Elaboration and physical-chemical, microbiological and sensorial characterization of sweet gluten-free cookies prepared with babassu mesocarp flour and rice flour

Preparación y caracterización físico-química, microbiológica y sensorial de galletas dulces sin gluten preparadas con harina de mesocarpió de babású y harina de arroz

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Resumo
Os cookies, em geral, são bem aceitos por todos os públicos, e pesquisas e investimentos nesse tipo de produto são de grande importância para atender às demandas do mercado. Existem poucas opções de produtos para pessoas com intolerância ao glúten no mercado, e a farinha de arroz e a farinha de mesocarpo de babaçu são boas opções para o desenvolvimento de novos produtos para esse público. O objetivo do presente trabalho foi preparar um biscoito doce sem glúten de alto valor nutricional, à base de farinha de mesocarpo de babaçu com substituição parcial pela farinha de arroz, para realizar a caracterização físico-química, microbiológica, aceitação sensorial e intenção de compra dos consumidores. Foram estudadas três formulações diferentes, com 50, 75 e 100% de farinha de mesocarpo de babaçu. Foram realizadas análises físico-químicas da umidade, atividade da água, cinzas, lipídios, proteínas e carboidratos (por diferença), a qualidade microbiológica também foi avaliada de acordo com a legislação e a aceitação sensorial por meio de um questionário com 80 provadores não treinados, quanto à aparência, cor, aroma, textura, doçura, sabor, impressão global e atributos de intenção de compra. Os resultados mostraram que os biscoitos com variações de farinha de mesocarpo de babaçu e farinha de arroz são comparáveis a outros estudos encontrados na literatura, em termos de características físico-químicas e microbiológicas. Na avaliação...
sensorial, os cookies receberam notas na faixa de aceitação para todas as formulações, demonstrando que o produto tem potencial para estar no mercado.

**Palavras-chave:** Linhaça; Biscoito enriquecido; Intolerância à gluten.

**Abstract**

Cookies, in general, are well accepted by all audiences, and research and investments in this type of product are of great importance to meet market demands. There are few product options for people with gluten intolerance on the market, and rice flour and babassu fruit mesocarp flour are good options for the development of new products for this audience. The objective of the present work was to prepare a gluten-free sweet biscuit with high nutritional value, based on babassu mesocarp flour with partial replacement by rice flour, to carry out the physical-chemical, microbiological characterization, sensory acceptance, and purchase intention of consumers. Three different formulations were studied, with 50, 75 and 100% babassu fruit mesocarp flour. Physical-chemical analyzes of moisture, water activity, ash, lipids, proteins and carbohydrates (by difference) were carried out, the microbiological quality was also evaluated according to the legislation and sensory acceptability through a questionnaire with 80 untrained tasters, as for the appearance, color, aroma, texture, sweetness, flavor, global impression and purchase intention attributes. The results showed that the cookies with variations of babassu fruit mesocarp flour and rice flour are comparable to other studies found in the literature, in terms of physical-chemical and microbiological characteristics. In the sensory evaluation, the cookies received grades in the acceptance range for all formulations, demonstrating that the product has the potential to be in the market.

**Keywords:** Flaxseed; Enriched biscuit; Gluten intolerance.

**Resumen**

Las cookies, en general, son bien aceptadas por todos los públicos, y la investigación y las inversiones en este tipo de producto son de gran importancia para satisfacer las demandas del mercado. Hay pocas opciones de productos para personas con intolerancia al gluten en el mercado, y la harina de arroz y la harina de mesocarpio de babasú son buenas opciones para desarrollar nuevos productos para esta audiencia. El objetivo del presente trabajo fue preparar una galleta dulce sin gluten de alto valor nutricional, a base de harina de mesocarpio de babasú con reemplazo parcial por harina de arroz, para llevar a cabo la caracterización físico-química, microbiológica, la aceptación sensorial y la intención de compra de los consumidores. Se estudiaron tres formulaciones diferentes, con 50, 75 y 100% de harina de
mesocarpio de babasú. Se realizaron análisis fisicoquímicos de humedad, actividad del agua, cenizas, lípidos, proteínas y carbohidratos (por diferencia), también se evaluó la calidad microbiológica de acuerdo con la legislación y la aceptación sensorial a través de un cuestionario con 80 catadores no capacitados, en términos de apariencia, color, aroma, textura, dulzura, sabor, impresión general y atributos de intención de compra. Los resultados mostraron que las galletas con variaciones de harina de mesocarpio de babasú y harina de arroz son comparables a otros estudios encontrados en la literatura, en términos de características físico-químicas y microbiológicas. En la evaluación sensorial, las cookies recibieron calificaciones en el rango de aceptación para todas las formulaciones, lo que demuestra que el producto tiene el potencial de estar en el mercado.

**Palabras clave:** Linaza; Galleta enriquecida; Intolerancia al gluten.

### 1. Introduction

Cookies have become one of the most popular and well-accepted snacks worldwide, among all age groups, for having a good source of energy and practicality (Cheng & Bhat, 2016), in addition to having a long shelf life, allowing them to be produced and distributed on a large scale (Chauhan, Saxena, & Singh, 2015).

This makes this product a good vehicle for the study of mixed flours, both for economic and nutritional reasons (Jan, Panesar, & Singh, 2018), since the consumer becomes more and more demanding, making the market develops and makes available products that meet all needs, such as gluten-free products, as an example. Among the flours that make it possible to produce gluten-free products are rice flour and babassu fruit mesocarp flour.

Rice flour is commonly used for these purposes, combined with other ingredients such as corn, starch and proteins, to improve its organoleptic properties, since it has a high carbohydrate content, but low protein content (Hamdani, Wani, & Bhat, 2020). Babassu fruit mesocarp, on the other hand, is a source of edible starch and represents a promising alternative for use in gluten-free products, since it has higher concentrations of fiber and ash when compared to wheat, thus producing food with a higher nutritional content (Silva, Barros, Pereira, Lemos, & Abreu, 2019).

Therefore, the development of cookies rich in essential compounds such as amino acids, minerals, fibers and fatty acids is of paramount importance, mainly due to the dietary restrictions of patients with celiac disease (Pagamunici et al., 2014).

Celiac disease (CD) is an inflammatory disease of the small intestine triggered by proteins from wheat, barley and rye gluten (de la Barca, Rojas-Martínez, Islas-Rubio, & Cabrera-Chávez,
2010), which attacks the intestinal mucosa, causing lesions and atrophies of the microvilli, damaging the nutrient absorption by the individual (Couri & Giada, 2016).

In addition, the consumer is also focused on healthier products, making the market seek the incorporation of substances in food, which can provide benefits to human health (Chauhan et al., 2015). As an example, flaxseed (*Linum usitatissimum* L), which has been incorporated as a functional food ingredient (Kasote, 2013), due to the large amount and variety of compounds such as alpha linolenic acid, lignans, dietary fibers, and phenolic compounds such as acids phenolics, flavonoids, phenylpropanoids and tannins (Kaur, Singh, & Kaur, 2016).

In Brazil, food enrichment must comply with the specific Resolution of the National Health Surveillance Agency - ANVISA, which defines “enriched food, any food to which nutrient substance is added, to reinforce its nutritional value, whether it be quantitatively replenishing the nutrients destroyed during food processing, either by supplementing them with nutrients at a higher level than its normal content” (Brasil, 1998).

In this sense, the present work had as objectives to elaborate a gluten-free cookie type biscuit, based on Babassu Fruit Mesocarp Flour (BMF) with a partial substitution for rice flour, and enriched with flaxseed flour, and to carry out its physical-chemical characterization, microbiological and sensory.

2. Material and Methods

This research consists of laboratory research, through physical-chemical analyzes based on the methodologies of the Adolf Lutz Institute (IAL, 2008), and field research, through sensory analysis following the methodology described in Peryam & Pilgrim (1957) and Meilgaard, Civille, & Carr (1991). The cookies were produced at the Cereals Technology Laboratory of the Federal University of Maranhão - Advanced Campus.

2.1 Material

Babassu Mesocarp Flour (BMF) was supplied by the Petrolina Coconut Breakers Association, located in the village of Petrolina, Imperatriz - Maranhão and the other ingredients such as rice flour, brown sugar, margarine, egg, flaxseed flour, vanilla essence and bicarbonate were purchased from local stores.
2.2 Methods

2.2.1 Development of formulations

Three formulations were developed as shown in Table 1, using as a base a conventional American recipe for cookies. The percentage of BMF and rice flour varied, while the other ingredients were kept constant for all formulations.

Table 1 - Cookie formulations containing variations of BMF and rice flour.

| Ingredients (g)          | Type 1 | Type 2 | Type 3 |
|--------------------------|--------|--------|--------|
| BMF                      | 50     | 75     | 100    |
| Rice Flour               | 50     | 25     | 0      |
| Brown Sugar              | 59     | 59     | 59     |
| Margarine                | 59     | 59     | 59     |
| Egg                      | 35     | 35     | 35     |
| Flaxseed Flour           | 15     | 15     | 15     |
| Vanilla essence          | 2      | 2      | 2      |
| Sodium bicarbonate       | 1      | 1      | 1      |

Source: Authors.

First, the ingredients were weighed, followed by the mixture of brown sugar with margarine, egg, vanilla essence and baking soda, in a planetary mixer (brand: Arno), for two minutes at low speed. Then, BMF and rice flour were added to the mixture, according to each formulation, and homogenized at medium speed for three minutes.

Then, the cookies went to the shaping process, with the aid of a pastry bag, obtaining the shape of a circular cookie, with an average diameter of 3 cm. After the shaping process, they were placed in aluminum trays with parchment paper, and taken to baking in a preheated industrial oven, at 150 ± 2 °C for 20min. After that, the cookies were cooled to room temperature, stored in airtight glass jars, and kept until analysis.

2.2.2 Microbiological analysis

Microbiological analyzes were performed in triplicate, following the methodology proposed by the American Public Health Association, which determines the most probable number (NMP) of coliforms at 45 °C g-1, mesophilic aerobes and Salmonella sp. (APHA, 2015).
2.2.3 Physical-chemical analysis

The cookies were evaluated for moisture content, using a moisture analyzer with an infrared scale (RADWAG - Model MAC 210), and water activity using the Aqualab equipment (AQUALAB® - Model 4TE). The total nitrogen content was determined by the Kjeldahl method, with a conversion factor of 6.25 (IAL, 2008). The ash content was analyzed using a muffle furnace at 550 °C until constant weight (IAL, 2008). The lipid content was determined by the Soxhlet direct extraction method (IAL, 2008). The total carbohydrates were calculated by difference (100g - total grams of moisture, protein, fat and ash), including the fraction of dietary fiber. All analyzes were performed in triplicate.

2.2.4 Sensory analysis

The sensory evaluation was carried out with 80 untrained tasters, at the Sensory Analysis Laboratory, in individual booths with white light. Each taster received three cookie samples of approximately 10g each, and glass with approximately 200ml of water. The samples were served in monadic sequential order, on napkins coded with random three-digit numbers.

All tasters received and signed the free and informed consent form - TCLE, in which they were informed about the composition of the product and risks for allergy sufferers.

Cookies were evaluated using a 9-point structured hedonic scale (9 = I liked it so much, 5 = I didn't like it; I didn't like it; 1 = I really liked it) (Peryam & Pilgrim, 1957) for the attributes: color, aroma, texture, flavor, and global impression. The purchase intention of the product was assessed using the 5-point structured buying attitude scale (5 = would certainly buy; 3 = I doubt if I would buy; 1 = certainly would not buy) (Meilgaard et al., 1991).

2.2.5 Statistical Analysis

It was considered an experiment in a completely randomized design, with biscuit formulations containing variations of BMF 50, 75 and 100%, and the physicochemical variables evaluated were: humidity, ash, lipids, proteins, carbohydrates and water activity. For the sensory analysis, the same BMF variations used in the physical-chemical analyzes were used, and the variables evaluated were: appearance, color, aroma, texture, sweetness, flavor, global impression and buying attitude. Shapiro-Wilk normality tests and Bartlett homogeneity
tests of variance were performed, both at 5% significance to verify the possibility of performing Analysis of Variance.

When assumptions are accepted in all cases, Analysis of Variance (more than two independent samples) at 5% significance is used (Callegari-Jacques, 2003). The significantly different variables between the samples follow the Tukey test at 5% significance. When the assumptions are rejected in all cases, Friedman's non-parametric test (more than two dependent samples) at 5% significance is used, where there are no assumptions about the data distribution (Gibbons & Chakraborti, 2010). The significantly different variables between the samples go to Dunn's test at 5% significance. All data were tabulated in the Excel 2016 spreadsheet and the tests performed in the SAS program (SAS, 2000).

3. Results and Discussion

3.1 Microbiological analyzes

The microbiological results showed absence for coliforms at 45 °C, aerobic mesophilic bacteria and Salmonella sp. Thus, it can be considered that the cookies are suitable for consumption, as they are within the standards established by the current legislation, demonstrating that they were handled in an appropriate manner (Brasil, 2001).

Dias, Santana, Pinto, & De Oliveira (2016) reported the absence of coliforms in an oatmeal cookie. A similar result was found in samples of whole type cashew nut cookies (Zuniga, Coelho, Ferreira, Resende, & Almeida, 2011), and in cookies and enriched snack-type (Krüger et al., 2003).

3.2 Physical and chemical analysis

The results of the physical-chemical analysis of the cookies made with different concentrations of BMF and rice flour are shown in Table 2.
Table 2 - Mean values ± standard deviations of physical and chemical characteristics.

| Formulations | Moisture | Ashes   | Lipids       | Proteins     | Carbohydrates * | Water activity |
|--------------|----------|---------|--------------|--------------|-----------------|----------------|
| Type 1       | 1.76±0.06a 1.07±0.18a | 20.09±0.77a 1.22±0.002a | 75.86±0.65a | 0.26±0.010c     |
| Type 2       | 4.53±0.13b 1.34±0.03a | 19.79±1.37a 1.07±0.100a | 73.27±1.48ab | 0.45±0.005b     |
| Type 3       | 5.51±0.31c 1.29±0.14a | 20.74±0.80a 1.05±0.080a | 71.41±0.79b | 0.51±0.006a     |

Means with different letters in the same column differed statistically (p <0.05) by the Tukey comparison test.

* The carbohydrate content was obtained from the difference calculation. Source: Authors.

It was possible to verify that there was no significant difference (p <0.05) between the different biscuit formulations containing variations of BMF regarding the content of ash, lipids and proteins (Table 2). Regarding the carbohydrate content, formulations with 50% and 100% showed a significant difference between them. However, the formulation with 75% did not differ significantly from the others regarding the carbohydrate content. This result is believed to have a direct influence on moisture values, as these were used as the basis for calculating the percentage of carbohydrates.

The ash contents were in the range of 1.07 to 1.29%, which are similar to other studies, such as Becker, Damiani, de Melo, Borges, & de Barros Vilas Boas (2014) that evaluated biscuits with flour from the buriti endocarp, which presented ash contents between 1.42 to 1.93%, being, therefore, according to Brazilian legislation that allows a maximum content of 3% of minerals for cookies and crackers (Brasil, 1978).

The humidity values varied from 1.76 to 5.51%. Values higher than those found by Mancebo, Rodriguez, & Gómez (2016) in his cookie based on rice flour, corn starch and pea protein. The water activity values were below 0.6, according to Brazilian legislation that allows a maximum moisture content of 14% for cookies and crackers (Brasil, 1978), thus disadvantaging the growth of contaminating microorganisms (Gava, 1998).

It was also observed that the results of moisture and water activity increased with increasing BMF concentration, respectively. This fact was also observed by Alexandre, Neto, Stanley, Oliveira, & Sabaa-Srur (2016) in their study on the development of pasta containing BMF and wheat flour, as it has sufficient dietary fiber content to cause an increase in moisture retention.

The lipid content, around 20%, was similar for the three formulations studied, due to the fact that BMF and rice flour are not a source of lipids, so the content of this came from margarine, which was kept in fixed concentrations.

The cookies had protein contents between 1.05 and 1.22%, which are lower than that
found by Becker et al. (2014), in the development of a gluten-free cookie type biscuit, based on buriti mesocarp flour, with a protein content of 2.26 to 2.39%. Low protein content was expected in this study, due to the lower protein content of rice and BMF flours used in the development of cookies (Maniglia & Tapia-Blácido, 2016; Puncha-Arnon & Uttapap, 2013).

On the other hand, a high amount of dietary fiber, from flaxseed and from BMF, deserves to be highlighted in cookies for people with celiac disease, since gluten-free products have low fiber content because they come from refined flours or starch (Maniglia & Tapia-Blácido, 2016; Pagamunici et al., 2014).

Thus, a cookie composed of this mix of flours would contain a pleasant amount of nutrients, supplying the amount of fiber in gluten-free products.

3.3 Sensory Analysis

The results of the cookies acceptance tests are shown in Table 3. For all the attributes evaluated, values were observed in the hedonic scale acceptance zone (above 6), except for the 100% formulation texture, which was in the indifference zone. Demonstrating that in general the cookies formulated with BMF were well accepted by consumers.

The color and aroma attributes did not differ statistically from each other, for the three samples of biscuits evaluated. It was observed that the formulations 50 and 100% showed significant differences regarding the attribute’s appearance, texture, sweetness and flavor. For the appearance and texture attributes, the 75% formulation was similar to the others. Texture is an important element in the quality of cookies, directly affecting consumer acceptance and sales (Becker et al., 2014). Ajila, Leelavathi, & Prasada Rao (2008) observed that the flour content of the mango peel is directly linked to the hardness of the biscuit, the same that occurred with the biscuits made with BMF. Silva et al. (2019) observed a significant difference between the cookie formulations with partial replacement by BMF, favoring the formulation with 50%.

For the sweetness and flavor attributes, formulations 75 and 100% did not differ significantly, only the formulation 50% obtained a significant difference in relation to the other samples.
Table 3 - Mean values ± standard deviations of the attributes related to the sensory analysis of biscuit with variations of BMF and rice flour.

| Formulation | Appearance | Color | Aroma | Texture | Sweetness | Flavor | Overall Impression |
|-------------|------------|-------|-------|---------|-----------|--------|-------------------|
| Type 1      | 7.23±1.63 a| 7.59±1.28 a| 7.24±1.54 a| 6.95±1.89 a| 7.35±1.74 a| 7.13±1.83 a| 7.21±1.60 a       |
| Type 2      | 6.58±1.89 ab| 7.58±1.40 a| 7.00±1.45 a| 6.26±1.81 ab| 6.40±1.85 b| 6.21±1.91 b| 6.42±1.66 b       |
| Type 3      | 6.49±1.92 b| 7.60±1.25 a| 7.36±1.36 a| 5.86±1.98 b| 6.39±1.93 b| 6.08±2.03 b| 6.40±1.69 b       |

Means with different letters in the same column differed statistically (p <0.05) by Dunn's comparison test.
Source: Authors.

Regarding the overall impression, it is possible to observe that all formulations were in the acceptability zone, however, formulation 1 was superior to the others. Formulations 2 and 3 did not show significant differences according to the test performed. A similar result was observed in the production of cookies based on babassu fruit mesocarp flour, where the greatest acceptance was for the formulation containing 50% BMF (Silva et al., 2019).

Table 4 - Mean values ± standard deviations for purchase intention regarding the sensory analysis of cookies with variations in BMF and rice flour.

| Formulation | Purchase Intention |
|-------------|-------------------|
| Type 1      | 2.84±1.21 a       |
| Type 2      | 2.81±1.22 a       |
| Type 3      | 2.10±1.20 b       |

Means with different letters in the same column differed statistically (p <0.05) by Dunn's comparison test.
Source: Authors.

Regarding the purchase intention (Table 4), all samples were in the acceptance zone, where the tasters stated that they would probably buy the product. The results showed that the formulations, as they present physical-chemical and sensory characteristics similar to commercial sweet cookies, can be an alternative for consumers with celiac disease. In addition to the addition of flaxseed, which increases the nutritional quality of the product.

4. Final considerations

According to the results obtained, it is clear that the physical-chemical characterization showed satisfactory and comparable values to other studies found in the literature. It can be observed that it is possible to obtain cookies with desirable technological characteristics through the incorporation of
BMF in the manufacture of cookie type cookies. The microbiological analysis showed that the cookies were suitable for consumption, as they did not present risks to the consumer's health, thus attesting the efficiency and hygiene during the preparation. As for the sensorial analysis, the cookies presented satisfactory acceptance, being the formulation with 50% of BMF the most accepted in relation the attributes appearance, colour, texture, flavour, global impression and buying attitude. Given, it is possible to produce gluten-free sweet biscuits with a high nutritional value, using BMF as a base with partial replacement by rice flour and enriched with flaxseed flour. However, rheological analyses can be performed to check and improve the texture of cookies, with the intention of producing a better quality product.

**Conflicts of interest**

All authors declare no competing interests.

**Acknowledgments**

Authors thank the University Federal of Maranhão for all the support during the experiments.

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