

## Sustainable and regional gastronomy: cassava leaves as a potential ingredient for gluten-free biscuits

Gastronomia regional e sustentável: folhas de mandioca como potencial ingrediente para biscoitos sem glúten

Gastronomía regional y sustentable: hojas de yuca como ingrediente potencial para galletas sin glúten

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### **Elisa Cristina Andrade Neves**

ORCID: <https://orcid.org/0000-0001-5849-9244>  
University of Campinas, Brazil  
Federal University of Pará, Brazil  
E-mail: [eneves@ufpa.br](mailto:eneves@ufpa.br)

### **Beatriz Andrade Moysés**

ORCID: <https://orcid.org/0000-0002-2694-7396>  
University of Campinas, Brazil.  
E-mail: [andradebiam@gmail.com](mailto:andradebiam@gmail.com)

### **Daniela Andrade Neves**

ORCID: <https://orcid.org/0000-0001-8007-8382>  
University of Campinas, Brazil  
E-mail: [dani\\_ela\\_neves@yahoo.com.br](mailto:dani_ela_neves@yahoo.com.br)

### **Pedro Henrique Campelo**

ORCID: <https://orcid.org/0000-0002-4048-3858>  
Federal University of Amazonas, Brazil  
E-mail: [pedrocampelo@ufam.edu.br](mailto:pedrocampelo@ufam.edu.br)

### **Maria Teresa Pedrosa Silva Clerici**

ORCID: <https://orcid.org/0000-0002-8445-336X>  
University of Campinas, Brazil  
E-mail: [mclerici@unicamp.br](mailto:mclerici@unicamp.br)

### **Abstract**

The addition of roots derivatives to bakery products may be a promising alternative to add value to these products. This study aimed to characterize and evaluate the effect of cassava leaves on the properties of gluten-free biscuits (GFB). The ingredients used were cassava flour, rice flour, *cuí*, tapioca flour, and 0 (F0), 3.69% (F1), 5.34% (F2), and 7.38% (F3) of cassava leaves (CCL). The results were evaluated by analysis of variance, followed by Scott-Knott test ( $p$ -value $<0.05$ ). The CCL presented 89.9% moisture, water activity of 0.98, and 2.75% protein; tryptophan was the limiting amino acid. No significant differences were observed between the F0-control and those made with the addition of CCL, which presented moisture and  $A_w$  below 3% and 0.30, and hardness between 45 to 60 N. The sensory acceptance scores were greater than 6, which indicates that it is possible to increase the added value of CCL in bakery products. The use of cassava leaves in biscuits showed good technological and sensory characteristics, making it possible to be an ingredient from regional by-products that can favor sustainable development, respect the concept of regionalization, and add income and employment.

**Keywords:** Sustainable food; Cassava by-products; Rich-protein leave; Celiac food; Cyanogenic compounds.

### **Resumo**

A utilização de derivados de raízes em produtos de panificação pode ser uma alternativa promissora para agregar valor a esses produtos. Este trabalho teve como objetivo caracterizar e avaliar o efeito das folhas de mandioca nas propriedades de biscoitos sem glúten (BSG). Os ingredientes utilizados foram farinha de mandioca, farinha de arroz, *cuí*, farinha de tapioca e 0 (F0), 3,69% (F1), 5,34% (F2) e 7,38% (F3) de folhas de mandioca (CCL). Os resultados foram avaliados pela análise de variância, seguida do teste de Scott-Knott ( $p$ -valor  $<0,05$ ). O CCL apresentou 89,9% de umidade, 0,98 de atividade de água e 2,75% de proteína; triptofano era o aminoácido limitante. Não foram observadas diferenças significativas entre o F0-controle e os com adição de CCL, que apresentaram umidade e  $A_w$  abaixo de 3% e 0,30, respectivamente, e dureza entre 45 a 60 N. Os escores de aceitação foram maiores que 6, o que indica que é possível aumentar o valor agregado do CCL em produtos de panificação. O uso de folhas de mandioca em biscoitos apresentou boas características tecnológicas e sensoriais, podendo ser um ingrediente de para elaboração de produtos regionais, que pode favorecer o desenvolvimento sustentável, respeitar o conceito de regionalização e agregar renda e emprego na cadeia produtiva da mandioca.

**Palavras-chave:** Sustentabilidade; Produtos de mandioca; Proteínas em folhas; Compostos cianogênicos.

### Resumen

La adición de derivados de raíces a los productos de panadería pueden ser una alternativa prometedora para dar un valor agregado a estos productos. Este estudio tuvo como objetivo caracterizar y evaluar el efecto de las hojas de yuca en las propiedades de galletas sin gluten (GFB). Los ingredientes utilizados fueron harina de yuca, harina de arroz, *cuí*, harina de *tapioca* (almidón de yuca), y hojas de yuca (CCL) en 0%(F0), 3.69% (F1), 5.34% (F2) y 7.38% (F3). Los resultados fueron evaluados por análisis de varianza, seguido de la prueba de Scott-Knott ( $p < 0.05$ ). Las CCL presentaron 89.9% de humedad, 0.98 de  $a_w$ , y 2.75% de proteína, con el triptófano como aminoácido limitante. No se observaron diferencias significativas entre las galletas F0-control y las elaboradas con adición de CCL, las cuales presentaron una humedad y  $a_w$  por debajo del 3% y 0.30, respectivamente, y una dureza entre 45 a 60N. Los puntajes de la aceptación sensorial fueron mayores a 6 puntos, lo que indica que es posible aumentar el valor agregado de las CCL en productos de panadería. El uso de hojas de yuca en la produjo galletas con buenas características tecnológicas y sensoriales, lo que las convierte en un ingrediente proveniente de subproductos regionales que puede favorecer al desarrollo sostenible, respetando el concepto de regionalización, y generar mayores ingresos y empleo.

**Palabras clave:** Comida sustentable; Coproductos de la yuca; Hoja rica en proteína; Comida para celíacos; Compuestos cianogénicos.

## 1. Introduction

The COVID-19 pandemic has brought a warning to world economies, especially about the food production chain. In times of crisis, countries tend to close their economies, mainly the food trade, to guarantee the internal supply of their population (Hobbs, 2020; Hoover, 2020). In times of a pandemic is a health risk related to the transport of these supplies. There is a possibility that food, packaging, and other equipment may be contaminated, offering risks to the local population's health (Laborde et al., 2020).

The consumption of gluten free foods is increasing in today's society and consumers are demanding more from their gluten free products (Muggah et al., 2016; Yilmaz & Koca, 2020). According to Di Cairano et al. (2018) and Valitutti et al. (2017) biscuits and crackers are considered as important sources of carbohydrates and can be used to make nutrients available to celiac individuals. Differently from the gluten-free breads, gluten-free biscuits (GFB) do not require the development of gluten, a three-dimensional network to retain gases, therefore their formulations allow the use of a larger variety of flours (Di Cairano et al., 2018), and however they show that products can have different flavors, colors and regional uses of ingredients. In addition to rice flour proteins, another alternative is the use of cassava leaves as an unconventional source of protein and dietary fiber.

Modesto Junior et al (2019) determined that the protein content in leaves of nine cassava varieties produced in Para, Brazil ranged from 19.73 to 29.47 g/100 g on a dry basis. Latif and Muller (2015) stated that the cassava leaves have the potential to be used for human consumption.

In Brazil, they are little explored for food purposes, being used only by the population of the northern region in the preparation of typical dishes. Due to the large production of cassava roots in Brazil, the increase in the consumption of leaves may also be an alternative for family farming, thus actions to improve its addition to high value-added products, such as bakery, are necessary, aiming at the intensification of the cassava production.

Besides the use of gluten-free flour from cereals, pseudo cereals and seeds, ingredients extracted from tuberous roots have been used in the production of GFB, such as cassava flour and cassava bran (Rodrigues et al., 2011) and cassava starch (Silva et al., 2015). Therefore, the use of cassava derivatives as a source of carbohydrates has great potential, especially in Brazil, which ranked fourth in the world production of cassava roots in 2017, with a stable production in recent years (19,000,000 tons) (FAOSTAT, 2020). Cassava flour also has the potential to be used in the preparation of GFB due to its higher starch levels (greater than 80%) and higher fiber content when compared to starch (Neves et al., 2017), as well as *cuí*, the co-product of tapioca flour processing, due to its pre-gelatinized starch content.

This study aimed to enable the use of cassava derivatives and cassava leaves as a protein source in gluten-free biscuits,

aiming to promote the insertion of these co-products in higher added value formulations.

## 2. Methodology

### 2.1 Material

The ingredients cooked cassava leaves (CCL); white cassava flour (WCF), used as a starchy base ingredient; tapioca flour (TF) and cui (C), a co-product of tapioca flour processing was purchased from the Ver-o-Peso Market complex (Belém-PA, Brazil, geographic coordinates, 01o27'21" S; 48o 30' 16" W). The other ingredients were purchased from the local market.

### 2.2 Characterization of rice flour and cassava derivatives

The cooked cassava leaves (CCL), white cassava flour (WCF), tapioca flour (TF), cui (C), and rice flour (RF) were characterized according to the following methodologies:

- Moisture determined in triplicate according to the Association of Official Analytical Chemists (AOAC, 2005) (method 925.09);
- Water activity ( $A_w$ ) determined in triplicate in the Aqualab apparatus (Aqualab, 4TEV, Decagon, Pullman, USA) using a dew point sensor;
- Color measurements in a CR-10 colorimeter (Konica Minolta, Tokyo, Japan) with D65 illuminant, using three light flashes on the CIE-Lab system;
- Cyanogenic compounds, determined in cassava derivatives according to Oliveira & Oliveira (2010);
- Paste properties of the starchy ingredients, determined in duplicate in a Rapid Visco-Analyzer (RVA 4500) using the Extrusion software from ThermoLine for Windows, version 2.3;
- Starch morphology assessed using an Olympus BX51 polarized light optical microscope with an Olympus E-330 digital camera at 40X magnification.

The cooked cassava leaves (CCL) were also characterized for:

- Proximate composition, in triplicate, according to AOAC official methods (AOAC, 2005): moisture (method 925.09); proteins (method 920.87); lipids (method 920.85); ash (method 923.03), and total dietary fiber (method 985-29). Digestible carbohydrate contents were calculated by difference [100 - (moisture + protein + lipid + ash + dietary fiber)];
- Amino acid profile according to White et al. (1986) and Hagen et al. (1993), and tryptophan contents according to Lucas and Sotelo (1980); Amino acid scoring pattern, calculated as a ratio of the amount of each essential amino acid (mg/g protein) in a sample and the amount of the respective amino acid in an ideal protein, according to the recommendations of FAO/WHO (1985) for older adults to 18 years.

### 2.3 Preparation of gluten-free biscuits

The gluten-free biscuits (GFB) were elaborated, adapted from Silva et al. (2015), replacing potato starch with cassava flour, considering as a starchy base the white cassava flour (WCF) (Table 1), being the control formulation (F0) and formulations with the addition of 3.69% (F1), 5.54% (F2), and 7.38% (F3) of CCL.

**Table 1.** Formulations of gluten-free biscuits control and with the addition of cooked cassava leaves

Ingredients	F0 (%)	F1 (%)	F2 (%)	F3 (%)
White cassava flour	24.39	23.60	23.60	23.60
Vegetable fat	22.87	22.13	22.13	22.13
Sugar	20.96	20.29	20.29	20.29
<i>Cuí</i>	7.62	7.38	7.38	7.38
Rice flour	6.10	5.90	5.90	5.90
<i>Tapioca</i> flour	5.72	5.53	5.53	5.53
Cocoa powder	3.80	3.69	3.69	3.69
Salt	0.19	0.18	0.18	0.18
Baking powder	0.13	0.12	0.12	0.12
Spices <sup>a</sup>	0.08	0.07	0.07	0.07
Ammonia carbonate	0.04	0.04	0.04	0.04
Water	8.10	7.38	5.53	3.69
Cooked cassava leaves	0.00	3.69	5.54	7.38

<sup>a</sup>Cinnamon powder, cloves powder, and nutmeg powder, in equal parts. Source: Authors.

For the preparation of GFB, the dry ingredients were mixed. Then, fat, water and cooked cassava leaves were added. Tapioca flour was added after a uniform and non-adherent dough was formed. The resulting dough was manually rolled out and cut into a circular shape ( $h = 6.5$  mm and  $\phi = 3.5$  cm), and the biscuits were baked at 160°C for 20 minutes. The baked GFBs were cooled for 1 hour, vacuum packed in multi-layer laminated packaging, and stored at 20°C.

## 2.4 Characterization of GFB

### 2.4.1 Physicochemical characterization

Moisture contents, water activity, color, and starch morphology were determined in ten GFB according to Section 2.2.

Hardness (expressed as N) was determined in ten GFB of each formulation using TA-XT Plus texture meter (Stable Micro Systems, England) using three-point bend rig (HDP/3PB) under the following conditions: 1 mm/s pre-test speed; 3 mm/s test speed; of 10 mm/s post-test speed; and penetration distance of 5 mm.

### 2.4.2 Sensory evaluation

The consumers' acceptance test of GFB (approved by the UNICAMP-Brazil Ethics Committee, CAAE 79825817.5.0000.5404), using 120 untrained volunteers. Consumers were asked to evaluate the GFB attributes, using a 9-point unstructured hedonic scale (1=disliked very much and 9=liked very much) according to Meilgaard et al. (2000). The consumption intention test was performed using a structured 5-point scale (1= would not consume and 5 = would certainly consume).

### 2.4.3 Nutritional characterization of the gluten-free biscuits

The nutritional characterization of the GFB were performed by theoretical calculations, based on the nutritional information presented on the ingredient labels and according to the formulations presented in Table 1, considering the weight loss between raw and baked biscuits and the final moisture of biscuits.

### 2.4.4 Amino Acid profile of control and gluten-free biscuits

The amino acid profiles and amino acid scoring were determined for both GFB control and GFB selected in the sensory evaluation according to the methodologies presented in Section 2.2.

## 2.5 Statistical analysis

The results were subjected to analysis of variance (ANOVA) using the SISVAR system, version 5.6, at a significance level of 5%. When significant, the Scott Knott test was used to determine the statistical differences between means (p-value<0.05).

## 3. Results and Discussion

### 3.1 Characterization of the ingredients

#### 3.1.1 Physicochemical characterization

As shown in Table 2, the water activity of starchy products was lower than 0.60 and of CCL were 0.98. For the preparation of GFB, CCL moisture content was used to adjust the amount of water to be added to each formulation.

**Table 2.** Moisture, water activity ( $A_w$ ), color parameters, and free cyanide contents of cassava derivatives and rice flour used in the manufacture of gluten-free biscuits<sup>a</sup>.

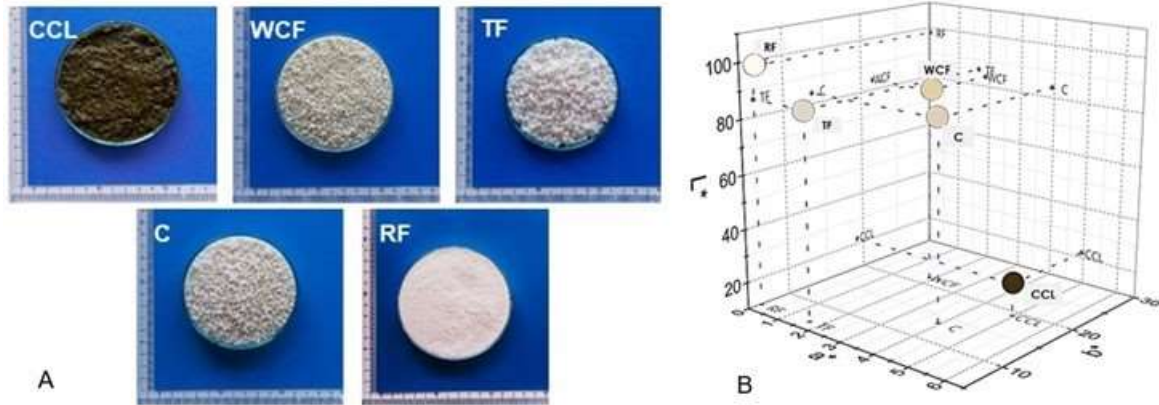
Ingredients <sup>b</sup>	Moisture (%)	$A_w$ 25°C	Color parameters			Free cyanide (mg HCN/kg)
			L*	a*	b*	
CCL	89.66±0.22	0.98±0	22.05±1.16	4.95±0.16	19.15±2.12	10.91
Starchy ingredients						
WCF	8.17±0.06 <sup>b</sup>	0.42±0.01 <sup>b</sup>	83.79±0.42 <sup>c</sup>	1.68±0.15 <sup>b</sup>	21.60±0.42 <sup>a</sup>	17.10
TF	3.80±1.42 <sup>c</sup>	0.40±0.01 <sup>c</sup>	86.28±0.81 <sup>b</sup>	1.65±0.02 <sup>b</sup>	6.48±0.57 <sup>c</sup>	< 10.00
C	4.80±0.08 <sup>c</sup>	0.35±0.02 <sup>d</sup>	83.90±0.34 <sup>c</sup>	3.94±0.13 <sup>a</sup>	13.54±0.01 <sup>b</sup>	< 5.00
RF	13.27±3.22 <sup>a</sup>	0.60±0.01 <sup>a</sup>	98.07±0.09 <sup>a</sup>	0.03±0.04 <sup>c</sup>	6.47±0.03 <sup>c</sup>	n.d.

<sup>a</sup>Values expressed as mean ± standard deviation; Different lowercase letters in the column differ from each other by the Scott-Knott test (p-value < 0.05); n.d. = not determined. <sup>b</sup>CCL= cooked cassava leaves; WCF= white cassava flour; TF= tapioca flour; C= cuí; RF= rice flour. Source: Authors.

According to results presented in Table 2 and Figure 1, the CCL was dark green tending to brown, and according to Butz et al. (2002), this color may indicate that chlorophyll degraded during the cooking process. WCF and cuí were cream color and darker when compared to TF and RF. The WCF was considered more yellowish (higher b\*), probably due to the presence of other nutrients rather than starch, such as proteins, lipids, and fibers, and was subjected to roasting at temperatures ranging between 90 and 150 °C (Neves et al., 2017). Tapioca flour, which was opaque white, was considered whiter and lighter

when compared to *cuí* (cream color).

**Figure 1.** Images of cassava derivatives and rice flour, considering (A) visual color and (B) color parameters ( $L^*$ ,  $a^*$ , and  $b^*$ ). Where: CCL= cooked cassava leaves; WCF= white cassava flour; TF= tapioca flour; C= *cuí*; RF= rice flour.



Source: Authors.

### 3.1.2 Cyanogenic compounds

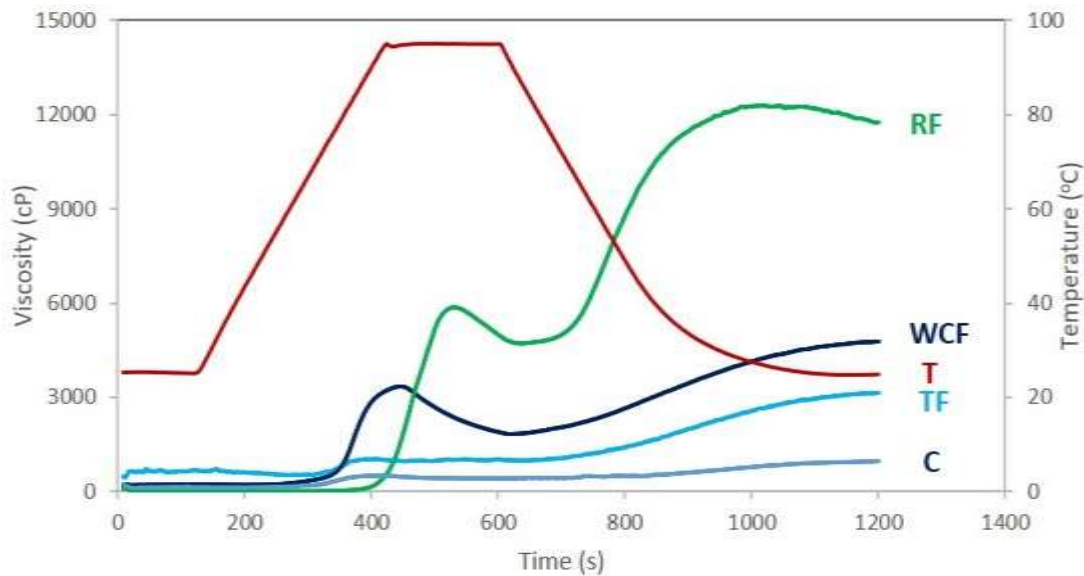
The cyanogenic compounds (Table 2) of CCL, TF and *cuí* were lower than 11 mg HCN/kg, while CCL and WCF presented levels lower than 20 mg HCN/kg with levels lower than those that could cause intoxication, as the World Health Organization stipulated, the lethal dose for ingesting cyanogenic compounds is 10 mg/kg of weight (Chisté et al., 2010). According to Cereda and Vilpoux (2013), the combination of the pressing and roasting steps of crushed cassava in the manufacture of flour leads to partial elimination of these compounds, as well as the cooking or drying of cassava leaves, which was also reported by Modesto Junior et al. (2019), Wobeto et al. (2004) and Ngudi et al. (2003).

### 3.1.3 Pasting properties

As can be seen in the pasting properties in Figure 2, rice flour showed higher viscosity and tendency to retrogradation (6892 cP) when compared to cassava flour (3058 cP). Due to the absence of the gluten network, gelatinization of the starch present in the ingredients is necessary to structure the biscuits during baking, as reported by Mancebo et al. (2015) and Witczak et al. (2016). An increase in viscosity of WCF was observed during heating ( $\mu_{max} = 3392$  cP and  $\mu_f = 4868$  cP), thus it has the potential to be used as a structural basis for the preparation of GFB together with rice flour.



**Figure 2.** Pasting properties of the starchy ingredients. RF= rice flour; WCF= white cassava flour; TF= tapioca flour; C= cuí; T=temperature.



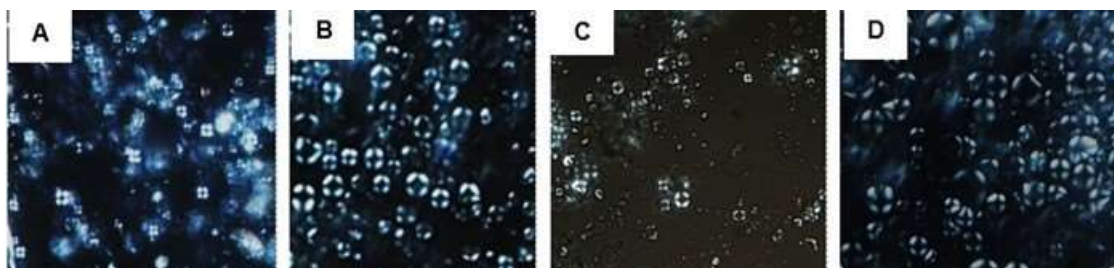
Source: Authors.

The cuí showed an initial viscosity of 196 cP and low final viscosity (1269 cP) (Figure 2), indicating that the product has pregelatinized starch. This behavior enables rapid water absorption during kneading of the GFB, contributing to the formation of the dough. The low retrogradation viscosity (699.62 cP) of starch present in cuí can reduce the hardness of GFB; otherwise, they would become brittle, which is one of the challenges in the manufacture of gluten-free product, especially when using rice flour as a starchy base. The TF presented the lowest viscosity when compared to WCF and RF, probably due to the starch gelatinization during processing with subsequent expansion (Neves et al., 2017).

### 3.1.4 Starch morphology

Despite the heat treatment, cassava root derivatives showed raw starch, what was observed by the birefringence, which demonstrates the presence of the well-defined Maltese cross. It was also observed the presence of raw starch in the rice flour (Figure 3). This result indicates that all starchy ingredients can contribute to the formation of the structure of GFB during kneading, but mainly during baking due to starch gelatinization, as reported by Witczak et al. (2016).

**Figure 3.** Light polarized light microscopy of (A) white cassava flour; (B) tapioca flour; (C) cuí; (D) rice flour.



Source: Authors.

### 3.2 Characterization of GFB made with cooked cassava leaves

#### 3.2.1 Moisture, water activity, and hardness

All GFB presented moisture and water activity (Table 3) lower than 4% and 0.4, respectively. The F0 exhibited higher moisture content and water activity when compared to the other biscuits. The F1 and F3 showed the lowest hardness while the F2 exhibited the highest hardness values, with no differences between moisture and water activity levels. GFBs with CCI showed hardnesses greater than those observed by Duta and Culetu (2015) (18.33 to 29.35 N), who studied GFBs made with oatmeal, while they were similar or superior to the GFB with rice flour and chocolate studied by da Silva and Conti-Silva (2018), which presented values between 31.8 to 49.2 N, indicating that compared to the two authors the use of mixed rice and cassava flour contributed to the increase in the hardness of the biscuits.

#### 3.2.2 Color measurements

All GFB of the present study were brown, as shown in Figure 4, probably due to the addition of chocolate to the formulations, as also reported by da Silva and Conti-Silva (2018) for GFB made with the addition of chocolate and inulin. According to Manley (2011), color changes in biscuits can also occur due to the Maillard reactions, starch dextrinization, and caramelization of sugars during baking.

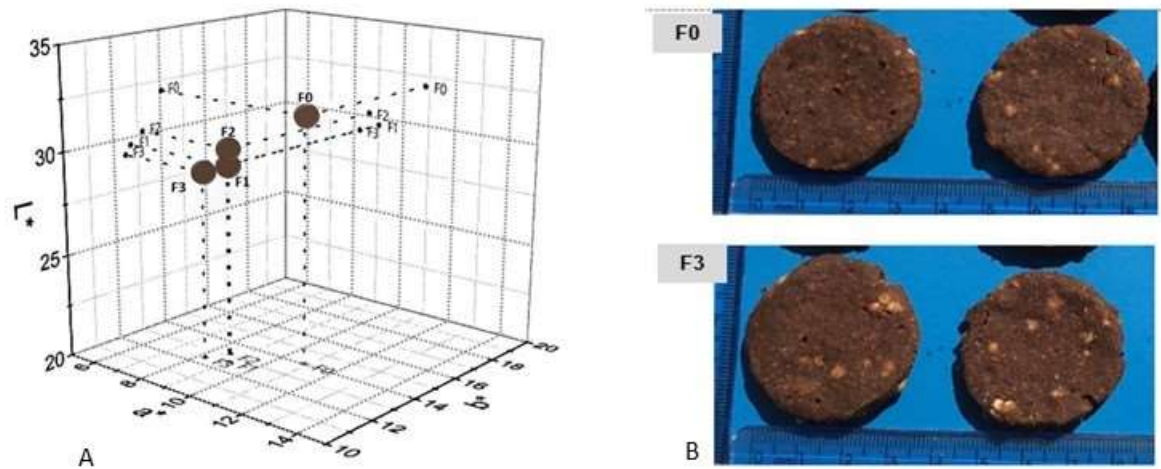
**Table 3.** Physicochemical parameters, acceptance scores, and consumption intent of GFB made with baked cassava leaves <sup>a,b</sup>.

Parameters	F0	F1	F2	F3
Moisture (%)	3.85±0.61 <sup>a</sup>	2.64±0.66 <sup>b</sup>	2.82±0.04 <sup>b</sup>	2.50±0.04 <sup>b</sup>
Water activity	0.31±0.01 <sup>a</sup>	0.28±0.01 <sup>b</sup>	0.28±0.01 <sup>b</sup>	0.27±0.01 <sup>b</sup>
Hardness (N)	52.23±2.02 <sup>b</sup>	45.04±5.46 <sup>c</sup>	58.84±6.26 <sup>a</sup>	46.99±3.8 <sup>c</sup>
Color parameters				
L*	31.99±0.04 <sup>a</sup>	29.62±0.18 <sup>c</sup>	30.16±0.36 <sup>b</sup>	29.18±0.36 <sup>c</sup>
a*	10.85±0.22 <sup>a</sup>	9.02±0.10 <sup>b</sup>	8.62±0.13 <sup>c</sup>	8.24±0.07 <sup>d</sup>
b*	14.05±0.11 <sup>a</sup>	12.70±0.30 <sup>c</sup>	13.21±0.17 <sup>b</sup>	12.48±0.32 <sup>c</sup>
ΔE* (color difference)	0.00	3.28	3.00	4.14
Acceptance test <sup>c</sup>				
Appearance	7.11±1.37 <sup>ns</sup>	6.92±1.49 <sup>ns</sup>	6.99±1.35 <sup>ns</sup>	6.76±1.56 <sup>ns</sup>
Color	7.29±1.41 <sup>ns</sup>	7.27±1.39 <sup>ns</sup>	7.34±1.34 <sup>ns</sup>	7.19±1.38 <sup>ns</sup>
Aroma	6.11±1.62 <sup>ns</sup>	6.39±1.57 <sup>ns</sup>	6.31±1.58 <sup>ns</sup>	6.28±1.5 <sup>ns</sup>
Crispness	7.56±1.46 <sup>a</sup>	6.93±1.88 <sup>b</sup>	7.46±1.48 <sup>a</sup>	7.52±1.4 <sup>a</sup>
Flavor	6.69±1.82 <sup>ns</sup>	6.56±1.71 <sup>ns</sup>	6.77±1.63 <sup>ns</sup>	6.76±1.47 <sup>ns</sup>
Overall acceptance	7.00±1.45 <sup>ns</sup>	6.60±1.45 <sup>ns</sup>	6.93±1.37 <sup>ns</sup>	6.94±1.25 <sup>ns</sup>
Consumption intent <sup>d</sup>	3.77±1.01 <sup>ns</sup>	3.44±1.02 <sup>ns</sup>	3.64±0.98 <sup>ns</sup>	3.61±0.89 <sup>ns</sup>

<sup>a</sup>Values expressed as mean ± standard deviation. Different lowercase letters on the same line differ from each other by the Scott-Knott test (P < 0.05). ns = not significant. Biscuits F0 = 0% CCL; F1 = 3.69% CCL; F2 = 5.54% CCL; F3 = 7.38% CCL <sup>c</sup>Acceptance test = 9 (liked very much) and 1 (disliked very much). <sup>d</sup>Consumption intent = 5 (would certainly consume) and 1 (would not consume). Source: Authors.



**Figure 4.** Color parameters ( $L^*$ ,  $a^*$ , and  $b^*$ ) according to the CIELab system of the biscuits (A) and images of the control (F0) and the biscuits made with 7.58% cassava leaves (F3) (B).



Source: Authors.

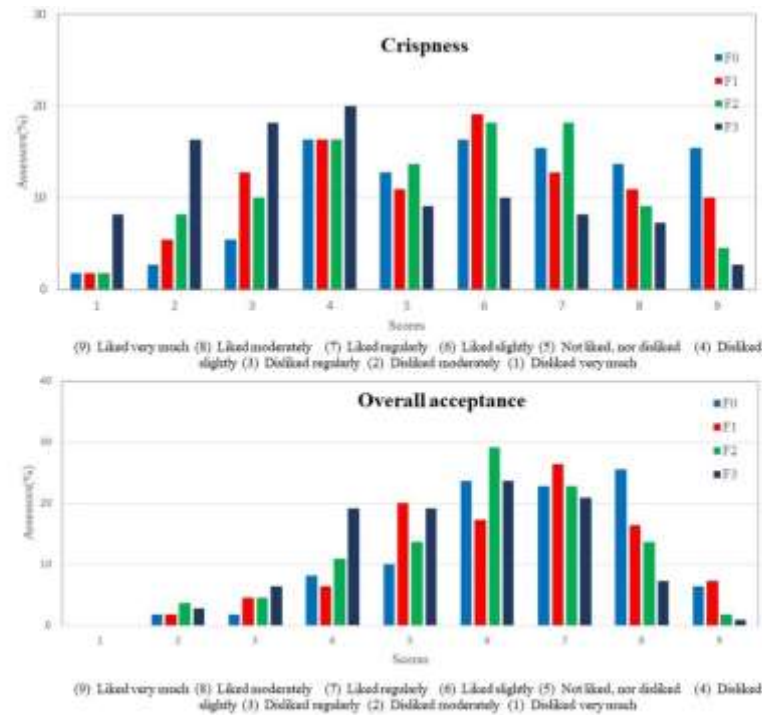
The GFB were darker when compared to the control (M0) probably due to the addition of cassava leaves with a dark olive-green color tending to brown (Figure 1 and Table 2), with higher  $a^*$  and  $b^*$  values (Figure 4), which may have contributed to significant differences in the color parameters  $L^*$ ,  $a^*$ , and  $b^*$  (Table 3) between the biscuits.

### 3.2.3 Sensory evaluation

According to the results in Table 3 and Figure 5, all GFB obtained scores higher than 6 in the acceptance test for all attributes. The addition of cassava leaves did not affect the acceptance as well as the consumption intent of the biscuits. The difference in color (Table 3) did not affect the acceptance of the GFB. A significant difference was observed only for the parameter crispness, and F1 and F3 were the least accepted among all formulations. As shown in Figure 5, the overall acceptance scores of GFB ranged from 5 (not liked nor disliked) to 8 (liked moderately), while the attribute crispness scored from 4 (disliked slightly) to 9 (liked very much).

The mean scores (Table 3) obtained in the acceptance test of GFB made with cassava leaves were higher than those reported by Mauro et al. (2010), who found scores of 6.53 and 6.22, and 7.07 and 6.61 for the attributes flavor and consistency of biscuits made with kale-stalk flour and spinach stalk, respectively. Rodrigues et al. (2011) evaluated the overall appearance, flavor, and texture of GFB made with cassava starch and bran and egg as a protein source and found values ranging from 6 (liked slightly) to 7 (liked moderately). According to the results of the characterization of the GFB, they presented good sensory acceptance for all attributes evaluated, regardless of the concentration of cassava leaves. Therefore, these results indicated that cassava derivatives, with emphasis on cassava leaves, associated with rice flour, have the potential for the production of gluten-free products.

**Figure 5.** Distribution of assessors for the sensory acceptance of CCL gluten-free biscuits for (A) crispness and (B) overall acceptance. Where F0= biscuit with 0% cooked cassava leaves; F1= biscuit with 3.69% cooked cassava leaves; F2= biscuit with 5.54% cooked cassava leaves; F3= biscuit with 7.38% cooked cassava leaves.



Source: Authors.

### 3.3 Nutritional information and amino acids profile of cassava leaves and gluten-free biscuits

The increased addition of cooked cassava leaves (CCL), which contained 2.75% protein, led to an increase in protein and fiber levels of the GFB when compared to F0 (Table 4). When comparing F3 with F0 (controls), the protein and fiber increases were 13.46% and 12.25%, respectively.

Whereas no differences were observed in the acceptance and consumption intent tests (Table 3), the F3 were selected for the analysis of amino acid profile, once they were made with the highest concentration of cooked cassava leaves (7.38%) thus presenting the highest protein levels (Table 4).

**Table 4.** Nutritional information of cooked cassava leaves and gluten-free biscuits<sup>a</sup>.

Values for 100g	CCL	F0	F1	F2	F3
Energy value (kcal/100g)	30.60	498	498	504	504
Carbohydrates (g)	4.70	63.98	64.78	64.01	64.1
Proteins (g)	2.75	2.12	2.14	2.39	2.45
Total fat (g)	1.20	25.96	26.29	26.33	26.38
Fiber (g)	1.32	4.0	4.05	4.39	4.49
Sodium(mg)	n.d.	90	90	80	80

<sup>a</sup>CCL= cooked cassava leaves; F0 = biscuit with 0% CCL; F1 = biscuit with 3.69% CCL; F2 = biscuit with 5.54% CCL; F3 = biscuit with 7.38% CCL. n.d. = not determined. Source: Authors.

According to the results in Table 5, the essential amino acids present in CCL were 43.64% of the total, and leucine, phenylalanine, and valine stood out with the higher ratios. Scores lower than 1 determine the limiting amino acids (FAO/WHO,

1985). When compared to the FAO/WHO (1985), tryptophan was the amino acids limiting (score <1).

The nonessential amino acids present in the highest concentration in the cooked cassava leaves were glutamic acid and aspartic acid, followed by alanine and arginine, while taurine were below the limit of quantification. Ngudi et al. (2003) found higher levels of the same essential amino acids in boiled leaves of different cassava varieties and stated that the variety and intensity of cooking may cause a reduction of these compounds in the leaves. For the 19 amino acids investigated in F0 and F3, only cystine and taurine were below the limit of quantification, while tryptophan was not detected in the formulation F3. Concerning the formulations F0 and F3, the essential amino acids corresponded to 40% and 36.55% of the total, respectively. The limiting amino acids (score <1) for F0 were cystine + methionine for F0 and for F3 were phenylalanine + tyrosine for F3.

**Table 5.** Amino acid profile of cooked cassava leaves (CCL), control (F0), and F3 biscuits<sup>a</sup>.

Amino acids <sup>b</sup>	CCL			F0			F3			Score FAO <sup>d</sup>
	mg/g sample	mg/g protein	S AA <sup>d</sup>	mg/g sample	mg/g protein	S AA <sup>d</sup>	mg/g sample	mg/g protein	S AA <sup>c</sup>	
<b>Essential amino acids (EAA)</b>										
VAL	0.16	65.35	3.63	0.80	57.55	3.20	0.90	62.50	2.31	18.00
TER	0.12	47.22	3.63	0.50	35.97	2.77	0.60	41.67	1.07	13.00
MET	0.05	18.13		0.20	14.39		0.20	13.89		
HIS	0.06	25.30		0.30	21.58		0.30	20.83		0.00
ILE	0.14	56.49	3.14	0.50	35.97	2.00	0.60	41.67	4.63	18.00
LEU	0.27	106.2	4.25	1.00	71.94	2.88	1.20	83.33	2.50	25.00
PHE	0.17	69.56		0.70	50.36		0.90	62.50		
TRP	0.01	5.48	0.84	1.10	79.14	12.17	n.q.	n.q.	n.q.	6.50
LYS	0.15	58.60	2.66	0.50	35.97	1.64	0.60	41.67	16.73	22.00
Total EAA	1.13	65.28		5.60	402.88		5.30	368.06	3.89	18.00
PHE+TYR	0.20	81.79	3.27	1.10	79.14	3.17	1.40	97.22	0.58	25.00
CYS+MET	0.08	29.09	1.21	0.20	14.39	0.60	0.20	13.89	2.31	24.00
<b>Conditionally essential amino acids (CEAA)</b>										
ARG	0.17	67.03		0.90	64.75		1.00	69.44		
GLY	0.16	63.24		0.60	43.17		0.70	48.61		
CYS	0.03	10.96		n.q.	n.q.		n.q.	n.q.		
PRO	0.13	53.96		0.70	50.36		0.80	55.56		
TYR	0.03	12.23		0.40	28.78		0.50	34.72		
Total CEAA	0.52	207.4		2.60	187.05		3.00	208.33		
<b>Nonessential amino acids (NEAA)</b>										
ALA	0.18	72.51		0.70	50.36		0.90	62.50		
ASP	0.24	94.86		1.50	107.91		1.50	107.91		
GLU	0.31	124.7		2.80	201.44		3.00	208.33		
SER	0.12	48.06		0.70	50.36		0.70	48.61		
TAU	n.q.	n.q.		n.q.	n.q.		n.q.	n.q.		
Total NEAA	0.85	65.28		5.70	410.07		6.10	423.61		
Total AA	2.50	1000		13.90	1000		14.40	1000		

<sup>a</sup> CCL= cooked cassava leaves; F0 = biscuit with 0% CCL; F3 = biscuit with 7.38% CCL; n.q. below the limit of quantification.

<sup>b</sup>Valine (VAL), threonine (TRE), methionine (MET), histidine (HIS), isoleucine (ILE), leucine (LEU), phenylalanine (PHE), tryptophan (TRP) and lysine (LYS), arginine (ARG), glycine (GLY), cysteine (CYS), proline (PRO), tyrosine (TYR), alanine (ALA), aspartic acid (ASP), glutamic acid (GLU), serine (SER), taurine (TAU), total amino acids (Total AA).<sup>c</sup> S AA= Score amino acid (mg/g sample protein)/mg/g protein FAO/WHO (1985)<sup>d</sup>Score adults = pattern requirements (mg/g protein) for adults (> 18 years) as suggested by FAO/WHO (1985). Source: Authors.

## 4. Conclusion

Cassava leaves and the mixture of starchy ingredients, such as cassava flour and cúí, used in GFB formulations, contributed to the production of gluten-free products for celiac. The sensory acceptance and the consumption intention scores were greater than 6 and 3, corresponding to “liked slightly” and “would consume, would not consume”, respectively, which indicates that it is possible to increase the added value of CCL in bakery products. In addition, the increase in cassava leaves consumption can also be an alternative for family farming, thus respecting the concept of regionalization, contributing to a healthy diet and taking into account the defense of species biodiversity and population habits, especially in developing countries.

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