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Development and physicochemical and microbiological evaluation of breads with macaúba pulp flour (*Acrocomia intumescens*)

Desenvolvimento e avaliação físico-química e microbiológica de pães com farinha de polpa de macaúba (*Acrocomia intumescens*)

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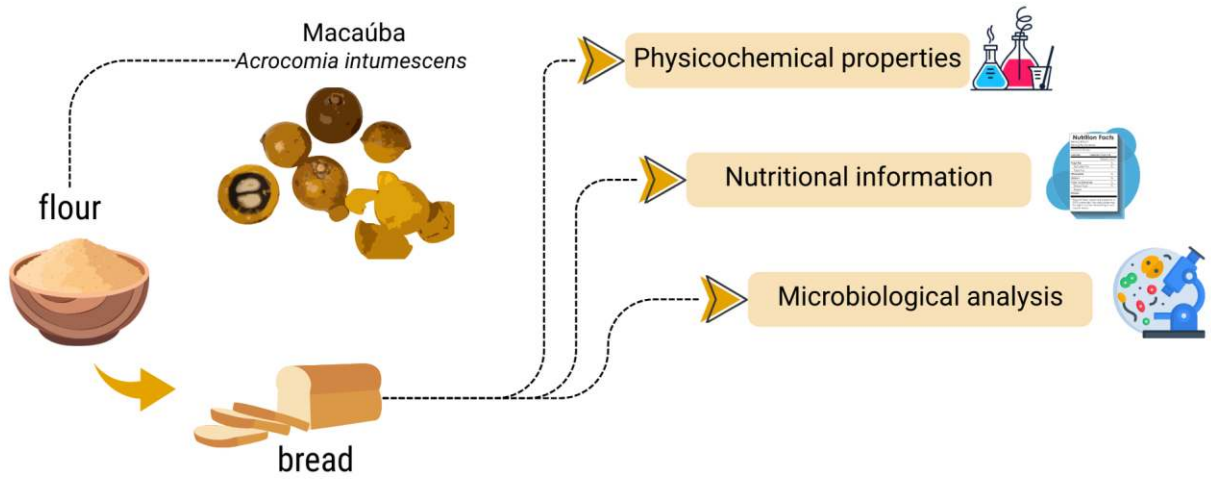
Abstract
The species *Acrocomia intumescens* (macauba) has significant economic importance and, during its industrial processing, especially for the extraction of fixed oil, it generates a significant amount of residues that can be used in the production of flour for incorporation into bakery products. This study aimed to develop bread formulations with macaúba pulp flour (10%, 13%, and 16%) and evaluate their physicochemical and microbiological characteristics. Bread formulations containing 13% and 16% macaúba pulp flour exhibited higher moisture content (~28%), while the 16% formulation also showed the highest carbohydrate content (~48%). All supplemented formulations demonstrated an increase in lipid content, ranging from 12.35% to 12.87% (~3% higher than the control), resulting in higher energy values. No significant differences were observed in pH (6.65 to 6.99), titratable acidity (0.42% to 0.49%), protein (9.70% to 10.82%), or ash content (2.74% to 2.92%) compared to the control. Microbiological analyses indicated that the breads were in adequate hygienic and sanitary conditions. The variations in the components indicate that the addition of macaúba pulp flour contributed to the physical-chemical characteristics of the final product.

Keywords: Macaúba. Macronutrients. Physicochemical and microbiological quality. Food technology. Sustainability.

Resumo
A espécie *Acrocomia intumescens* (macaúba) possui expressiva importância econômica e, durante o seu beneficiamento industrial, especialmente para a extração do óleo fixo, gera uma quantidade significativa de resíduos que podem ser aproveitados na produção de farinha para incorporação em produtos de panificação. Este estudo teve como objetivo desenvolver formulações de pães com farinha de polpa de macaúba (10%, 13%, e 16%) e avaliar suas características físico-químicas e microbiológicas. As formulações de pães contendo 13% e 16% de farinha de polpa de macaúba apresentaram maior teor de umidade (~28%), enquanto a formulação de 16% também apresentou o maior teor de carboidratos (~48%). Todas as formulações suplementadas demonstraram aumento no teor de lipídios, variando de 12,35% a 12,87% (~3% maior que o controle), resultando em valores energéticos mais elevados. Não foram observadas diferenças significativas no pH (6,65 a 6,99), acidez titulável (0,42% a 0,49%), proteína (9,70% a 10,82%) ou teor de cinzas (2,74% a 2,92%) em comparação com o controle. As análises microbiológicas indicaram que os pães apresentavam condições higiênico-sanitárias adequadas. As variações nos componentes indicam que a adição da farinha da polpa da macaúba contribuiu para as características físico-químicas do produto final.

Palavras-chave: Macaúba. Macronutrientes. Qualidade físico-química e microbiológica. Tecnologia de alimentos. Sustentabilidade.

Graphical Abstract



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ROYALDATASET

1. Introduction

The Cariri Cearense bioregion, located in the Northeast of Brazil, is known for its typical fruit trees that are rich in nutrients and bioactive compounds, representing a significant agroindustrial potential (Sousa et al., 2021a). Among these species, *Acrocomia intumescens*, an oil palm of significant economic and nutritional value, stands out. Attention is mainly focused on the fruit, the macaúba, which has peculiar sensory characteristics, such as flavor, core and aroma (Leal et al., 2021).

Macaúba holds significant socioeconomic value and is considered a promising source of raw material for food, medicinal and industrial applications (Leal et al., 2021). Both the pulp and the almond are highly nutritious, containing high levels of fiber, minerals, vitamins and especially crude proteins. The extraction of the oil, highly prized by the cosmetics industry, is one of the main ways of using this fruit (Silva et al., 2022).

The macauba oil extraction process generates significant waste, which can be repurposed in the food industry, either as animal feed due to its high protein content or as an ingredient in the formulation of foods such as cakes, biscuits, cookies and breads (Paulo et al., 2020). The economic value of this byproduct lies in its nutritional content, which includes vitamins, minerals, fibers and antioxidant compounds with recognized importance for human health (Sousa et al., 2021b).

Maximizing the use of such waste aims to add value to by-products that are normally discarded, transforming them into food ingredients, reducing waste and creating new food sources (Silva et al., 2021). One promising alternative is the production of flours from this waste, with potential for commercialization or use in food enrichment. This practice has gained prominence, especially in the formulation of bakery products (Sousa et al., 2021a).

Several studies have shown that vegetable residue or fruit and vegetable flours can enrich or fortify bakery foods, especially breads (Lima et al., 2020; Salgado et al., 2022). Bread is a widely consumed product, present in both snacks and main meals, and is valued for its appearance, aroma, flavor, affordable price, and wide availability (Paulo et al., 2020).

Due to its high consumption, bread represents an excellent alternative for incorporating food waste that contributes to nutritional enrichment, especially those rich in fiber and protein (Salgado et al., 2022; Tavares et al., 2023). Given the nutritional value of macaúba, the preparation of flours from its pulp and its application in bread formulations constitutes a promising strategy to add functional properties to the product (Sousa et al., 2021a).

Thus, this study aimed to develop bread formulations using macaúba pulp flour and evaluate their physical-chemical and microbiological characteristics.

2. Material and Methods

2.1. Plant material and botanical identification

The fruits of the species *Acrocomia intumescens* (macauba) were obtained in an area of Chapada do Araripe (Sitio Boa Esperança) in the city of Barbalha, CE, Brazil. An exsiccate (#9710) of the species is deposited in the Herbarium Caririense Dárdano Andrade Lima (HCDAL) of the Universidade Regional do Cariri (URCA).

2.2. Preparation of pulp flour

In the preparation of macauba pulp flour, the methodology proposed by Sousa et al. (2021a) was followed. The steps for obtaining the flour are shown in the flowchart from **Fig. 1**. The fruits were cleaned under running water and immersed for 15 min in a 1% sodium hypochlorite solution. The skins and seeds were manually removed using stainless steel knives. The pulp was cut into small pieces, placed on trays, and dehydrated in an oven at 60 °C for 24 h. Then, the pulp was crushed in an industrial blender (Model LSB-25 SN 010097, Skymsen) and subjected to oil extraction using a hydraulic press (Model RP0004, Ribeiro) for 2 h. The macaúba pulp flour was obtained from the defatted cake resulting from oil extraction. The residual cake was crushed and sieved through a 0.250 mm mesh sieve (60 mesh) to obtain the flour, which was then packed in plastic packaging and stored at room temperature (~25°C) with a relative humidity of 38%.

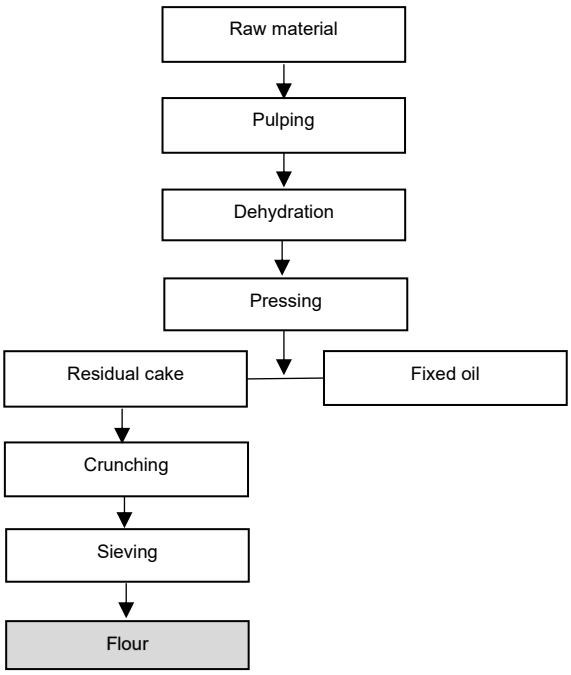


Fig. 1 Flowchart of the process steps for obtaining flour from macauba pulp

2.3. Breads formulation with pulp flour

Four bread formulations were prepared: a control sample (P0) without macaúba flour and formulations P1, P2, and P3 with flour proportions of 10%, 13%, and 16%, respectively. The flour percentages in the formulations were calculated based on the mass of the standard formulation.

The choice of these specific substitution levels was based on the balance between mass stability and nutritional enrichment. Levels ranging from 10% to 20% are commonly used in recent studies (Cacak-Pietrzak et al., 2024; Zhang et al., 2021). Therefore, 10% and 13% were chosen to explore the impacts of low to intermediate substitution, while 16% represents a maximum level that still maintained adequate handling of the mass and acceptable bread characteristics.

The bread formulations were made with basic ingredients such as sugar (30 g), yeast (15 g), salt (4 g), egg (45 g), butter (50 g), powdered milk (16 g) and water (150 mL), remaining constant in all samples. The variation between the formulations occurred in the proportion between wheat flour and macaúba pulp flour, as described in **Table 1**.

Table 1 Bread formulations using macaúba pulp flour as compared to a control sample

Formulation	Wheat flour (g)	Macaúba pulp flour (g)
P0 (0%)	250	0
P1 (10%)	210	40
P2 (13%)	198	52
P3 (16%)	186	64

The dough was prepared using the direct method, where all ingredients, previously weighed, were processed in an electric mixer (Model OBAT610 PT, Oster) at high speed for 25 min. After processing, the dough was allowed to rest for 30 min in a controlled temperature environment of 37 °C. Next, the dough was weighed, divided, and shaped into approximately 60 g portions each. The portions were placed on baking trays greased with oil for the resting and fermentation period in a controlled chamber at 37 °C. Following fermentation, the breads were brushed with egg wash and placed in a preheated oven at 180 °C (Model CS-4 S, Venâncio) for 10 min. After baking, the breads were cooled to room temperature and stored in plastic bags.

2.4. Physicochemical properties of flour and breads

Physicochemical analyses of pulp flour and bread were performed in triplicate, as recommended by the Instituto Adolfo Lutz (2010). Moisture content was determined by the desiccation method through direct drying in an oven (Memmert, Model 400 S/N B4980457) at 105 ºC for 24 h. Lipid content was measured using the Soxhlet extraction method with hexane, employing intermittent flow. Protein content was assessed using the Kjeldahl method, where the sample was digested and distilled, with a conversion factor of 6.5 to account for nitrogen content. Ash content was determined by incineration in a muffle furnace (Solidsteel, Model SSFMr) at 550°C. Carbohydrates were calculated by difference, subtracting the moisture, protein, lipid, and ash content from the total sample weight. The pH was measured using a potentiometer, and acidity (expressed as % oleic acid equivalent) was determined by the NaOH titration method.

2.5. Nutritional information of the breads

The total energy value (TEV) was calculated based on a 50 g portion (1 slice) relative to a 2,000 kcal/day diet. The energy value, expressed in kcal/100g, was calculated by multiplying the percentage of each macronutrient (proteins, carbohydrates, lipids) by the respective energy provided (% proteins × 4 kcal + % lipids × 9 kcal + % carbohydrates × 4 kcal) (Silva et al., 2022).

2.6. Microbiological analysis of breads

The microbiological analyses carried out on the breads followed the standards for the food group “biscuits and crackers, without filling, with or without topping, including bread, biscuits and similar products” (Brazil, 2019). The analyses focused on the following microorganisms: *Escherichia coli*, molds and yeasts.

Escherichia coli: Detection was performed using the selective culture medium EC Broth, with incubation at 44–45°C, the optimal temperature for the growth of thermotolerant bacteria. The presence of *E. coli* was confirmed by the production of gas in the fermentation tubes. After incubation, the number of positive tubes with gas production was recorded, and the result was expressed as the Most Probable Number (MPN/g or mL), based on specific tables for the dilutions used (American Public Health Association, 2001).

Molds and yeasts: Counting was performed using the standard plating method. For this, 10 g of the sample were homogenized in 90 mL of sterile peptone water, followed by serial dilutions. The samples were plated on potato dextrose agar acidified with 10% tartaric acid solution and incubated at 25 ± 1 °C for 5 to 7 days. The results were expressed in Colony Forming Units per gram (CFU/g) (American Public Health Association, 2001).

2.7. Statistical Analysis

Data were expressed as means ± standard deviation. Statistical analysis was performed using analysis of variance (ANOVA), followed by Tukey’s post hoc test at a 5% significance level, using GraphPad Prism version 5.0. Differences were considered statistically significant at $p < 0.05$.

3. Results and Discussion

3.1. Physico-chemical characterization of flour and breads

Macauba pulp flour presented a moisture content of 5.62% (**Table 2**). This low value is desirable, as it indicates good hygroscopic and microbiological stability, since reduced moisture is associated with lower water activity, which limits microbial multiplication and decreases the occurrence of degradative physicochemical reactions (Le Lay et al., 2016). Similar results were reported for pequi (5.11%) and babassu (6.87%) pulp flours, confirming the tendency of flours to present low moisture contents, a characteristic that contributes to extended shelf life and reduced risk of microbial contamination (Sousa et al., 2021a).

Table 2 Nutritional composition of macauba pulp flour and bread in different flour concentrations per 100 g of product.

Parameters	Flour	Formulations			
		P0	P1	P2	P3
Moisture (%)	5.62±0.07	25.21±0.27^{b}	26.48±0.50^{b}	27.22±0.13^{a}	28.29±0.53^{a}
pH	5.32±0.02	6.99±0.02^{a}	6.79±0.00^{a}	6.65±0.03^{a}	6.67±0.04^{a}
Titratable acidity (%)	1.37±0.90	0.46±0.14^{a}	0.42±0.00^{a}	0.49±0.02^{a}	0.49±0.00^{a}
Carbohydrates (%)	76.38±1.00	50.99±1.20^{b}	48.79±1.00^{b}	48.01±0.80^{b}	46.33±1.50^{a}
Lipids (%)	9.56±0.24	10.06±0.24^{b}	12.56±1.31^{a}	12.35±0.08^{a}	12.87±0.04^{a}
Proteins (%)	5.67±0.57	10.82±0.08^{a}	9.30±0.49^{a}	9.68±0.21^{a}	9.70±0.13^{a}
Ashes (%)	2.77±0.01	2.92±0.08^{a}	2.87±0.02^{a}	2.74±0.06^{a}	2.80±0.00^{a}
TEV (kcal/100g)	-	117.30±0.10^{a}	128.10±0.55^{b}	129.40±1.15^{b}	133.90±0.10^{c}

(TEV) Total Energy Value. (P0) standard formulation. (P1) 10% formulation. (P2) 13% formulation. (P3) 16% formulation. Results are expressed as mean ± standard deviation. Means followed by the same letter in the same row does not differ statistically by Tukey's test ($p < 0.05$).

In the breads, an increase in moisture content was observed with the addition of macauba flour, being more pronounced in formulations with higher substitution levels. This effect can be attributed to the higher water retention capacity of macauba flour, possibly related to its protein content, since proteins have a high affinity for water molecules, favoring retention and positively influencing product texture. Similar moisture increases with the incorporation of alternative flours were reported in cookies formulated with guavira residue flour (8.36–11.95%) (Medino et al., 2019), in sequilhos prepared with macauba almond flour (5.05–8.13%) (Silva et al., 2022), and in cookies with partial replacement of rice flour by soy flour (8.68% in the control to 9.30% with 20% soy flour) (Adeyeye, 2020).

The pH and titratable acidity of macauba pulp flour were 5.32 and 1.37%, respectively. In breads, no statistically significant differences were observed in these parameters compared with the control formulation. The pH values obtained classify both the flour and the breads as low-acid products (pH > 4.5), according to the classification of Silva et al. (2021). This characteristic is desirable in bakery products, as environments with lower acidity hinder microbial growth, thereby favoring stability and safety. These results are consistent with findings by Souza et al. (2014), who emphasized the importance of pH in extending the microbiological shelf life of bakery products.

Previous studies also corroborate the pH and acidity findings of the present work. Barbosa et al. (2013) observed that sliced breads with partial replacement of wheat flour by cashew flour presented pH values similar to those of the control formulation. Similarly, Santos et al. (2018) reported acidity values between 0.20% and 0.47% in whole-grain breads with papaya flour, which are in line with the values obtained in this study.

The carbohydrate content of macaúba pulp flour was 76.38%. This value is consistent with the general composition of flours, which are typically rich in carbohydrates, as these compounds constitute the main fraction of plant dry matter (Lafia et al., 2020; Silva et al., 2022). Comparable values were reported for babassu pulp flour (75.85%), while pequi pulp flour presented a lower content (59.07%) (Sousa et al., 2021b).

In breads, carbohydrate contents ranged from 46.33% to 50.99%. A significant reduction was observed in the formulation with the highest level of substitution by macaúba flour. This decrease may be associated with the relative increase in other components, particularly lipids, since variations in lipid, protein, moisture or ash fractions generally alter the final carbohydrate content of food matrices (Silva et al., 2022).

Similar behavior has been reported in other products formulated with alternative flours. Cake formulations containing mangaba pulp flour showed carbohydrate contents between 45.89% and 51.53% (Silva et al., 2021). Likewise, wholemeal breads enriched with papaya flour presented values between 40.63% and 42.01%, whereas breads produced with malt bagasse ranged from 58.11% to 58.68% (Kuiavski et al., 2020; Santos et al., 2018). These results demonstrate that the incorporation of non-conventional flours can significantly affect the carbohydrate profile of bakery products, depending on the nutritional composition of the added raw material.

Macaúba flour had a protein content of 5.67%. In comparison, flours obtained from pequi and buriti pulp had contents of 10.76% and 2.32%, respectively (Sousa et al., 2021b). In breads made with the addition of macaúba flour, the protein contents did not differ significantly from the standard formulation, indicating that the inclusion of this flour did not promote significant changes in the protein content of the products. This result is relevant, as the partial substitution of wheat flour with macaúba flour allowed for the diversification of the product without compromising the protein levels, which is an essential component for the nutritional quality of bread. These results indicate that breads enriched with the flour can maintain protein levels comparable to those of traditional formulations.

On the other hand, studies such as that by Borges et al. (2011) reporting a significant increase in protein content in savory breads added with whole linseed flour, with values ranging from 13.73% to 14.19%, when compared to the control. These results demonstrate that the impact of adding flours on protein content depends directly on the nutritional composition of the ingredient used (Silva et al., 2022).

The lipid content in the flour was 9.56%. Since the pulp is naturally rich in fixed oil, it is common for the resulting flour to have a high lipid content (Leal et al., 2021). However, the oil extraction process contributed to reducing this content, resulting in a flour with a lower lipid content. This characteristic is advantageous, as it provides greater stability to the product, reducing susceptibility to lipid oxidation and, consequently, extending its shelf life (Sousa et al., 2021a). In addition, the use of the pulp through oil extraction allows for more efficient use of the raw material, since the waste generated in this process can be used in the preparation of new products, in this study, it was used for the production of flour and bread.

All formulations with the addition of macauba flour (10%, 13%, and 16%) showed significantly higher lipid content than the control formulation, possibly due to the residual oil present in the defatted cake from the industrial pulp processing. This result is consistent with the observations of Sousa et al. (2021b), which indicate that lipid content in defatted flour is due to the incomplete removal of oil during the extraction process. On the other hand, studies with bakery products made with alternative flours indicate that lipid content can vary considerably when partially replacing wheat flour, depending on the composition of the raw material used (Silva et al., 2022). In the present study, the increase in lipids, together with the higher total energy value observed in P2 and P3 formulations, suggests that these products may be particularly suitable for consumers with higher energy demands, such as athletes or individuals with greater caloric requirements.

The ash content found in the flour was 2.77%. This value was relevant and similar to that observed for other flours. Ash contents ranging from 2.17% to 3.85% have been reported in pequi and buriti pulp flour and babassu almond flour (Sousa et al., 2021a). This parameter is relevant because it can be used as a general measure of the quality of the minerals contained in the product, determination of foods rich in minerals, and is also a starting point for the analysis of specific minerals (Silva et al., 2021).

The ash contents in the formulations with the addition of flour did not differ statistically from the control formulation, indicating that the substitution made did not significantly impact the mineral content of the breads. Similar contents were observed in studies with whole-wheat breads added with papaya flour, whose ash contents ranged from 1.99% to 2.11%; with chia flour in potato bread, with values from 1.66% to 2.20%; and with the addition of malt bagasse, whose contents ranged from 1.85% to 2.26%. In contrast, formulations with higher proportions of malt bagasse presented higher contents, ranging from 3.6% to 4.5% (Kuiavski et al., 2020; Pereira et al., 2013; Santos et al., 2018).

The energy value of the formulations showed statistically significant variation depending on the level of substitution of wheat flour for macauba flour. These values represent, respectively, 5.86%, 6.40% and 8.28% of the recommended daily caloric intake for an adult, considering a 2.000 Kcal diet. The observed variations may be associated with the reduction in the carbohydrate content and the increase in the lipid content in the formulations, considering that these macronutrients have different caloric values. In a similar study, in breads added with cashew nut flour, energy values varied from 296.51 to 336.69 kcal/100 g between treatments (Barbosa et al., 2013). Therefore, the development of breads with macaúba flour not only enhances the nutritional diversity of bakery products but also adds value to a regional and sustainable raw material. Such formulations may be targeted at consumers who seek innovative products with differentiated nutritional profiles, especially those interested in foods from Brazilian sociobiodiversity.

3.2 Microbiological analysis of breads

Microbiological analyses are essential to assess the hygienic-sanitary quality of food and to guarantee its safety, in addition to providing important information for estimating shelf life and preventing risks to public health. In this study, the breads presented satisfactory microbiological quality, complying with the limits established by Good Manufacturing Practices (**Table 3**). These results indicate that the processing conditions adopted were adequate and ensured product safety.

Table 3 Microbiological quality of breads under different concentrations of macaúba pulp flour

Microorganisms	Unit	Formulations				AML
		P0	P1	P2	P3	
Molds and yeasts	CFU/g	< 10	< 10	< 10	< 10	10^4
Thermotolerant coliforms	MPN/g	< 3	< 3	< 3	< 3	10^2

(P0) standard formulation. (P1) 10% formulation. (P2) 13% formulation. (P3) 16% formulation. CFU: Colony Forming Units. MPN: Most Probable Number. AML: Allowed Microbiological Limit (Ministério da Saúde, 2019).

Foodborne diseases (FBDs) are commonly associated with pathogenic microorganisms or their toxins, representing a serious risk to consumer health (Lima et al., 2020). The presence of *Escherichia coli*, for example, is indicative of fecal contamination and reflects inadequate hygiene practices during processing, which may occur due to handlerds, insects or contaminated water (Silva et al., 2022). In addition, molds can produce mycotoxins, toxic compounds that cause both acute poisoning and long-term health effects, such as DNA damage, immune system impairment and carcinogenicity. Although yeasts are generally considered non-pathogenic, under favorable conditions they may act as opportunistic agents, causing infections and physiological imbalances (Galvão et al., 2019).

Similar results were reported by Silva et al. (2022), who evaluated sequilhos-type biscuits enriched with macaúba almond flour and verified that all formulations met the microbiological standards required by Brazilian legislation, confirming that the products were safe for consumption. This evidence reinforces the importance of adopting good manufacturing practices and highlights that the incorporation of non-conventional flours, such as macaúba derivatives, does not compromise the microbiological safety of bakery products when adequate processing conditions are maintained.

4. Conclusion

The incorporation of *Acrocomia intumescens* (macaúba) flour into bread formulations demonstrated that protein content remained stable, even at higher substitution levels, which is nutritionally relevant, since protein value was not compromised. A gradual reduction in carbohydrates, along with an increase in lipids and total energy value, were also observed, suggesting a distinct nutritional profile that may meet the needs of consumers interested in foods with higher energy density, such as athletes or populations with greater caloric demands. The increase in moisture content in the formulations may positively contribute to the texture and softness of the bread, characteristics that are desirable from a sensory perspective. The formulated maintained microbiological quality within the limits established by good manufacturing practices. It is recommended that future research include sensory analyses in order to assess consumer acceptance. In this context, the use of macaúba in baking represents an innovative and sustainable alternative that contributes to valuing Brazilian sociobiodiversity and developing new products aimed at consumers seeking variety and differentiation in the bread market.

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Authors' Contributions

P. P. G. M.: Investigation, Data Curation, Writing and Editing. C. L. S. F.: Investigation, Data Curation, and Writing. D. L. L. S.: Data Curation. A. R. J. M.: Data Curation. M. K. S. B. F.: Data Curation. N. M. G. C.: Data Curation. M. P. Q. R.: Data Curation. C. G. G.: Data Curation. E. O. S.: Data Curation, Editing, and Supervision. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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