: UNICAMP.

Bellotti, A., Campo, B. V. H., & Hyman, G. (2012). Cassava production and pest management: present and potential threats in a changing environment. *Tropical Plant Biology*, 5(1), 39-72. http://dx.doi.org/10.1007/s12042-011-9091-4.

Box, G. E. P., Hunter, W. G., & Hunter, J. S. (1978). *Statistics for experimenters: an introduction designs, data analysis and model building*. New York: John Wiley & Sons Inc.

Brasil, Ministério da Saúde. (2012, Novembro 12). Regulamento técnico sobre informação nutricional complementar (RDC n. 54, de 12 de nov. de 2012). *Diário Oficial [da] República Federativa do Brasil*.

Cagnon, J. R., Cereda, M. P., & Pantarotto, S. (2002). Cultura de tuberosas amiláceas latino-americanas. *Fundação Cargill*, 2(1), 13-37.

Cereda, M. P., & Lopes, A. M. (2003). Determinação do potencial de intoxicação em ratos, de linamarina extraída de mandioca. In *5º Simpósio Latino Americano de Ciência de Alimentos* (CD-ROM). Campinas: UNICAMP.

Chisté, R. C., & Cohen, K. O. (2011). Total and free cyanide content in the processing stages of tucupi. *Revista do Instituto Adolfo Lutz*, 70(1), 41-46.

Chisté, R. C., Cohen, K. O., & Oliveira, S. S. (2007). Study of the physical-chemical properties of tucupi. *Food Science and Technology*, 27(3), 437-440.

Dutcosky, S. D. (2013). *Análise sensorial de alimentos* (4. ed.). Curitiba: Editora Champagnat.

Essers, S. A. J. A., Bosveld, M., Van Grift, R. M. D., & Voragen, A. G. J. (1993). Studies on the quantification of specific cyanogenes in cassava products and introduction of a new chromogen. *Journal of the Science of Food and Agriculture*, 63(1), 287-296. http://dx.doi.org/10.1002/jsfa.2740630305.

Ghimire, A., Sen, R., & Annachhatre, A. P. (2015). Biosolid management options in Cassava starch industries of Thailand: Present practice and future possibilities. *Procedia Chemistry*, 14(1), 66-75. http://dx.doi.org/10.1016/j.proche.2015.03.011.

Haridy, S., Gouda, S. A., & Wu, Z. (2011). An integrated framework of statistical process control and design of experiments for optimizing wire electrochemical turning 85 process. *International Journal of Advanced Manufacturing Technology*, 53(1), 191-207. http://dx.doi.org/10.1007/s00170-010-2828-7.

Nambisan, B. (2011). Strategies for elimination of cyanogens from cassava for reducing toxicity and improving food safety. *Food and Chemical Toxicology*, 49(3), 690-693. http://dx.doi.org/10.1016/j.fct.2010.10.035. PMid:21074593.

Ngiki, Y. U., Igwebuike, J. U., & Moruppa, S. M. (2014). Utilization of cassava products for poultry feeding: a review. *International Journal of Science and Technology*, 2(6), 48-59.

Pará. Agência de Defesa Agropecuária do Estado do Pará – ADEPARÁ. (2008, Junho 24). Regulamento técnico do padrão de identidade e qualidade do tucupi para comercialização (Instrução Normativa n. 001/2008, de 24 de junho de 2008). *Diário Oficial do Estado do Pará*. cad. 8, p. 1.

Pholdee, N., & Bureerat, S. (2014). Comparative performance of metaheuristic algorithms for mass minimisation of trusses with dynamic constraints. *Advances in Engineering Software*, 75(1), 1-13. http://dx.doi.org/10.1016/j.advengsoft.2014.04.005.

Statsoft. (2004). *Statistica: data analysis software system, version 7*. Tulsa: Statsoft. Retrieved from www.statsoft.com

Stone, H., & Sidel, J. L. (2004). *Sensory evaluation practices* (3. ed.). New York: Academic Press.

Uchechukwu-Agua, A. D., Caleb, O. J., & Opara, U. L. (2015). Postharvest handling and storage of fresh cassava root and products: a review. *Food and Bioprocess Technology*, 8(1), 729-748. http://dx.doi.org/10.1007/s11947-015-1478-z.

United States Department of Agriculture – USDA. (1963). *Composition of foods: raw, processed, prepared* (Agricultural Handbook; 8). Washington: USDA.

Vanderzant, T., & Splittstoesser, E. F. (1992). *Compendium of methods for the microbiological examination of foods* (3th ed.). Washington: American Public Health Association – APHA.