









## Characterization and classification of cassava flour produced in Barreirinhas, Maranhão

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### Abstract

Cassava flour is a strategic food for Brazilian food security due to its low cost, good acceptance, and versatility, as well as the cultural custom of consumption, especially in the North and Northeast regions. However, as in Barreirinhas, Maranhão, its artisanal production lacks standardization and sanitary control. This study aimed to characterize and classify nine samples of artisanal flours sold and produced in Barreirinhas, based on physical, physicochemical, and microbiological analyses. The classification followed Normative Instruction 52/2011 of the Brazilian Ministry of Agriculture, Livestock, and Supply. The present study revealed significant differences in the acidity levels between the dry and water flour groups, 1.87 and 1.32 meq NaOH 0.1 N 100 g<sup>-1</sup>, respectively, and reinforced the non-uniformity in the parameters evaluated between the groups. Principal component analysis explained 70.3% of the total variance. Two samples had *Bacillus cereus* above the legal limit ( $1.0 \times 10^4$  and  $1.5 \times 10^4$  colony-forming unit g<sup>-1</sup>), and four had a high load of molds and yeasts ( $> 10^3$  colony-forming unit g<sup>-1</sup>). Total coliforms were detected in two samples (23 most probable number g<sup>-1</sup>), while *Salmonella spp.* was absent and *Escherichia coli* was under 3 most probable number in all samples. The results show variations in quality and reinforce the need to train producers and adopt good manufacturing practices to guarantee food safety and strengthen the traditional production chain.

**Keywords:** cassava flour; nutritional composition; food safety; microorganisms; physicochemical characteristics.

**Practical Application:** This study not only classifies cassava flour based on current legislation but also provides a detailed characterization of its microbiological and physicochemical properties.

## 1 INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the primary sources of carbohydrates in tropical countries. It is strategic for the food security of millions of people due to its low price, good acceptance, and versatility. According to the Food and Agriculture Organization (FAO, 2023), Brazil ranks fifth in the world in terms of gross production of this crop. Cassava also represents a symbol of identity for various indigenous peoples, with its name deriving from the Tupi legend “mani-oca” (Mani’s house). Its use as cassava flour dates back to the colonial period in Brazil, when it was considered the primary source of energy for expeditions into the Brazilian hinterland (Denardin & Komarcheski, 2015; Pinto, 2002; Xavier et al., 2020).

In Brazil, around 40% of root production is used to make flour, a widely consumed food with high nutritional value, especially in the North and Northeast regions (Dias & Leonel, 2006). According to the latest Family Budget Survey from Brazil (POF 2017–2018), cassava flour is a staple in the daily diet of people in the North and Northeast regions, where the daily per capita consumption can reach 38 g (Instituto Brasileiro de Geografia e Estatística [IBGE], 2020). In addition to being consumed fresh

or as flour, cassava is used to produce traditional beverages (tiquira and cauim) and products such as cassava starch, contributing to a diversity of culinary and sociocultural practices in Brazil (Xavier et al., 2020).

In Maranhão, around 72% of the cassava grown is used for artisanal flour production, usually carried out in “flour houses” by family farmers (Araujo, 2023). The city of Barreirinhas stands out in this scenario as an important producer, combining traditional processing practices with economic and even tourist impact in the region. However, the production process, which is mostly manual and non-standardized, occurs in poor sanitary conditions and without adequate microbiological control (Santos et al., 2023; Silva, Cardoso, et al., 2017).

The sale of flour at fairs and markets also exposes the food to additional risks due to inadequate storage and handling practices, compromising its safety. Although cassava flour is essential for Brazilian food security and has been redefined by new gastronomic trends, few studies address its nutritional composition, microbiological quality, and socioproductive aspects in traditional contexts such as Barreirinhas, Maranhão, in an integrated perspective (Pena et al., 2020; Santos et al., 2014).

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Given this, the present study aimed to characterize and classify artisanal cassava flour produced in the city of Barreirinhas according to Normative Instruction (IN) 52/2011 of the Brazilian Ministry of Agriculture, Livestock, and Supply (IN 52/2011), integrating physicochemical and microbiological analyses. The approach seeks to fill gaps in the scientific literature, support quality control strategies, and contribute to strengthening the traditional production chain in the state.

### 1.1 Relevance of work

This study is significant for investigating the quality of cassava flour produced in Barreirinhas, Maranhão, a staple food widely consumed in the state, yet supported by limited scientific evidence. The research identified shortcomings in sanitary control and product standardization by characterizing its physicochemical, nutritional, and microbiological aspects. The findings can inform capacity-building initiatives for producers and foster improvements in the local production chain, enhancing traditional knowledge while promoting advances in public health, nutrition, and the state's economic value. Furthermore, the study holds potential to contribute to future regulatory changes proposed by federal institutions for products of a similar nature.

## 2 MATERIAL AND METHODS

The study was conducted in two stages, the first in Barreirinhas, Maranhão, to collect samples. The second happened in the city of São Luís, Maranhão, to perform the analyses at the Bromatology Laboratory of the Federal University of Maranhão (UFMA) and the Chemical Technology Laboratory of the same institution.

### 2.1 Samples

Nine samples of cassava flour were purchased at the Barreirinhas Municipal Market (latitude: 2°44'49" South; longitude: 42°49'33" West) in October 2024, with the only criterion being that it had to be produced in Barreirinhas, regardless of the group.

The samples were stored in their original 1 kg plastic packaging. The packaging was identified by labels filled with codes in letters and numbers (a1, a2, a3, a4, a5, b1, b2, d1, and d2), with the letters identifying the place/village of production and the numbers corresponding to the samples.

### 2.2 Classification of flours

The flours were classified into dry (FS), water (FD), or bijusada (FB) groups depending on visual aspects, color, granules/flakes, and seller references (Brasil, 2011).

The classes were determined using the values retained in 1 mm and 2 mm granulometric sieves subjected to mechanical movement, for classification into coarse, medium, and fine (Álvares, 2014; Brasil, 2011).

For the type, the number of shells and inter-shells found in 10 g samples using metal tweezers was multiplied by 10 and

compared to the references established in the Brazilian legislation. The values obtained classified the samples as type 1, 2, 3, single type, or off type (Álvares, 2014; Brasil, 2011).

When foreign matter (any material not constituting the product, such as hair, glass, plastic, or paper) was identified, the sample was considered off type, regardless of the other parameters evaluated (Álvares, 2014; Brasil, 2011).

### 2.3 Physicochemical analyses

#### 2.3.1 Moisture (AOAC 925.10)

The moisture content was determined in triplicate by direct drying in an oven (Solab SL100) at 105°C for 3 h, until constant weight. The samples were cooled in a desiccator and weighed on an analytical balance (Marteá AY220) (Association of Official Analytical Chemists [AOAC], 2006).

#### 2.3.2 Titratable acidity (AOAC 942.15)

The total titratable acidity was determined in triplicate by dissolving 3 g of the sample in 100 mL of distilled water for 30 min. Afterward, four drops of 1% phenolphthalein were added for titration with 0.1 N NaOH (AOAC, 2006).

#### 2.3.3 Proteins (AOAC 920.87)

The protein percentage was determined in duplicate using the Kjeldahl method. Five grams of the sample were digested in H<sub>2</sub>SO<sub>4</sub> in a digester block (Tecnal 008/50-04). Subsequently, the amount of nitrogen distilled and captured in 0.02 M HCl solution was titrated with 0.02 M NaOH solution until a persistent color change occurred. The volume was used to calculate the percentage of nitrogen. The percentage of protein was obtained by multiplying the percentage of nitrogen by the vegetable protein factor of 5.75 (AOAC, 2006).

#### 2.3.4 Lipids (AOAC 920.85)

The lipid content was determined in triplicate by hot extraction in a Soxhlet apparatus, using PA hexane as the solvent. Approximately 3 g of the sample was weighed, placed in filter paper cartridges, and subjected to extraction for 6 h. The solvent was removed by evaporation, and the bottles containing the extract were dried in an oven (Solab SL100) at 105°C until a constant weight was achieved (AOAC, 2006).

#### 2.3.5 Ash (AOAC 923.03)

The ash content was determined by analyzing triplicate samples of 5 g incinerated at 550°C for 4 h, then cooled in a desiccator, and weighed on an analytical balance (Marteá AY220) (AOAC, 2006).

#### 2.3.6 Crude fiber (AOAC 920.86)

To determine fiber content, 3 g of duplicate samples were initially digested in an acid solution (1.25% H<sub>2</sub>SO<sub>4</sub> for 30 min). After washing with boiling distilled water, the process was repeated

with a basic solution (1.25% NaOH for 30 min). The residue from the digestions was dried in an oven at 105°C until a constant weight was achieved (Álvares, 2014; AOAC, 2006).

### 2.3.7 Starch

The starch content was determined by acid hydrolysis and titration with Fehling's solution in duplicate. Approximately 5 g of the sample was hydrolyzed with concentrated HCl and heated for 30 min. After neutralization with 10 N NaOH, the extract was titrated hot with Fehling's solution until it turned brick red, using methylene blue as an indicator. The glucose content obtained was converted to starch using a factor of 0.9 (Instituto Adolfo Lutz, 2008).

### 2.3.8 Carbohydrates

Carbohydrate values were assigned based on the difference in moisture, ash, crude fiber, protein, and lipid values, expressed in g 100 g<sup>-1</sup>, according to the following formula:

$$\text{Carbohydrates} = 100 - (\text{ash} + \text{moisture} + \text{proteins} + \text{lipids} + \text{crude fiber})^*$$

\* Values expressed in g 100 g<sup>-1</sup>

### 2.3.9 Energy

For the energy values per 100 g of food, 4 kcal was multiplied by each gram of carbohydrates and proteins, and 9 kcal by each gram of lipids.

### 2.3.10 Microbiological analyses

Molds and yeasts, total coliforms, coliforms at 45°C, *Escherichia coli*, *Salmonella* spp., and *Bacillus cereus* were tested by the protocols described by Silva, Junqueira, et al. (2017).

### 2.3.11 Molds and yeasts

The mold and yeast count was performed using the surface plating method with Acidified Potato Dextrose Agar (APDA) medium. Samples diluted in a decimal series were inoculated and incubated at 25°C for 5 days. The results were expressed in colony-forming units per gram (CFU g<sup>-1</sup>).

### 2.3.12 Total coliforms, coliforms at 45°C, and *E. coli*

Total coliforms, coliforms at 45°C, and *E. coli* were determined using the most probable number (MPN) method. The analysis was performed in three stages: presumptive, with incubation in Lauryl Sulfate Tryptose (LST) broth for 24–48 h at 35°C; confirmatory, with subculturing in EC broth and incubation at 45°C for 24 h; and isolation on EMB agar for the identification of typical *E. coli* colonies, subsequently confirmed by biochemical tests.

### 2.3.13 *Salmonella* spp.

The search for *Salmonella* spp. was carried out using the protocols starting with pre-enrichment in 1% peptone water, followed by selective enrichment in Tetrathionate broth for 24 h

at 37°C. Subsequently, seeding was performed on Xylose Lysine Deoxycholate (XLD) agar and *Salmonella* Shigella (SS) agar for 24 h at 37°C. Biochemical and serological tests confirmed suspicious colonies.

### 2.3.14 *Bacillus cereus*

The *B. cereus* count was conducted using the surface plating method using Mannitol Egg Yolk Polymyxin (MYP) agar. The plates were incubated at 30°C for 24 h. The characteristic colonies were quantified and identified through biochemical tests.

### 2.3.15 Statistics

The data were expressed as median and interquartile range (IQR). Analysis of variance (Kruskal-Wallis) was applied, followed by Dunn's post-test with Bonferroni correction for multiple comparisons between samples, and Mann-Whitney test for comparison between dry and water groups. A significance level of 5% was adopted. Principal component analysis (PCA) was performed to explore possible groupings among flour samples based on the physicochemical variables evaluated. The data were centered and scaled for this analysis, using the correlation matrix between variables. The data were organized in Microsoft Excel® and analyzed using RStudio® software (Version 2025.05.1 + 513).

## 3 RESULTS AND DISCUSSION

### 3.1 Classification criteria and physicochemical characteristics of flours

Most flours were classified as being in the FD group, with the absence of flours from the FB group being reported (Table 1). The greater presence of FD flours can be attributed to the preference of this group by consumers for *in natura* consumption, mainly due to the acidity attribute resulting from fermentation (Brasil, 2011; Chisté & Cohen, 2011).

For the classes, only sample d2 was in the “fine” class; the others were in the “coarse” class. When the samples were purchased, it was mentioned that the a2 sample was meant to be a thickener and not for *fresh* consumption, which may explain the granules being smaller than 1 mm. It is known that the greater the surface area of contact between water and starch granules, the greater their capacity for gelatinization, which makes them a good thickener (Brasil, 2011; Brito et al., 2015).

Sample d2 was considered a “single type,” as it was from the FS group and the “fine” class. In addition, samples a2 and a5 were off type, with peel/inter-peel counts higher than those required by regulation (Figure 1). The high number of peels and inter-peels may be related to flaws in the cassava peeling stage, resulting in the presence of these components in the final product (Brasil, 2011; Santos et al., 2023).

Although most of the flours did not contain any foreign matter from parts that were not part of the food or from the raw material used to produce it, sample a1 was off type because it happened to have an undefined hair (Brasil, 2011). Foreign matter and dirt, such as insect fragments, charred particles,

larvae, plastic, and various plant materials, have been reported more frequently in other studies. The artisanal production process and poor packaging may explain these results (Santos et al., 2023; Sousa et al., 2021).

The variance between the attributes studied was assessed to better explore the uniformity between the parameters assessed

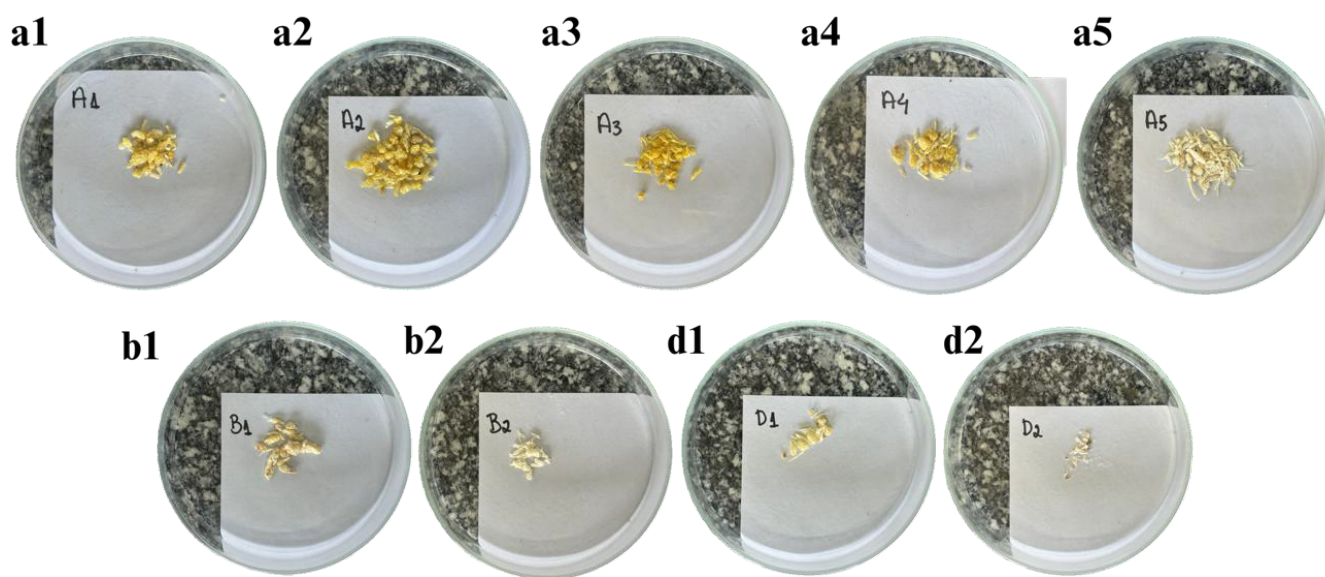
within the different FS and FD groups (Tables 2 and 3). The results reinforce the lack of standardization in the production process and highlight the particularities of each producer.

The moisture content obtained is within the standards required by current legislation (Brasil, 2011). Pinto et al. (2020) also obtained favorable results, with moisture content ranging from

**Table 1.** Classification of flours produced in Barreirinhas according to Normative Instruction 52/2011.

Sample	Group	Class	Type	Foreign matter	Off type
a1	FD	Coarse	3	Yes	Yes
a2	FD	Coarse	Off type	No	Yes
a3	FD	Coarse	3	No	No
a4	FD	Coarse	3	No	No
a5	FS	Coarse	Off type	No	Yes
b1	FD	Coarse	3	No	No
b2	FS	Coarse	1	No	No
d1	FD	Coarse	2	No	No
d2	FS	Fine	Single type	No	No

FD: water flour group; FS: dry flour group.



**Figure 1.** Peels and inter-peels from 10 g of samples in nine different cassava flours from Barreirinhas, Maranhão.

**Table 2.** Physicochemical characterization of dry group flours produced in Barreirinhas, Maranhão.

Variable	a5	b2	d2	<i>p</i> -value
Moisture	6.91 (0.23) <sup>a</sup>	9.38 (0.05) <sup>b</sup>	7.30 (0.05) <sup>ab</sup>	.04
Ash	0.42 (0.01) <sup>a</sup>	1.18 (0.01) <sup>b</sup>	1.04 (0.02) <sup>ab</sup>	.02
Lipids	0.54 (0.08)	0.86 (0.09)	0.78 (0.06)	.06
Acidity	2.23 (0.08) <sup>a</sup>	1.83 (0.20) <sup>ab</sup>	1.51 (0.15) <sup>b</sup>	.03
Proteins	1.92 (0.19)	1.42 (0.12)	2.05 (0.18)	.16
Fibers	1.47 (0.09)	1.79 (0.02)	1.77 (0.15)	.18
Starch	65.89 (0.00)	59.07 (0.00)	61.70 (2.13)	.09
Carbohydrates	86.47 (0.18)	83.57 (0.22)	85.60 (0.17)	.10
Energy	358.66 (0.62)	347.77 (0.29)	358.16 (0.58)	.16

Values expressed as median (IQR). Different letters in the same row indicate a statistically significant difference ( $p < .05$ ) in Dunn's post-test with Bonferroni correction. Acidity values are expressed in meq NaOH 0.1 N 100 g<sup>-1</sup>, energy in kcal, and other variables in g 100 g<sup>-1</sup>.

6.8 to 10.6%. Adequate values in this product type are essential to ensure that they are microbiologically stable (Chisté et al., 2006). It should be noted that the statistical differences found in the moisture percentages between the samples may reflect inadequate packaging or shorter drying times during production.

Although there are no stipulated values for the percentage of lipids and proteins in the Brazilian regulation, Chisté et al. (2006) stated that cassava flour is a product with a low lipid content and showed these contents to vary between 0.17 and 0.20%. Variations in protein, lipid, fiber, and ash contents are largely related to the intrinsic characteristics of the cassava used to produce the flour or ingredients added, such as coconut, oils, or fats, to make the flour “coconutty” or “buttery,” as the sellers refer to it when offering the product (Brasil, 2011).

According to Tabela Brasileira de Composição de Alimentos (TACO), roasted cassava flour has an energy value of 365 kcal per 100 g, a value compatible with that found in this study. This finding reinforces the idea that flour is an efficient source of energy (Núcleo de Estudos e Pesquisas em Alimentação [NEPA] & Universidade Estadual de Campinas [UNICAMP], 2011). Artisanal cassava processing practices, such as the type of fermentation, the way the flour is roasted, and its granulometry, influence its nutritional composition, giving the final product distinct sensory characteristics and a non-uniform chemical composition (Chisté & Cohen, 2011; Widowati et al., 2025).

There were no significant variations in starch content between the flours in the FS group; however, it should be noted that the values are lower than those described in Brazilian regulation (Table 2) (Brasil, 2011). The reduced starch values can be attributed to removing starch from the pressed pulp of roots to produce by-products and pressing and fermenting the mass (Chisté & Cohen, 2010; Santos et al., 2023).

When comparing the physicochemical characteristics of the FS and FD flours, there was a statistical difference only for acidity, with the FS group being more acidic ( $p = .02$ ) (Table 4). Both groups were classified as having “low acidity” as they have values of less than 3 and 5 meq NaOH 0.1 N 100 g<sup>-1</sup>, respectively. Pinto et al. (2020) reported values of 2.38 and 4.65 meq NaOH 0.1 N 100 g<sup>-1</sup> for the same group of flours from Maranhão.

The difference between flour groups lies in the fermentation process. Dias and Leonel (2006) associated higher acidity levels with fermentation during processing, even though this was not

evident in this study. This result may be linked to the successive washing of the dough in the production process before roasting, which can reduce the acidity of the final product.

Chisté et al. (2006), when evaluating the quality of dry group from supermarkets and markets in the city of Belém, identified acidity and starch content values that did not comply with Brazilian legislation (Brasil, 2011), suggesting a failure in the safety and processing of the flours. Higher acidity values may be related to the fermentation of the dough due to interruptions in the processing of the dry flour. Given the fermentation process of the flours in the water group, the higher acidity value of the dry group may suggest flaws in the processing flow of these flours (Chisté et al., 2007; Vilpoux, 2003).

In a complementary way, Chisté & Cohen (2011) evidenced a preference of consumers for flours fermented for up to 96 h, which consequently have high acidity, a typical characteristic of water flour.

The crude fiber values obtained for the groups are within the standards established in IN 52/2011 (Brasil, 2011). However, sample a1 presented values above the 2.3 g 100 g<sup>-1</sup> limit for the FD group. Other authors also reported higher values for fiber content, ranging from 0.57 to 2.44 for the dry group and 1.95 to 2.75 for the water group (Brasil, 2011; Dias & Leonel, 2006).

The discussion about the limits established for this nutrient came to the fore in Brazil in 2020, resulting in a relaxation of the limits for dry flour through Normative Instruction 58/2020,

**Table 4.** Comparison between the physicochemical characteristics of dry and water cassava flours produced in Barreirinhas, Maranhão.

Variable	FS	FD	<i>p-value</i>
<b>Moisture</b>	7.30 (2.15)	7.02 (1.55)	.10
<b>Ash</b>	1.04 (0.74)	0.68 (0.15)	.17
<b>Lipids</b>	0.78 (0.22)	0.72 (0.26)	.62
<b>Acidity</b>	1.83 (0.58)	1.13 (1.18)	.02
<b>Proteins</b>	1.80 (0.46)	1.61 (0.34)	.28
<b>Fibers</b>	1.70 (0.22)	2.15 (0.62)	.13
<b>Starch</b>	61.70 (6.18)	66.12 (8.62)	.15
<b>Carbohydrates</b>	85.60 (1.97)	86.12 (2.58)	.21
<b>Energy</b>	357.82 (8.13)	357.97 (10.70)	.64

FD: water flour group; FS: dry flour group.

Values are expressed as median (IQR). Acidity values are expressed in meq NaOH 0.1 N 100 g<sup>-1</sup>, energy in kcal, and other variables in g 100 g<sup>-1</sup>.

**Table 3.** Physicochemical characterization of water group flours produced in Barreirinhas, Maranhão.

Variable	a1	a2	a3	a4	b1	d1	<i>p-value</i>
<b>Moisture</b>	6.74 (0.08) <sup>ab</sup>	6.15 (0.10) <sup>ab</sup>	5.47 (0.08) <sup>a</sup>	7.77 (0.08) <sup>ab</sup>	8.42 (0.10) <sup>b</sup>	7.24 (0.08) <sup>ab</sup>	< .01
<b>Ash</b>	0.94 (0.03) <sup>a</sup>	0.56 (0.02) <sup>b</sup>	0.68 (0.01) <sup>ab</sup>	0.72 (0.02) <sup>ab</sup>	0.56 (0.01) <sup>b</sup>	0.68 (0.04) <sup>ab</sup>	< .01
<b>Lipids</b>	0.22 (0.13) <sup>a</sup>	1.60 (0.26) <sup>b</sup>	0.68 (0.12) <sup>ab</sup>	0.77 (0.03) <sup>ab</sup>	0.69 (0.07) <sup>ab</sup>	0.57 (0.12) <sup>ab</sup>	.02
<b>Acidity</b>	1.98 (0.08) <sup>a</sup>	1.51 (0.41) <sup>a</sup>	1.12 (0.17) <sup>a</sup>	0.70 (0.04) <sup>a</sup>	0.67 (0.07) <sup>a</sup>	2.06 (0.29) <sup>a</sup>	.01
<b>Proteins</b>	2.08 (0.47)	1.10 (0.50)	0.95 (0.02)	2.12 (0.51)	1.54 (0.11)	1.59 (0.06)	.31
<b>Fibers</b>	3.75 (0.03)	0.68 (0.15)	2.08 (0.04)	2.30 (0.02)	2.23 (0.06)	1.58 (0.20)	.06
<b>Starch</b>	67.28 (1.27)	58.89 (0.97)	77.73 (3.38)	70.69 (4.16)	59.89 (2.00)	65.05 (1.18)	.09
<b>Carbohydrates</b>	84.34 (0.56)	88.16 (0.08)	89.28 (0.23)	85.64 (0.53)	85.88 (0.19)	86.38 (0.30)	.07
<b>Energy</b>	347.43 (0.09)	373.44 (3.68)	366.34 (0.26)	357.97 (0.08)	355.86 (0.34)	356.98 (1.50)	.08

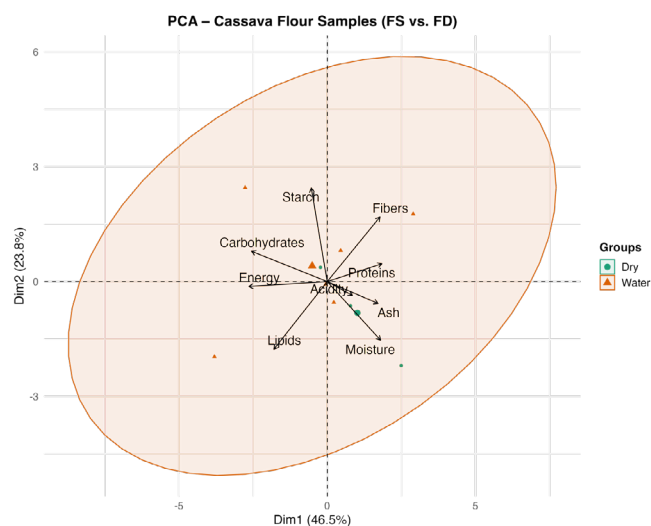
Values are expressed as median (IQR). Different letters in the same row indicate a statistically significant difference ( $p < .05$ ) in Dunn's post-test with Bonferroni correction. Acidity values are expressed in meq NaOH 0.1 N 100 g<sup>-1</sup>, energy in kcal, and other variables in g 100 g<sup>-1</sup>.

changing the limits to up to 4 g 100 g<sup>-1</sup>. The World Health Organization (WHO) recommends a daily intake of 25 g of dietary fiber, and cassava flour can provide up to 13.2% of this recommendation (Oliveira et al., 2021; WHO, 2023).

The exploration of the physicochemical data by PCA shows that the first two principal components jointly explained 70.3% of the total variance, with 46.5% attributed to Dimension 1 and 23.8% to Dimension 2. Figure 2 shows a tendency for the samples to be grouped according to the groups defined by MAPA, with a partial separation between FS and FD.

The samples from the FS group were predominantly positioned in the quadrant associated with higher moisture and ash contents. In contrast, the FD group samples were distributed in the opposite direction, more related to higher starch, carbohydrate, and energy contents.

The acidity variable made a moderate contribution in separating the groups along Dim 1, while protein and fiber contents had a greater influence on Dim 2. These results corroborate the patterns identified in the univariate analyses and reinforce the discriminatory role of variables such as moisture, starch, and energy as possible indicators in differentiating between groups of cassava flours.



PCA: principal component analysis; FS: dry flour group; FD: water flour group.

**Figure 2.** Principal component analysis of the dry and water cassava flours produced in Barreirinhas, Maranhão.

It is worth noting that some samples were positioned differently on the graph, which may indicate variations in the production process or single characteristics of the cassava, as previously discussed.

### 3.2 Presence of microorganisms

In the analysis of molds and yeasts, sample d1 had the highest CFU count (Table 5). Some studies on cassava flour sold at street markets have also shown high fungi counts and indicate the presence of genera such as *Aspergillus* and *Penicillium*. Although flour is considered a stable food due to its low moisture content, the presence of these microorganisms is worrying due to the toxins produced during storage (Ferreira Neto et al., 2004; Rodrigues et al., 2015; Sousa et al., 2021).

This contamination seems associated with the moment the flour is sold, mainly due to cultural habits in the North and Northeast regions, where consumers often put their hands into the bags to dry the flour. In addition, the flour is often packaged under inadequate conditions, remaining open for long periods in raffia bags placed near pathways and drains. These conditions, added to the aggravating factor of the hot and humid climate, significantly favor the development of microorganisms, such as fungi, which can proliferate even in foods with low moisture and water activity (Rodrigues et al., 2015; Santos et al., 2014).

Total coliforms were identified in samples b2 and d2 with values of 23 MPN g<sup>-1</sup>. As for coliforms at 45°C and *E. coli*, the results were less than 3 MPN g<sup>-1</sup> for all the samples, in line with the recommendations of Brasil (2022), which determine values between 10 and 10<sup>2</sup> MPN g<sup>-1</sup> for *E. coli* (Table 5). For assessing food safety, the presence of this microorganism is an important indicator of poor hygienic and sanitary conditions in production processes, suggesting a failure in the processing chain and even fecal contamination in food when *in natura* (Silva, Junqueira, et al., 2017).

All the samples were free of *Salmonella spp.* per 25 g of sample, following the legislation (Table 5). The WHO ranks *Salmonella spp.* among the leading causes of diarrheal diseases worldwide. It reinforces the importance of good hygiene and food safety practices throughout the production chain. For this reason, the presence of this pathogen is not tolerated even at low levels. Other studies that evaluated the quality of cassava derivatives, such as flour and starch, also did not find *Salmonella spp.* in the samples, but they emphasize the importance

**Table 5.** Microbiological profile of dry and water flours produced in Barreirinhas, Maranhão.

Samples	Molds and yeasts (CFU g <sup>-1</sup> )	Total coliforms (MPN g <sup>-1</sup> )	Coliforms at 45°C (MPN g <sup>-1</sup> )	<i>Escherichia coli</i> (MPN g <sup>-1</sup> )	<i>Salmonella</i> (presence/25 g)	<i>Bacillus cereus</i> (CFU g <sup>-1</sup> )
a1	1.00 × 10 <sup>2</sup>	< 3	< 3	< 3	Absent	Absent
a2	5.00 × 10 <sup>2</sup>	< 3	< 3	< 3	Absent	Absent
a3	8.00 × 10 <sup>2</sup>	< 3	< 3	< 3	Absent	1.0 × 10 <sup>3</sup>
a4	6.00 × 10 <sup>2</sup>	< 3	< 3	< 3	Absent	1.0 × 10 <sup>4</sup>
a5	3.00 × 10 <sup>2</sup>	< 3	< 3	< 3	Absent	1.5 × 10 <sup>4</sup>
b1	< 10 × 10 <sup>1</sup>	< 3	< 3	< 3	Absent	Absent
b2	3.00 × 10 <sup>3</sup>	23	< 3	< 3	Absent	Absent
d1	1.04 × 10 <sup>4</sup>	< 3	< 3	< 3	Absent	Absent
d2	2.60 × 10 <sup>3</sup>	23	< 3	< 3	Absent	Absent



of quality control in production (Chisté et al., 2007; Dósea et al., 2010).

Flours a3, a4, and a5 showed colony growth typical of *B. cereus*, quantified in  $1.0 \times 10^3$ ,  $1.0 \times 10^4$ , and  $1.5 \times 10^4$  CFU g<sup>-1</sup>, respectively (Table 5). Based on the legislation, samples a4 and a5 are beyond the allowable limit of  $1.0 \times 10^3$  CFU g<sup>-1</sup> for this microorganism. The presence of *B. cereus*, evidenced in the samples, may indicate a potential fault in the handling or storage of the flours. Levels in the order of less than  $10^1$  CFU g<sup>-1</sup> have been observed in studies involving the same food matrix (Chisté et al., 2007; Santos et al., 2023; Sousa et al., 2021).

*B. cereus* contamination in food can mainly cause two distinct types of food poisoning: diarrheal and emetic, the latter being potentially more serious due to the heat-stable cereulide toxin. The presence of *B. cereus* and its ability to form heat-resistant spores make it a persistent concern in flour, as even after heat treatment in the roasting process, the product can remain contaminated (Ehling-Schulz et al., 2019).

Notably, the emetic toxin produced by *B. cereus* is thermostable and can resist the heat applied during flour roasting. This characteristic increases the risk even after processing, especially in foods with low acidity and stored at room temperature. The European Food Safety Authority (EFSA) recommends paying special attention to this microorganism in starch and popular consumer products (EFSA Panel on Biological Hazards, 2016).

These findings reinforce the need for effective implementation of good manufacturing practices in “flour houses,” as recommended by the Codex Alimentarius (FAO & WHO, 2023). Studies show that, even in traditional production contexts, the adoption of simple hygiene and control measures can significantly reduce the microbial load of products (Silva, Cardoso, et al., 2017).

## 4 CONCLUSIONS

This study revealed that three out of nine samples were off type due to the presence of foreign matter or higher peel/inter-peel counts. Also, there was a wide heterogeneity in the characterization of the cassava flour produced in Barreirinhas, with significant variations between samples in physicochemical parameters such as acidity between the dry and water groups.

The microbiological analysis identified contamination by *B. cereus* at levels above the legal limits in two samples and a high fungal load in part of the flours analyzed. Although all the samples were free of *Salmonella* spp. and *E. coli* within the legal standards, the data obtained may indicate flaws in the handling, storage, and marketing stages.

The multivariate analysis showed a grouping pattern between flours from the dry and water groups, suggesting that variables such as moisture, starch, and energy can perform as discriminants.

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