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Physicochemical and functional characterization of babassu flour: a flour rich in dietary fiber, resistant starch and phenolic compounds

Caracterização físico-química e funcional de farinha de babaçu: uma farinha rica em fibra alimentar, amido resistente e compostos fenólicos

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Babassu flour is a byproduct of babassu oil extraction. It is a nutritionally rich food, but it is still little used in human nutrition. There is little information about the characteristics of this flour, which would allow indications for its use as an ingredient in food formulations. Therefore, this work aimed to evaluate the physicochemical, functional, and pasting properties of babassu flour. Babassu flour was obtained from the mesocarp of babassu fruit dried at 60 °C for 6 hours, ground in an industrial blender, and sieved (0.6 mm) to standardize its particle size. The produced flour was characterized as a fine powder with a high content of resistant starch (98% RS). This flour did not present amylase inhibitors and showed a low content of phytic acid and trypsin inhibitors, which evidences the high nutritional quality of the babassu flour. The flour also showed interesting functional characteristics with pasting properties indicating heat, shear, and storage stability at low temperatures. Babassu flour also showed a very high content of phenolic compounds (8031.61 mg GAE/100 g) with good antioxidant activity (62.28%). Therefore, it is possible to suggest that babassu flour is a promising food ingredient that can used as a bioactive component for developing nutraceutical foodstuffs.
Keywords: *Attalea speciosa*, starch digestibility, anti-nutritional factors.

A farinha de babaçu é um subproduto da extração de óleo de babaçu. Ela é um alimento rico nutricionalmente, mas ainda é pouco utilizada na alimentação humana. Existe pouca informação sobre as características desta farinha, que possibilite indicações de uso como ingrediente em formulações alimentícias. Portanto, este trabalho teve como objetivo avaliar as propriedades físico-químicas, funcionais e de pasta da farinha de babaçu. A farinha de babaçu foi obtida a partir do mesocarpo do fruto de babaçu seco à 60 °C por 6 horas, moído em liquidificador industrial e peneirado (0,6 mm) para padronização de sua granulometria. A farinha produzida caracterizou-se como um pó fino com alto teor de amido resistente (98% AR). Esta farinha não apresentou inibidores de amilase e apresentou baixo teor de ácido fítico e inibidores de tripsina, o que evidencia a alta qualidade nutricional da farinha de babaçu. A farinha também apresentou características funcionais interessantes com propriedades de pasta indicando estabilidade ao calor, cisalhamento e armazenamento em baixas temperaturas. A farinha de babaçu também apresentou alto teor de compostos fenólicos (8.031,61 mg GAE/100 g) com boa atividade antioxidante (62,28%). Portanto, é possível sugerir que a farinha de babaçu é um ingrediente alimentar promissor que pode ser utilizado como componente bioativo no desenvolvimento de alimentos nutracêuticos.
Palavras-chave: *Attalea speciosa*, digestibilidade de amido, fatores antinutricionais.

1. INTRODUCTION

Babassu (*Attalea speciosa* Mart. ex Spreng.) is a palm tree widely distributed in Latin America, occurring in four Brazilian biomes: Amazon, Atlantic Forest, Cerrado, and Caatinga. Babassu coconut is composed of four parts. The fibrous outer layer of the fruit, named epicarp, comprises the coconut peel and represents 11-14% of the babassu weight. The mesocarp is an intermediate layer between the epicarp and endocarp, rich in starch and fiber (20-23% of the fruit weight). The endocarp represents 57-63% of the fruit and corresponds to an inner woody layer covering the almond [1-3]. The almond comprises 7-9% of the fruit and is used for extraction of a babassu oil with application in human nutrition or, more commonly, for the production of biofuel and lubricants [4-6].

The babassu mesocarp is a by-product of extracting oil from the babassu almond. It is generated during the separation of the almonds from the babassu coconut. Babassu mesocarp flour is rich in nutrients, fiber, and bioactive compounds. Babassu flour is still neglected as an ingredient in foodstuff production, despite its importance as animal feed and raw material for biomass [2, 6, 7].

Indeed, there are some reports about the use of babassu flour in the production of breads, biscuits, cakes, cookies, cereal bars, pasta, and dairy desserts [2, 6, 8]. However, there needs to be more information about the complete physicochemical and functional properties of babassu flour. Thus, this study aimed to evaluate the physicochemical and functional characteristics of babassu flour, including the determination of starch digestibility and paste properties that have never been reported for this food product.

2. MATERIAL AND METHODS

Babassu fruits were acquired from rural producers in the Ariquemes region – RO, Brazil. The reagents used were of analytical grade.

2.1 Obtention of the babassu flour

The fruits were sanitized and peeled, the mesocarp was separated from the endocarp and dried in an oven at 60 °C for 6 hours. Babassu flour was obtained by milling the dried mesocarp in an industrial blender and sieving the produced flour (28 mesh, 0.6 mm opening) to standardize its granulometry.

2.2 Granulometry

The granulometry of the babassu flour was determined according to Rigon et al. (2022) [9]. 100 g of flour was sieved using seven analytical sieves (1.19, 0.500, 0.250, 0.177, 0.149, 0.119 and 0.074 mm) on a sieve shaker for 10 min. Particle size was expressed as the percentage of flour retained on each sieve.

2.3 Proximate composition

Moisture, ash, protein, and lipid contents of the babassu flour were evaluated according to the methodologies described by AOAC (2006) [10]. Moisture content was analyzed in an infrared analyzer (Q533M, Quimis, Diadema, SP, Brazil). Nitrogen content was determined by the micro Kjeldahl method, with a conversion factor for proteins of 6.25. Ash content was determined after calcination in a muffle for 3 hours at 550 °C. The lipid content was determined using a Soxhlet extractor with hexane as the solvent. Total carbohydrates were estimated by difference.

2.4 Total dietary fiber and starch digestibility

The total dietary fiber content was determined by the enzymatic-gravimetric method (nº 985.29) from AOAC (2006) [10].

In vitro starch digestibility was analyzed in raw and cooked samples according to Englyst et al. (1992) [11], with modification. One gram of babassu flour was added to 20 mL of 0.1 M sodium acetate buffer (pH 5.2) containing 4 mmol L^{-1} $CaCl_2$. After 5 min of incubation at 37 °C, 5 mL of an enzyme mixture (pancreatin, amyloglucosidase, and invertase) was added, and the system was kept at 37 °C. Aliquots of 0.5 mL were withdrawn at intervals of 0, 20, and 120 min and transferred to tubes containing 4 mL of absolute ethanol. The samples were centrifuged at 3000 x *g* for 5 min, and the supernatant was used for glucose determination. After 120min of incubation, the mixture was boiled for 30 min for enzyme inactivation. After that, 10 mL of 7 mol L^{-1} KOH solution was added, and the system was stirred at 25 °C for 30 min, followed by treatment with amyloglucosidase at 60 °C for 30 min. The amount of glucose released in each period was quantified using the glucose oxidase-peroxidase reagent. The correspondent content of starch was calculated by multiplying the glucose content by 0.9, the factor to convert from free glucose, as determined, to anhydrous glucose as occurs in starch. From these results, three starch fractions were obtained: (1) rapid digestible starch (RDS), corresponding to the glucose release from 0 to 20 min of enzyme digestion; slowly digestible starch (SDS), corresponding to the glucose released from 20 to 120 min of reaction; and resistant starch (RS), corresponding to the starch remaining after 120 min of digestion.

2.5 Total titratable acidity and pH

The titratable acidity and pH of the babassu flour were evaluated according to the methodologies of Instituto Adolfo Lutz (2008) [12]. The pH was measured in 10 g of sample suspended in 100 mL of distilled water, under stirring. For total titratable acidity, 10 g of sample were weighed and 100 mL of distilled water added to the sample. The titration was performed with 0.01 M sodium hydroxide solution up to pH 8.3 (using a pH meter). The result was expressed in mL of NaOH/100 g of sample.

2.6 Water absorption (WA) and water solubility (WS)

WA and WS were determined according to the methodology of Anderson et al. (1969) [13], with some modifications. To determine WA and WS, 0.5 grams of babassu flour was dispersed with 6 mL of distilled water and incubated at room temperature (25 °C) for 30 min, under stirring. After incubation, the mixture was centrifuged at 2057*x* g for 10 min, and the supernatant was carefully transferred to previously weighed Petri dishes. The wet material was weighed, and the water was evaporated in an oven at 105 °C until weight stabilization. The dried residue was weighed, and the WA and WS were determined as: WA (g/g) = Weight of wet sediment / (Weight of dry sample – Weight of dry solids in supernatant); WS (%) = (Weight of dry solids in supernatant / Weight of dry sample) × 100.

2.7 Pasting properties

The pasting properties of babassu flour were evaluated in a Rapid Visco Analyzer (RVA-S4A, Newport Scientific, Warriewood, NSW, Australia) using 3 g of sample (14% moisture) in 25 g of distilled water. A programmed heating and cooling cycle was employed at a constant shear rate, during which the sample was kept at 50 °C for 1 min, heated to 95 °C, and cooled to 50 °C for 2 min. The parameters recorded were pasting temperature, peak time, peak viscosity, breakdown viscosity, final viscosity, and setback viscosity.

2.8 Phenolic content and antioxidant activity

The phenolic compounds from babassu flour were extracted by mixing 5 g of the sample with 50 mL of 70% ethanol solution, followed by incubation at room temperature (25 °C) for 24h in the dark. After incubation, the mixture was centrifuged for 10 min at 3000 *x* g, and the supernatant was used for phenolics quantification.

The quantification of total phenolic compounds in the extract was performed according to the colorimetric analysis described by Singleton and Rossi (1965) [14], with adaptations by Haminiuk et al. (2011) [15], using the Folin-Ciocalteu reagent and gallic acid as standard. The absorbance was measured at 765 nm in a UV-VIS spectrophotometer (IL-227, Kasuaki, São Paulo, SP, Brazil), and the results were expressed as milligrams of gallic acid equivalents per 100 g of flour (mg GAE/100 g).

The antioxidant activity was determined by the DPPH (2.2-diphenyl-1-picrylhydrazyl) method according to the methodology proposed by Mensor et al. (2001) [16].

2.9 Antinutritional factors

The phytic acid content was determined by the method described by Latta and Eskin (1980) [17] with modifications for DOWEX-AGX-4 resin [18]. A standard curve of phytic acid (Sigma, P8810) was done, and the results were expressed as milligrams of phytic acid per gram of the sample (mg PA/g).

The trypsin inhibitory activity was determined according to Kakade et al. (1974) [19], as described by Lopes et al. (2012) [20]. Casein was used as a substrate for trypsin. The trypsin inhibition unit (TIU) was defined as the ratio between the units observed at maximum activity and the activity of the samples containing the inhibitors.

The α-amylase inhibitor activity was determined according to Deshpande et al. (1982) [21], as described by Lopes et al. (2012) [20]. Corn starch was used as a substrate for the enzyme. One unit of α-amylase inhibitor was defined as the quantity of inhibitor that inhibits one unit of α-amylase.

3. RESULTS AND DISCUSSION

3.1 Granulometry and chemical composition of the babassu flour

Granulometry determination (Table 1) revealed that babassu flour presents small particle sizes, with 85% of the particles smaller than 0.25 mm, of which 50% smaller than 0.074 mm. Flour granulometry plays an essential role in the production of bakery products (cakes, cookies, bread, and pasta), as the particle size is related to the uniformity of the final product, influencing the water absorption capacity of the flour and determining the food processing conditions [9].

Table 1: Granulometry of babassu flour.

Sieve opening (mm)	**1.190**	**0.500**	**0.250**	**0.177**	**0.149**	**0.119**	**0.074**	**Bottom pan**
Retained flour (%)	0	5	10	10	5	10	10	50

Based on the granulometry values, babassu flour is classified as very fine flour, similar to the classification of wheat flour [22]. This result suggests that depending on the technological and functional properties of babassu flour, it could successfully replace wheat flour in gluten-free food formulations.

The chemical composition of the babassu flour is shown in Table 2. As can be seen, babassu flour presented carbohydrates as the major component, with small amounts of ash, proteins, and lipids. These results are in agreement with the results reported by other authors [23-25]. The difference in the proximate composition may be related to the industrial process of obtaining the flour, to the inherent characteristics of the babassu variety or to the different climatic conditions in which the plants were cultivated.

It is important to know the centesimal composition of a raw material, since each component provides the food with a specific nutritional and/or sensory characteristic. For example, proteins and lipids can provide texture and nutritional value, complex carbohydrates provide texture and satiety, while simple carbohydrates are related to sweet taste.

Table 2: Proximate composition (%) of babassu flour.

Moisture	Ash	Proteins	Lipids	Total Carbohydrates
12.76±0.42	1.33±0.09	1.39±0.24	0.17±0.03	84.77±0.39

Data were reported as mean ± standard deviation.

3.2 Total dietary fiber and starch determination

The total dietary fiber content (Table 3) of the babassu flour is in agreement with the results already reported by other authors, with values of TDF ranging from 2.5 to 17.8% [2, 24-26]. Compared to other commonly consumed flours, babassu flour has TDF values higher than those reported for rice (0.75-4.89%, [27-31]), wheat (2.3-11.4%; [31-34]), corn (2.0-13.25%; [29, 31, 35]), and cassava (5.2-5.4%; [36, 37]). From a technological point of view, the content of dietary fiber can interfere with the rheological and functional properties of the flour and will dictate the possible applications of this food ingredient [31]. Generally, fiber-rich food components present high water retention capacity and potent ability to absorb potentially hazardous molecules, contributing to gastrointestinal health. It is already been reported that an increase in dietary fiber intake could contribute to the prevention and management of diseases such as functional constipation, irritable bowel syndrome, Crohn’s disease, ulcerative colitis, and colon cancer [38, 39].

Table 3: Total dietary fiber (%), total starch, and starch digestibility (%) of babassu flour.

TDF	TS	RDS	SDS	RS
15.01±0.06	65.63±0.51	0.50±0.03	0.79±0.05	64.33±0.45

Data were reported as mean ± standard deviation. TDF = total dietary fiber; TS = total starch; RDS = rapidly digestible starch; SDS = slowly digestible starch; RS = resistant starch.

Furthermore, a high fiber intake was shown to be a promising non-conventional therapeutic strategy in the prevention and management of several metabolic disturbs, contributing to the reduction of blood glucose, blood pressure, and serum lipids and helping to reduce the physiologic negative impact of chronic diseases such as cardiovascular disease, obesity, and diabetes [6]. In this sense, babassu flour is a potential ingredient candidate for developing functional and nutraceutical food products.

Babassu flour also showed a high content of starch (Table 3), which agrees with the literature data [24, 25, 40]. Considering the digestibility properties of the babassu flour, the results evidenced a higher content of resistant starch, with 98% of the total starch being resistant to enzyme digestion. This result can be a direct effect of the high content of TDF since dietary fibers increase the viscosity of the gastrointestinal contents and cause a steric hindrance that blocks the contact of the digestive enzymes with the starch granules [33]. Therefore, the resistance of the babassu flour to the α-amylase hydrolysis indicates that it will remain undigested in the small intestine but make its way to the large intestine, where it could act as a prebiotic component, serving as substrate for the gut microbiota [41]. This is another important result that suggests that babassu flour is a promising nutraceutical food ingredient to be used as a prebiotic. Additionally, the reduced release and diffusion rate of glucose will provide a slowly energetic intake, which could be beneficial to controlling diabetes and obesity and relieving functional constipation [42, 43].

3.3 Acidity, functional, and pasting properties of the babassu flour

The total titratable acidity and pH determination are interrelated parameters widely used to depict the acidity of food components and are a result of the composition of the food matrices [44]. The values of titratable acidity and pH of the babassu flour (Table 4) indicated a slightly acidic material, which can be explained by the composition of the babassu mesocarp in terms of organic acids [45]. Organic acids influence the food matrix's organoleptic characteristics and dictate the final product's microbial stability and shelf-life quality. In general, titratable acidity provides a better understanding of the impact of acidity on the organoleptic features, whereas pH is better for predicting the potential for microorganism growth in food matrices [44]. The results show that the pH value (Table 4) of the babassu flour is within the acceptable pH range of 5-7 for developing bakery products [46].

WA and WS of the babassu flour are presented in Table 4. WA represents the ability of the components present in the flour to bind to water molecules. The presence of starch, proteins, and fibers increases the water-binding capacity of flour. In contrast, the presence of lipids and larger particles reduces the water-binding ability of the food matrix. Compared to other flours currently used in the food industry, the WA values of babassu flour are similar to those reported by rice, wheat, corn, and chickpea [47-50] and higher than that observed for cassava flour [46].

Table 4: Total titratable acidity, pH, water absorption (WA), and water solubility (WS).

Total titratable acidity (mL/100 g)	**pH**	**WA (g/g)**	**WS (%)**
0.9±0.08	5.55±0.04	2.05±0.19	11.28±0.35

Data were reported as mean ± standard deviation.

WS represents the number of water-soluble molecules present in the flour. In addition to the composition of the flour, parameters such as temperature, pH, pressure, particle size, and the presence of other compounds in the medium will change the flour's solubility [49, 51, 52]. Babassu flour has presented WS values higher than those observed for cassava [46], rice [50], chickpea, and wheat flours [47].

The pasting properties of babassu flour (Table 5) mainly reflect the gelatinization of the starch present in this starch-rich food matrix (≈66% starch). However, it is important to note that non-starch carbohydrates and proteins are also affected by temperature and contribute to gelling and pasting properties through swelling, denaturation, and unfolding. The degree of starch damage during flour processing also influences the pasting properties [53].

Table 5: Pasting properties parameters of babassu flour suspensions.

PV (mPa.s)	**BD (mPa.s)**	**FV (mPa.s)**	**SB (mPa.s)**	**PT (°C)**
1402.5 ± 20.51	194 ± 36.77	1651 ± 22.63	442.5 ± 38.89	77.23 ± 0.04

Data were reported as mean ± standard deviation. PV = peak viscosity; BD = breakdown; FV = final viscosity; SB = setback; PT = pasting temperature.

Peak viscosity refers to the maximum viscosity obtained by the slurry during heating before granules break down. Babassu flour showed a relatively lower peak viscosity when compared to potato and taro flours [53, 54]. However, the peak viscosity was similar to those observed in rice and soybean flours [50, 53] and higher than those reported for wheat, quinoa, and corn flours [53, 54]. The peak viscosity is directly related to the swelling of starch granules, as the greater the swelling, the greater the amount of water that enters into the granule, reducing the available water in the medium, with the consequent increase in the sample viscosity [20, 53]. In this sense, proteins and non-starch carbohydrates (dietary fiber) interacting with starch granules will reduce the flour's swelling ability, resulting in a food matrix showing lower viscosity.

Breakdown measures how easily the swollen starch granules break, and the water inside the granules is released back into the medium, reducing viscosity [48]. Setback is the difference between the final viscosity and the trough viscosity. This parameter determines the retrogradation tendency of the cooked flour paste under cooling [48]. Setback represents the reorganization of amylose molecules and the presence of fragmented granules incorporated into the leached amylose network. Lower setback suggests the absence or lesser number of fragmented granules due to the lower expansion capacity of the granules [55]. Altogether, these two pasting properties determine the stability of pasting flours [20]. Babassu flour showed low breakdown and setback values, thus signaling thermal stability and reduced tendency to retrogradation. The low retrogradation tendency of babassu flour indicates that its inclusion in foodstuffs formulations may contribute to improving the stability of the final product during storage at low temperatures.

Pasting temperature is the temperature at which the viscosity increases during heating. It indicates the minimum temperature required for cooking the flour. Higher pasting temperature means the presence of starch granules more resistant to expansion and rupture [48]. The pasting temperature observed for babassu flour (Table 5) was higher than that reported for rice, potato, quinoa, wheat, cassava, and lentil flours [54, 56, 57], and it may be a result of the presence of proteins and the high content of fibers in the babassu flour, which limits the hydration and swelling of the starch granule. The proteins could bind tightly to the starch molecules, slowing down the stretching of starch chains. The fibers present in the babassu flour may create a "protective" matrix around the starch granules that prevents water diffusion. Additionally, the proteins and fibers attached to the starch surface could hold the water molecules on the starch granule surface, delaying the entry of water inside the granules and reducing the swelling of the starch grains [58].

3.4 Antinutritional factor, phenolic content, and antioxidant activity of the babassu flour

The nutritional quality of food components is highly influenced by antinutritional factors such as enzyme inhibitors and phytates, which limit the bioavailability of essential nutrients [59]. As can be observed in Table 6, the babassu flour did not present α-amylase inhibitors and little amount of protein inhibitor and phytic acid. Phytates comprise a complex class of natural components highly present in seeds and are considered antinutritional components because they reduce the absorption of minerals, especially calcium, iron, and zinc [60]. Considering the babassu flour, is it known that the phytates strongly interact with Cu^{+2} and Fe^{+3} [61], as well as interact with amino acids from the side chains of enzymes such as pepsin, pancreatin, and α-amylase, inhibiting enzymatic action and affecting protein and starch digestibility [62].

Table 6: Total phenolic, antioxidant activity, phytic acid, trypsin, and α-amylase inhibitor activity of babassu flour.

α-amylase inhibitor activity (IU/100 mg)	**trypsin inhibitor activity (IU/mg)**	**phytic acid (mg/g)**	**total phenolic (mg GAE/100 g)**	**antioxidant activity (%)**
–	0.90±0.04	2.68±0.37	8031.61±87.80	62.28±0.02

Data were reported as mean ± standard deviation.

The presence of protease inhibitors that disturb the work of digestive enzymes could also affect protein digestibility. However, the low content of trypsin inhibitors indicates that the antinutrients would not affect the nutrient availability of the babassu flour, contributing to guaranteeing a high nutritional quality of this flour.

Regarding the content of phenolic compounds, babassu flour showed a high content of phenolics (Table 6), values higher than those reported by other authors studying babassu flour (98.3 – 1360 mg GAE/100 g of flour; [7, 63]) and could be a result of the differences in plant characteristics, cultivation conditions (soil and climate) and extraction methods employed in each research. It is known that phenolic-rich food products may have a protective role against the development of metabolic pathologies such as diabetes and cardiovascular diseases, as well as exert a neuroprotective potential and a preventive effect against oxidative stress [64, 65]. Considering the antioxidant potential of babassu flour (Table 6), it is possible to suggest that it is a promising food ingredient to be used as a bioactive component for developing nutraceutical foodstuffs.

4. CONCLUSION

Babassu flour showed high nutritional quality due to a high dietary fiber and resistant starch content, phenolic compounds with high antioxidant activity, and absence or low values of anti-nutritional compounds. In addition, the produced flour presented a very fine granulometry, with a paste stable to heating, shearing, and cooling. Therefore, babassu flour is a promising food matrix to be used as a bioactive ingredient for producing nutraceutical foodstuffs with excellent nutritional quality and technological properties.

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