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Food Research International

Influence of spontaneous fermentation of *manipueira* on bioactive amine and carotenoid profiles during *tucupi* production

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ABSTRACT

Tucupi is a kind of broth that is common to the Amazonian region of Brazil, which is produced after the spontaneous fermentation and boiling of *manipueira*, a liquid by-product extracted from cassava roots during cassava flour production. The bioactive amines formation and their alterations during *tucupi* processing were investigated for the first time. In addition, the physicochemical changes, instrumental color parameters and carotenoid profile were monitored during *tucupi* production. Regarding the changes on the profile of carotenoids, all-*trans*-β-carotene was the major carotenoid, followed by its 9- and 13-*cis* isomers, and this profile was not altered throughout the process. Spermidine (polyamine), putrescine, tyramine and histamine (biogenic amines) were the bioactive amines identified during the *tucupi* production process, but at low levels (0.76–7.24 mg/L). Principal Component Analysis suggested three distinct stages during *tucupi* production: the first stage was characterized by the *manipueira* up to 12 h of fermentation with the highest values of pH, total and reducing sugars, and total starch; the second stage refers to the end of the fermentation process (16 to 24 h), in which biogenic amines (histamine and tyramine) were produced; and the third stage was characterized by the final product (*tucupi*), with the highest values of total acidity, soluble solids, polyamines and tyramine. Therefore, the results suggest the need of an accurate control of the fermentation of the *manipueira* to avoid or minimize the formation of biogenic amines.

1. Introduction

Cassava (*Manihot esculenta* Crantz) is the fifth most important basic crop in the world, with a production of about 285 million tons of unprocessed roots per year (FAOSTAT, 2017). According to the Brazilian Institute of Geography and Statistics (IBGE, 2017), the Brazilian cassava production in 2017 was 21 million tons, mostly from the Northern region, where it is widely used in the diet, mainly as cassava flour. Cassava is also used in the production of several typical foods all over Brazil. Moreover, in the Amazonia region, a food product called *tucupi* is a highly appreciated broth and widely used as an ingredient of several Brazilian Amazonian traditional dishes. Nowadays, it has been used in signature dishes from great chefs worldwide due to its exotic sensorial characteristics.

Tucupi is a by-product obtained from cassava flour processing. The roots are crushed and pressed, resulting in solids for flour production

and a liquid fraction called *manipueira* is obtained for *tucupi* production. After undergoing spontaneous fermentation at ambient conditions and cooking, the *manipueira* is transformed into *tucupi* (Chisté, Cohen, & Oliveira, 2007). However, despite the wide use of *tucupi*, its processing occurs in a rudimentary way, with a lack of technological information regarding the main stages of production from fermentation of *manipueira* to the final product.

There are scarce studies on *tucupi* quality. The main information available is related to changes in pH, total acidity, soluble solids, microbial quality and total and free hydrocyanic acid (HCN) (Campos, Carmo, Carvalho, & Mattietto, 2016; Campos, Carmo, Carvalho, & Mattietto, 2017; Campos, Carvalho, & Mattietto, 2016; Chisté et al., 2007; Chisté & Cohen, 2011). The concern associated with HCN relies on the fact that cassava roots contain cyanogenic glycosides with potential to release HCN during processing (Shigaki, 2015). In fact, *tucupi* was reported to contain total and free cyanide contents at levels varying

from 55.58 to 157.17 and 9.47 to 46.86 mg HCN/kg, respectively (Chisté et al., 2007). Campos, Carmo, Carvalho, & Mattietto (2016), Campos, Carvalho, and Mattietto (2016)) determined a range below the values reported by Chisté et al. (2007), but still there is no standardization for *tucupi* commercialization. These studies stressed out the importance of controlling *tucupi* production in order to warrant its quality and safety.

In food products, especially fermented ones, the profile and levels of bioactive amines may be related to the quality and safety during food production and storage (Brito, Chisté, Pena, Glória, & Lopes, 2017; EFSA, 2011; Martuscelli, Arfelli, Manetta, & Suzzi, 2013). It is well known that bioactive amines exhibit relevant roles in human health. The term bioactive amines or biologically active amines refer to polyamines and also biogenic amines. The polyamines spermine and spermidine are involved in several cellular functions including DNA stabilization, regulation of gene expression, channel functions and cell proliferation. Therefore, they play important roles in the immune system, digestive tract and neuroprotection (Ramani, Bandt, & Cynober, 2014; Sharma, Kumar, & Deshmukh, 2018). The biogenic amines are produced and accumulate during fermentation processes, mostly through amino acid decarboxylation by microbial enzymes. They are usually associated with neuro- and vaso- activities and can result in adverse effects on human health. Histamine and tyramine are the amines most commonly associated with food intoxications (EFSA, 2011; Gardini, Özogul, Suzzi, Tabanelli, & Özogul, 2016; Glória, 2005; Ordonez, Callejon, Morales, & Garcia-Parrilla, 2013).

Some studies have shown the predominance of lactic bacteria in cassava-based fermented products (Lacerda et al., 2005; Oguntoyinbo & Dodd, 2010), and these microorganisms are able to produce biogenic amines (Linares, Martín, Ladero, & Alvarez, 2011). *Tucupi* is a suitable medium for the production of biogenic amines, therefore, the levels and contents of amines can be used as quality and safety indexes (Martuscelli et al., 2013). No information was found regarding amines in *tucupi*. The knowledge of the levels of amines in *tucupi* is needed from the functional, toxicological and quality control point of views.

Scarce information is also available regarding the functional properties of *tucupi*. Since yellow-colored cassava roots are used in *tucupi* production, it is likely that carotenoids are present. The major carotenoids found in cassava roots are all-*trans*-β-carotene and its 13-*cis* and 9-*cis* isomers (Failla et al., 2012; Kimura, Kobori, Rodriguez-amaya, & Nestel, 2007) which are pro-vitamin A carotenoids. Ingestion of carotenoid-containing foods has been associated with low incidence of cancer, cardiovascular disease among other chronicle degenerative diseases (Koko, Kouame, Anvoh, Amani, & Assidjo, 2014; Rao & Rao, 2007). Therefore, the consumption of cassava roots or derived products may represent low-cost access to this important vitamin for economically disadvantaged populations. Nevertheless, as far as our knowledge is concerned, the influence of fermentation of *manipueira* during *tucupi* production was not yet reported in the literature.

Due to the lack of information on the levels of carotenoids and bioactive amines in *tucupi*, despite their relevance as indexes of quality and safety, the objective of this study was to investigate the occurrence and profile of these compounds during *manipueira* fermentation and *tucupi* production. The results were evaluated by two multivariate exploratory techniques, aiming the characterization of the main stages of *tucupi* production.

2. Material and methods

2.1. Chemicals

Bioactive amine standards – spermidine trihydrochloride, putrescine dihydrochloride, agmatine sulfate, cadaverine dihydrochloride, 5-hydroxytryptamine - serotonin, histamine dihydrochloride, tyramine hydrochloride, 2-phenylethylamine hydrochloride and tryptamine, as well as all-*trans*-β-carotene, methanol and *tert*-butyl methyl ether

(HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Petroleum ether, ethyl ether, and acetone were of analytical grade (Synth, São Paulo, SP, Brazil). Ultrapure water was from Milli-Q System (Millipore Corp., Milford, MA, USA). Mobile phases and samples for HPLC analysis were filtered through 0.45 μm membranes from Millipore Corp. (Milford, MA, USA) and Filtrilo (Colombo, PR, Brazil), respectively.

2.2. Cassava roots

Cassava roots (*Manihot esculenta* Crantz) (60 kg) of yellow pulp varieties, commonly used in the production of cassava flour, known as "brava" (above 100 mg HCN/kg of fresh root without bark) (Cagnon, Cereda, & Pantarotto, 2002) were purchased in triplicate ($n = 3$) in the *Ver-O-Peso* market located in Belém, Pará, Brazil (latitude 01°27′21″ and longitude 48°30′16″). These roots were collected and processed in April, May and June (20 kg, each) of 2016.

2.3. Tucupi production

The roots were washed, peeled with stainless steel knives and washed again. The peeled roots were ground by means of grinders and the obtained mass (15 kg for batch) was manually pressed for the extraction of the liquid fraction – *manipueira* (5 L). These steps (peeling, grinding and pressing) were carried out at the own place of the purchase of samples, using the same equipment and processing conditions of *tucupi* producers of the *Ver-o-Peso* market. The extracted *manipueira* was transported to the laboratory, in plastic containers (5 L) at ambient temperature. Sample transportation from the Market to the laboratory took, on average, 30 min.

In the laboratory, the *manipueira* (5 L) was placed in a stainless steel container, that was previously disinfected with sodium hypochlorite solution (100 mg/L), washed again with potable water. The *manipueira* was then submitted to a spontaneous fermentation process by incubation at 30 °C (ambient temperature of the region) during 24 h in an oven (Q-316 M5, Quimis, Brazil). During fermentation, aliquots of 250 mL were withdrawn for the analytical determinations, at 4 h-intervals, until the end of the *tucupi* production. After the fermentation, the *manipueira* was boiled at ≈100 °C for 10 min to obtain *tucupi*. The definition of the process conditions for *tucupi* production were based on the study by Chisté and Cohen (2011). *Tucupi* was obtained from the three different *manipueira* batches ($n = 3$).

All the analyses were carried out in triplicate (n = 3) immediately after sample collection at the nine sampling times along *tucupi* production: ground cassava (1st sample), in the newly extracted *manipueira* (2nd sample) and *manipueira* after 4, 8, 12, 16, 20 and 24 h of fermentation (3rd to 8th samples); and the fermented product after boiling (*tucupi*) (9th sample).

2.4. Analytical determinations

2.4.1. Physicochemical characterization during tucupi production

The moisture content (g/100 g) was determined by oven drying at 105 °C, total acidity was determined by titration with 0.1 N NaOH (results expressed as g/100 mL of lactic acid), pH values were obtained by direct reading with a potentiometer, total starch (g/100 g), and the reducing and total sugars (g/100 g) were determined by the Lane-Eynon method and all these methods were carried out according to the Association of Official Analytical Chemists (AOAC, 1997). The soluble solids contents were determined by direct measurement in a digital refractometer (Q767B0, Quimis, Brazil), and the values were expressed as °Brix. The color parameters were measured by a colorimeter (CR-400, Konica Minolta Sensing, Japan) with the following parameters: diffuse illumination/0° viewing geometry (specular component included) and light source D65. The values obtained in the CIELAB system were $L*$ (lightness), the chromatic coordinates $a*$ (red–green

component) and $b*$ (yellow–blue component). The values of $C_{ab}*$ (chroma) and $h_{ab}°$ (hue angle) were calculated according to Eqs. (1) and (2).

$$C_{ab}^* = \sqrt{(a^*)^2 + (b^*)^2} \tag{1}$$

$$h_{ab}° = 180 + \arctan\left(\frac{b^*}{a^*}\right) \tag{2}$$

when $[-a^*, +b^*]$ (quadrant II).

2.4.2. Analysis of carotenoids

The extraction of carotenoids from cassava (10 g) and *tucupi* (10 mL) was carried out according to Rodriguez-Amaya and Kimura (2004). After exhaustive extraction, an aliquot of the carotenoid extract was evaporated under N_2 flow, dissolved in methyl *tert*-butyl ether (MTBE) and used for HPLC analysis.

HPLC-DAD analysis was performed in an Agilent HPLC (Agilent 1260 Infinity model, Santa Clara, CA, USA) equipped with a quaternary pump (G1311C), a Rheodyne injection valve with a 20 μL loop, an oven (G1316A) and a DAD detector (G1328C). The carotenoids were separated on a C30 YMC column (5 μm, 250 × 4.6 mm) using as mobile phase a linear gradient of methanol/MTBE from 95:5 to 70:30 in 30 min, followed by 50:50 in 20 min (Chisté & Mercadante, 2012). The flow rate was 0.9 mL/min, and the column oven was set at 29 °C. The carotenoids were identified according to the elution order on C30 column, and co-chromatography with standards. The carotenoids were quantified at 450 nm, using external six-point analytical curves (in duplicate) for all-*trans*-β-carotene (1.56–50 μg/mL) ($r^2 \geq 0.98$). The contents of carotenoids ($n = 3$, wet basis) were expressed as μg/mL of *manipueira* and *tucupi*.

The National Academy of Sciences, Institute of Medicine (NAS-IOM) conversion factor was used to calculate the vitamin A value, with 12 μg of dietary all-*trans*-β-carotene corresponding to 1 μg of retinol activity equivalent (RAE), and the activity used was 100% for all-*trans*-β-carotene and 50% for each *cis* isomer of β-carotene (NAS-IOM, 2001).

2.4.3. Bioactive amines determination

The bioactive amines were extracted from cassava samples (5 g) with 7 mL of 5% trichloroacetic acid (TCA) mixed in a shaker for 5 min followed by centrifugation at 11,180 x g at 4 °C for 10 min. Extraction was repeated twice and the supernatants were combined in a volumetric flask and filtered through qualitative paper and 0.45 μm membrane (Brito et al., 2017). *Manipueira* and *tucupi* were centrifuged and filtered through qualitative paper and 0.45 μm membrane. Nine free bioactive amines standards (spermidine, putrescine, agmatine, cadaverine, serotonin, histamine, tyramine, tryptamine, and

phenylethylamine) were determined by ion-pair reverse phase HPLC. A Shimadzu LC-10 CE with SIL-10 CE VP automatic injector (Shimadzu, Kyoto, Japan) was used, and the amines were separated using a Novapak C18 column (3.9 × 300 mm, 4 μm, 60 Å, Waters, MA, USA) and a gradient elution of 0.2 M sodium acetate and 15 mM sodium octanesulfonate with pH adjusted to 4.9 (mobile phase A) and acetonitrile (mobile phase B) (Brito et al., 2017). The bioactive amines were identified by comparison of retention times and co-elution with standards. The quantification was carried out by fluorimetry (340 and 445 nm of excitation and emission, respectively), after post-column derivatization with *o*-phthalaldehyde, by using analytical curves (measurements in duplicate) for each amine ($r^2 \geq 0.99$). The contents of bioactive amines ($n = 3$, wet basis) were expressed as mg/100 g of sample.

2.5. Statistical analysis

All the results were submitted to analysis of variance using one-way ANOVA, and the means were compared by Tukey's test at 95% significance, using Statistica 7.0 software. Two multivariate exploratory techniques, Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA), were applied to evaluate the production of *tucupi* using Statistica 7.0. For PCA, pH, total acidity, total sugar, reducing sugars, total starch, chroma ($C_{ab}*$), $L*$, $h_{ab}°$, carotenoids and bioactive amines were used as active variables in the derivation of the principal components, and the supplementary variable (chromatic coordinates $a*$ and $b*$) was projected onto the factor space. PCA analysis was performed using the covariance matrix. For HCA, the hierarchical tree was obtained considering the same active variables applied to PCA and the processing steps of *tucupi* production were joined by Ward's method as the linkage rule, considering the Euclidian distances as the coefficient of similarity.

3. Results and discussion

3.1. Changes on the physicochemical characteristics

The cassava roots used for extraction of *manipueira* in the three experiments of *tucupi* production presented similar chemical composition to that reported in the literature (Koko et al., 2014; Ladeira, Souza, & Pena, 2013) regarding the following: moisture content (70.68 ± 3.38 g/100 g), pH (5.5 ± 0.3), total acidity (0.24 ± 0.05 g/100 g lactic acidic), reducing sugars (0.93 ± 0.41 g/100 g), total sugars (2.20 ± 1.13 g/100 g), soluble solids (7.24 ± 0.14 °Brix), total starch (20.42 ± 5.60 g/100 g). The variations observed on these parameters are acceptable, and depend on the degree of root maturation, climatic conditions, cultivation practices, among other factors

Table 1

Physicochemical characterization and color parameters during *tucupi* production.

Properties	Manipueira	Fermentation time (h)*						After boiling (Tucupi)
		4	8	12	16	20	24	
pH	6.01 ± 0.28[a]	5.86 ± 0.22[a]	5.20 ± 0.12[b]	4.50 ± 0.06[c]	4.20 ± 0.06[cd]	4.07 ± 0.07[d]	3.97 ± 0.07[d]	4.10 ± 0.11[d]
Total acidity (g lactic acid/100 mL)	0.02 ± 0.01[b]	0.02 ± 0.01[b]	0.28 ± 0.04[b]	0.68 ± 0.24[ab]	0.89 ± 0.35[ab]	1.16 ± 0.49[a]	1.33 ± 0.39[a]	1.27 ± 0.42[a]
Soluble solids (°Brix)	6.90 ± 1.40[b]	6.82 ± 1.49[b]	6.61 ± 1.26[b]	6.31 ± 1.28[b]	6.00 ± 1.31[b]	6.10 ± 1.19[b]	5.94 ± 1.26[b]	9.34 ± 3.64[a]
Total sugars (g/100 g)	3.58 ± 0.37[a]	3.50 ± 0.35[a]	3.50 ± 0.12[a]	2.70 ± 0.24[ab]	2.08 ± 0.39[bc]	1.62 ± 0.25[c]	1.29 ± 0.21[c]	1.23 ± 0.55[c]
Reducing sugars (g/100 g)	1.17 ± 0.22[ab]	1.37 ± 0.28[ab]	1.46 ± 0.31[ab]	1.63 ± 0.32[a]	1.55 ± 0.16[ab]	1.34 ± 0.10[ab]	0.99 ± 0.18[b]	1.34 ± 0.06[ab]
Total starch (g/100 g)	13.05 ± 1.86[a]	2.92 ± 0.54[b]	2.34 ± 1.05[b]	2.23 ± 1.21[b]	2.50 ± 0.65[b]	2.37 ± 1.11[b]	2.63 ± 1.62[b]	3.97 ± 1.88[b]
Color parameters								
$L*$	37.51 ± 3.20[a]	34.32 ± 3.53[a]	29.01 ± 3.50[a]	30.70 ± 2.25[a]	32.64 ± 4.14[a]	33.53 ± 1.90[a]	32.76 ± 1.46[a]	30.37 ± 0.73[a]
$a*$	−3.77 ± 0.99[a]	−3.99 ± 1.19[a]	−4.22 ± 1.29[a]	−4.50 ± 1.33[a]	−4.83 ± 1.40[a]	−4.55 ± 1.69[a]	−4.29 ± 0.94[a]	−2.80 ± 0.55[a]
$b*$	16.13 ± 5.74[a]	13.59 ± 1.62[a]	14.98 ± 1.09[a]	16.39 ± 0.14[a]	17.23 ± 1.20[a]	20.62 ± 1.93[a]	20.13 ± 6.35[a]	15.71 ± 0.23[a]
$C_{ab}*$	16.46 ± 5.68[a]	14.02 ± 1.80[a]	15.39 ± 1.22[a]	16.82 ± 0.26[a]	17.71 ± 1.33[a]	21.00 ± 2.22[a]	20.49 ± 6.29[a]	15.95 ± 0.10[a]
$h_{ab}°$	100.85 ± 2.79[a]	103.56 ± 1.68[a]	102.42 ± 1.71[a]	102.34 ± 1.55[a]	103.18 ± 1.03[a]	100.71 ± 2.74[a]	100.19 ± 2.30[a]	98.37 ± 3.15[a]

* Means (± standard deviations) with different superscript letters in the same row are statistically different ($p \leq 0.05$, Tukey test).

(Fasuyi & Aletor, 2005).

The changes on the physicochemical and color characteristics during *tucupi* production are shown in Table 1. During spontaneous fermentation the main changes included significant increase in total acidity and decrease in pH values, which is typical of *manipueira* fermentation for the production of *tucupi* (Chisté et al., 2007; Chisté & Cohen, 2011; Campos, Carvalho, & Mattietto, 2016). The increase in acidity could play a role on the preservation of the fermented product. Total sugar contents decreased 64% of initial levels after 24 h fermentation. There was an initial increase in reducing sugars, possibly due to the hydrolysis of non-reducing sugars, favored by the reduced pH and increased acidity of the medium (Ferreira, Caliari, Júnior, & Beleia, 2013).

Regarding soluble solids, their contents did not change throughout *manipueira* fermentation, as it was observed by total sugars, which can be attributed to the production of soluble substances, such as organic acids during fermentation (Chisté & Cohen, 2011; Campos, Carmo, et al., 2016; Campos et al., 2017). The production of these organic acids during fermentation of *manipueira* is attributed to hydrolysis of carbohydrates by fermentation microorganisms, such as lactic bacteria (Fayemi & Ojokoh, 2014; Oguntoyinbo & Dodd, 2010).

The high starch content observed in the *manipueira* before fermentation, compared to the values during fermentation (Table 1), is attributed to the fact that the starch was suspended in *manipueira* during homogenization of the sample, while in the collection of the samples during fermentation, the starch was decanted. During sample collection, the homogenization was not carried out, as this procedure is not common during fermentations by *tucupi* producers. Additionally, the homogenization could interfere with the fermentation process due to the aeration of *manipueira.*

During the extraction of *manipueira,* most of the starch contained in the mass of ground cassava is carried with the liquid, since filtration is done in meshes with openings which are not sufficient to retain starch particles (9–17 μm) (Ladeira et al., 2013; Leonel, 2007). In this study, no significant difference ($p > 0.05$) was observed for starch during the fermentation process of the *manipueira*. Therefore, the separation of starch at the beginning of the process, which would involve an additional step in the process, is not necessary. However, if desired, the separation may be carried out before or after fermentation, without interfering with the final characteristics of *tucupi*, because starch is not contributing to the fermentation process.

The yellowish color of *manipueira* (Table 1), characterized by $a*$ and $b*$ values in the second quadrant ($-a$, $+b$), did not change ($p > 0.05$) during and after fermentation, as well as the other color parameters, including $L*$ (luminosity) and $C_{ab}*$(vividness or dullness of a color). This result suggests that carotenoids remained unchanged (Meléndez-Martínez, Britton, Vicario, & Heredia, 2006).

3.2. Changes in carotenoid contents

The chromatogram obtained from HPLC-DAD analysis of carotenoids of the cassava roots used in the production of *tucupi* showed the presence of three carotenes: 9-*cis*-β-carotene, all-*trans*-β-carotene and 13-*cis*-β-carotene, with all-*trans*-β-carotene as the major compound (Fig. 1). The same carotenoid profile of cassava roots was already described in the literature (Failla et al., 2012; Rodriguez-Amaya & Kimura, 2004). During the entire fermentation process of *manipueira,* and also after boiling during production of *tucupi,* the chromatographic profile remained the same, and no other compound associated with carotenoid degradation was observed (data not show). Therefore, this is the first time that the carotenoid profile was monitored and quantified (Fig. 2) to characterize the changes during *tucupi* production.

In the first stage of *tucupi* production, the *manipueira* was submitted to a spontaneous fermentation and after 24 h, all-*trans*-β-carotene concentration remained constant (1.03–1.65 μg/mL), as well as the isomers 9-*cis* (0.53–0.79 μg/mL) and 13-*cis* (0.30–0.38 μg/mL). The

statistical test used (*Tukey* HSD) did not show significant differences on carotenoid contents ($p > 0.05$) during the processing stages. However, a slight increase on the levels were observed after boiling. In order to obtain *tucupi*, the fermented *manipueira* is submitted to boiling for 10 min at approximately 100 °C (Chisté & Cohen, 2011). Therefore, the small changes observed are possibly due to the concentration of the product, by the partial removal of water which takes place during the boiling process.

The *trans-cis* isomerization of β-carotene during fermentation followed by boiling was also observed when preparing fufu (cassava flour stirred with boiled water over a low-heat fire to give a hard mass) (Thakkar, Huo, Maziya-Dixon, & Failla, 2009). These authors reported a minimal loss of β-carotene (8%) after 3 days of fermentation at room temperature during the production of *gari,* a fermented cassava product, which is widely consumed in many West African countries. Conversely, Chávez et al. (2007) reported a loss of β-carotene by 66% during preparation of *gari* due to the long fermentation time of cassava roots (7 days).

In relation to the vitamin A activity, β-carotene and its *cis*-isomers are the compounds responsible for the vitamin A activity in *manipueira* and *tucupi*, since they have at least one unsubstituted β-ionone ring with an attached polyene side chain of at least eleven carbons, which are the structural requirements for provitamin A carotenoids. As observed for the contents of carotenoids, the vitamin A activity (expressed as retinol activity equivalent, RAE) was similar during the processing steps of *tucupi*, with values varying from 0.12 μg RAE/mL *(manipueira)* to 0.18 μg RAE/mL (after 24 h of fermentation), with a slight increase (0.35 μg RAE/mL) after boiling (*tucupi*); also due to the concentration of the product.

3.3. Changes in bioactive amines

This is the first study on the identification and quantification of bioactive amines in tucupi. Among the nine investigated amines, only four were detected during tucupi production, the polyamine spermidine and the biogenic amines putrescine, tyramine and histamine (Table 2). Manipueira was characterized by the presence of only spermidine and putrescine. This result is similar to reports for bioactive amines in boiled cassava (Glória, 2005), which showed only spermidine (0.16–0.27 mg/100 g) and putrescine (0.08–0.61 mg/100 g).

Throughout fermentation, there was no significant change on spermidine and putrescine levels (p > 0.05). However, the presence of tyramine and histamine was detected from 16 h of fermentation until the end of tucupi production and the levels of histamine remained constant (p ≤ 0.05) despite the slight decrease observed after boiling; whereas the levels of tyramine increased significantly (p ≤ 0.05). The presence of the spermidine and putrescine is expected in foods as they are inherent to plants and all living organisms. Spermidine is involved in cell growth, renewal and metabolism; whereas putrescine is an obligate precursor in the formation of polyamines (Kalač, 2014; Kalač & Krausová, 2005).

According to the literature, there are several microorganisms involved in the beginning of the fermentation of cassava roots, among them, *Lactobacillus, Pediococcus, Clostridium, Propionibacterium* and *Bacillus* sp. (Lacerda et al., 2005; Oguntoyinbo & Dodd, 2010). However, as fermentation goes on, *Lactobacillus* becomes prevalent, including *Lactobacillus plantarum, L. fermentum,* L. delbrueckii and *L. manihotvorans* (Elijah, Atanda, Popoola, & Uzochukwu, 2014; Lacerda et al., 2005). These bacteria are capable of producing tyramine and histamine as a protection from the acidic media (decreased pH), which can be detrimental to bacteria survival (Glória, 2005). Fermentation of foods provides favorable conditions for biogenic amines generation and these conditions are both the availability of free amino acids and the presence of microorganisms with decarboxylase activity (EFSA, 2011). Therefore, these bacteria are able to decarboxylate amino acids, such as tyrosine and histidine to form tyramine and histamine, respectively,
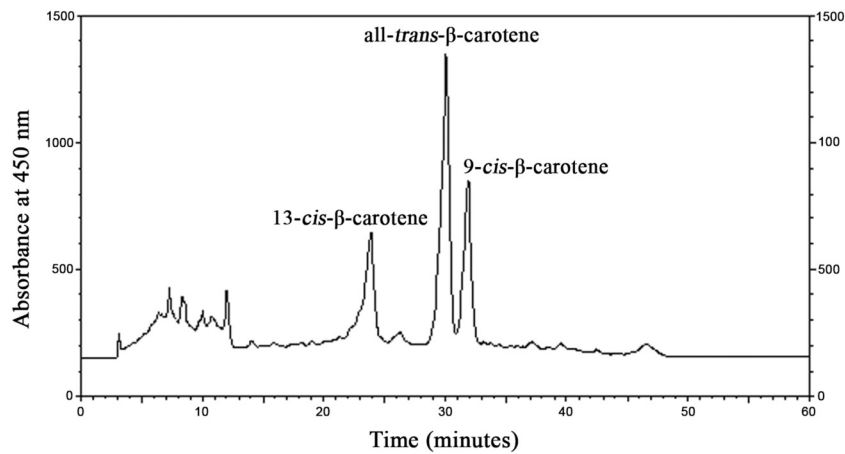
Fig. 1. *HPLC-DAD chromatogram at 450 nm of carotenoids extracted from cassava roots used for* tucupi *production.*
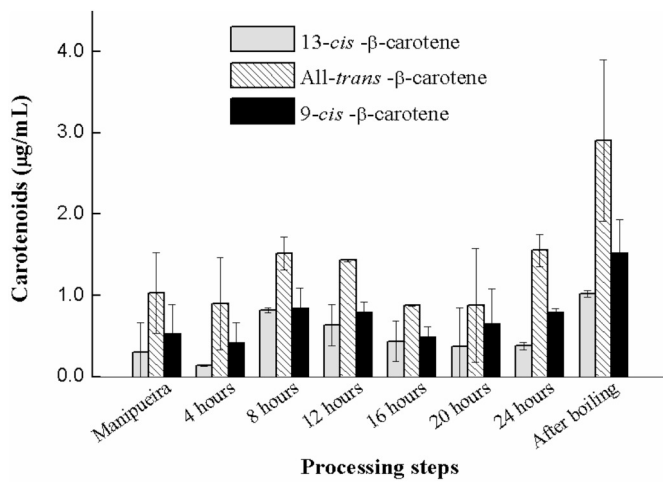

Fig. 2. Changes on carotenoids during *tucupi* production. There was no significant difference ($p > 0.05$) between the contents of 9-cis-β-carotene, all-trans-β-carotene and 13-cis-β-carotene during the production process.

which can buffer pH, ensuring bacteria survival (Glória, 2005; O'Sullivan et al., 2015).

The presence of tyramine, histamine and putrescine can be indexes of quality and safety of fermented products as these amines can result from low quality raw material and/or inadequate hygienic-sanitary conditions (Glória, 2005; Mohedano, López, Spano, & Russo, 2015). In the first 12 h of fermentation, only spermidine and putrescine were detected (Table 2). However, from 16 h of fermentation until the end of the process, the contribution of histamine and tyramine to total levels became evident. Furthermore, boiling did not decrease the contents of biogenic amines, which can be due to their high stability to heating

processes (Gonzaga, Lescano, Huamán, Salmón-Mulanovich, & Blazes, 2009; Tapingkae, Tanasupawat, Parkin, Benjakul, & Visessanguan, 2010). This fact represents concern since these biogenic amines have been widely involved in food poisoning episodes (EFSA, 2011). Therefore, there is a need to understand why these amines are formed to prevent their format. The levels of histamine present in *tucupi* are below the limits established for fish – 100 mg/kg (Glória, 2005). However, according to EFSA (2011), the no adverse health effects levels for histamine are: 50 mg histamine for healthy individuals, but below detectable limits for those with histamine intolerance; and for tyramine are 600 mg tyramine for healthy individuals not taking monoamino oxidase inhibitor (MAOI) drugs, but 50 mg for those taking third generation MAOI drugs and 6 mg for those taking classical MAOI drugs. Therefore, histamine (2.18 mg/kg) and tyramine (6.40 mg/kg) levels are worrisome based on the no observed adverse effect level (NOAEL) for individuals with histamine intolerance and also for individuals under classical MAOI drugs.

It is always difficult to establish a critical threshold of biogenic amine in foods due to the intrinsic intra- and inter-individual variations in sensitivity or to the concomitant interference of inhibitors of the detoxification pathways such as antidepressants drugs or alcohol (Spano et al., 2010). Furthermore, the presence of other biogenic amines such as putrescine and cadaverine, can enhance the toxicity of histamine (Emborg & Dalgaard, 2006). The presence of histamine in a meal can cause symptoms like headache, nasal secretion, bronchospasm, tachycardia, hypotension, edema (eyelids), urticaria, pruritus, flushing and asthma. Tyramine intoxication can result in migraine headache and hypertensive crisis (EFSA, 2011).

Chisté et al. (2007) and Campos, Carmo, et al. (2016) evaluated commercial *tucupi* and concluded that there is no standardization in the *tucupi* production process. It was observed large variations in physical-chemical and microbiological characteristics indicating deficiencies during processing. Factors such as poor sanitary conditions of the

Table 2
Bioactive amines (mg/L) during *tucupi* production.

Bioactive amine	Manipueira	Fermentation time (h)						After Boiling (*Tucupi*)
		4	8	12	16	20	24	
Polyamine								
Spermidine	3.89 ± 1.34[a]	3.12 ± 0.23[a]	4.37 ± 1.68[a]	4.43 ± 1.11[a]	5.43 ± 1.64[a]	4.64 ± 2.35[a]	5.10 ± 2.16[a]	7.24 ± 1.80[a]
Biogenic amine								
Putrescine	3.88 ± 1.00[a]	3.42 ± 0.01[a]	3.66 ± 1.36[a]	3.20 ± 0.85[a]	4.18 ± 1.04[a]	3.95 ± 1.42[a]	4.20 ± 1.05[a]	5.40 ± 1.71[a]
Histamine	nd	nd	nd	nd	0.97 ± 0.67[a]	2.02 ± 1.50[a]	4.08 ± 2.91[a]	2.26 ± 1.68[a]
Tyramine	nd	nd	nd	nd	0.76 ± 0.16[d]	2.54 ± 0.29[c]	5.48 ± 0.32[b]	6.60 ± 0.23[a]

Means (± standard deviations) with different superscripts letters in the same row are statistically different ($p \leq 0.05$, Tukey test; *nd = not detected (detection limit – 0.4 mg/L)*.
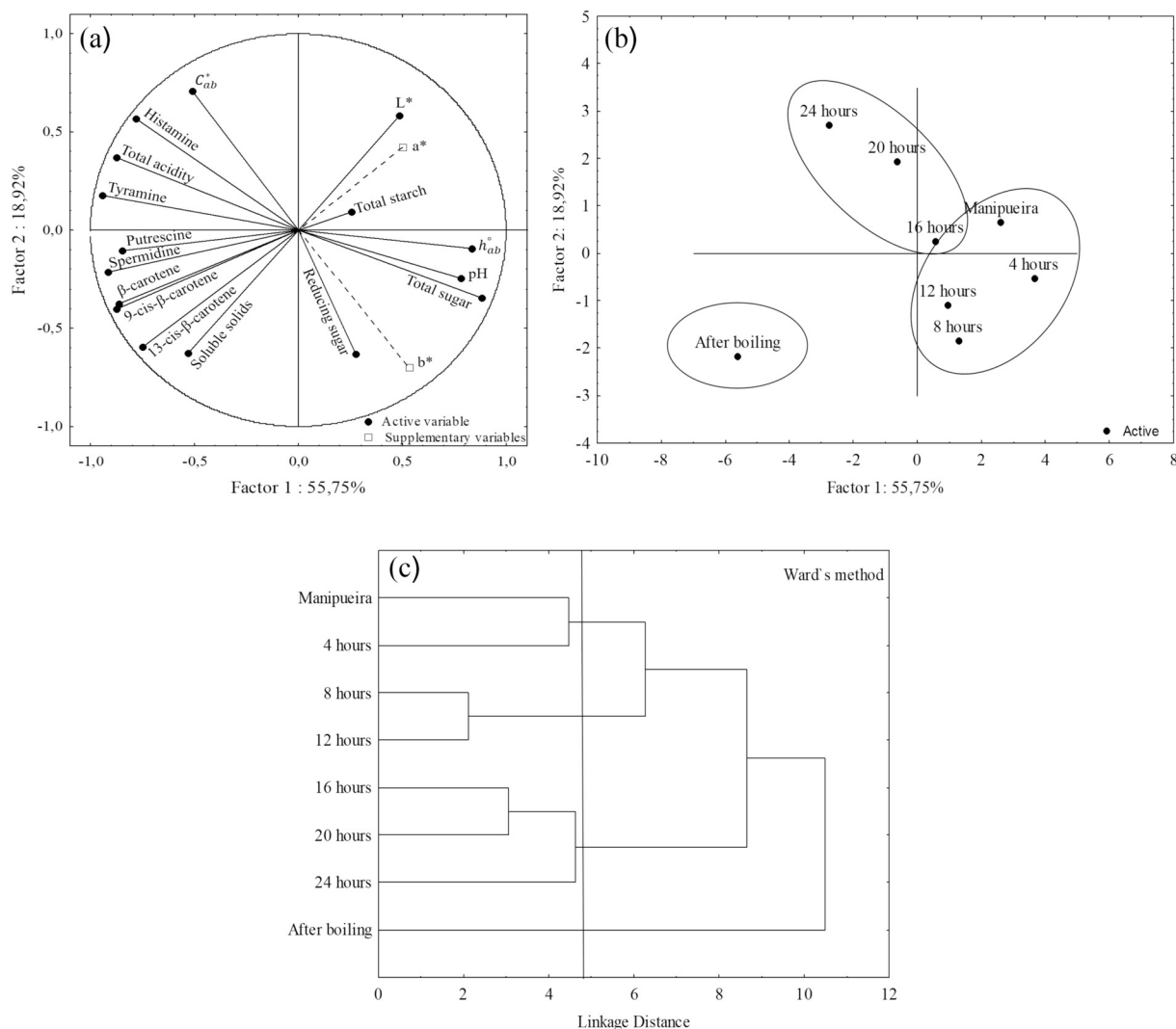
Fig. 3. Classification of the different stages of *tucupi* production as affected by the physicochemical characteristics, contents of carotenoids and bioactive amines. (a) Projection of the variables by Principal Component Analysis (PCA); and (b) Dispersion of the samples from the different stages of *tucupi* production by PCA (c) Dendrogram by HCA analysis.

production units may affect the quality and food safety standards. Therefore, the implementation of Good Manufacturing and Handling Practices during *tucupi* production and quantification of biogenic amines would be relevant to warrant product quality and safety.

3.4. Classification of changes during tucupi production by multivariate statistical analysis

In the PCA, the first two components accounted for 77% of the explained variance, taking into consideration the physicochemical characteristics (pH, total acidity, reducing and total sugar, and total starch), color measurements ($L*$, $C_{ab}*$ and $h_{ab}°$), and contents of carotenoids and bioactive amines (Fig. 3).

In Fig. 3a, negative correlations were observed between pH and the contents of tyramine ($r = -0.66$), histamine ($r = -0.73$), putrescine ($r = -0.48$) and spermidine ($r = -0.72$). These correlations are in agreement with Glória (2005) since in an acidic medium, the production of amines is stimulated as a mechanism of bacteria protection to the acid environment. Indeed, the pH levels observed in this group (4.5–3.97) (Table 1) are highly favorable to the production of biogenic amines.

When grouping the different stages of *tucupi* production (Fig. 3b), as evidenced by HCA (Fig. 3c) based on similarities, the first group was

formed by *manipueira* and the samples obtained after 4 h, 8 h and 12 h of fermentation due to high pH values, total and reducing sugars, and total starch at this stage of processing, as expected. The second group included *manipueira* after 16 h, 20 h and 24 h of fermentation, which were characterized by the formation of tyramine and histamine (biogenic amines), as well as the highest values of $C_{ab}*$ and total acidity. Finally, the sample, composed by the final step of *tucupi* production (boiled *manipueira* after 24 h of fermentation) was discriminated from the other samples (Fig. 3b and c) due to the highest levels of soluble solids, spermidine, putrescine and carotenoids (13-*cis*-β-carotene, all-*trans*-β-carotene and 9-*cis*-β-carotene).

PCA classified the *tucupi* production process into different stages according to the parameters evaluated (physical-chemical characteristics, bioactive amines, and carotenoids). The separation in groups allows identifying the group that should have greater control, mainly in relation to biogenic amines and the carotenoids, which are the focus of this work, due to the interest for nutrition and human health.

4. Conclusion

For the first time, the influence of fermentation of *manipueira* to produce *tucupi* on the bioactive amine and carotenoids profile was assessed. The profile of all-*trans*-β-carotene, 9-*cis*-β-carotene and 13-*cis*-β-

carotene were monitored in the cassava roots and during *tucupi* production and none remarkable alteration was observed. One polyamine and one biogenic amine were identified in the *manipueira* (spermidine and putrescine) and two biogenic amines were formed during the fermentation process of *tucupi* (tyramine and histamine). PCA analysis evidenced three distinct moments in the *tucupi* production: the first one consists of the *manipueira* until 12 h of fermentation, the second refers to the end of the fermentation process (16 to 24 h) and finally the third moment refers to the product after boiling (*tucupi*). These results suggest the need of an accurate control of the hygienic-sanitary conditions during fermentation of *manipueira* in order to produce *tucupi* with very low levels of biogenic amines to ensure a high quality product. Based on these results, studies are needed regarding the molecular identification of the microorganisms responsible for the spontaneous fermentation of cassava. Such knowledge would allow the development of the starter cultures adequate for the production of high quality and safe *tucupi*.

Conflict of interest

The authors have no conflict of interest.

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