

## Physicochemical and structural properties of native starch from *Matayba guianensis* Aubl. seeds (Sapindaceae)

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### Abstract

This study aimed to characterize the physicochemical, crystalline, morphological, biological, *in vitro* digestibility, and microbiological properties of starch extracted from *Matayba guianensis* seeds. Chemical composition analysis revealed a starch yield of 85% and a total protein content of 12%. The starch exhibited good paste clarity (66%), high lightness ( $L^* > 70\%$ ), and relevant mineral contents, particularly nitrogen, iron, copper, and zinc. Morphological evaluation indicated typical native starch patterns, while biological assays demonstrated notable *in vitro* digestibility and low cytotoxicity, with 94.56% cell viability. In addition, the material complied with microbiological standards required for food-grade biopolymers. Overall, these findings highlight the technological potential of *Matayba guianensis* seed starch, supporting its exploitation as a novel and promising starch source and as a functional ingredient for food industry applications.

**Keywords:** proteins, minerals, Sapindaceae, color, microbiological analysis, digestibility.

## Propriedades físico-químicas e estruturais do amido nativo das sementes de *Matayba guianensis* Aubl. (Sapindaceae)

### Resumo

O objetivo deste estudo foi caracterizar as propriedades físico-químicas, cristalinas, morfológicas, biológicas, digestibilidade *in vitro* e microbiológicas do amido extraído das sementes de *Matayba guianensis*. A análise da composição química revelou rendimento de amido de 85% e teor de proteínas totais de 12%. O amido apresentou boa claridade de pasta (66%), alta luminosidade ( $L^* > 70\%$ ), além de teores relevantes de minerais, com destaque para nitrogênio, ferro, cobre e zinco. A avaliação morfológica indicou padrões característicos de amidos nativos, enquanto os ensaios biológicos demonstraram digestibilidade *in vitro* significativa e baixa citotoxicidade, com 94,56% de viabilidade celular. Adicionalmente, o material atendeu aos padrões microbiológicos exigidos para biopolímeros de uso alimentício. De forma geral, os resultados obtidos evidenciam o potencial tecnológico do amido de *Matayba guianensis*, caracterizando-o como uma nova e promissora fonte de amido, com aplicação como ingrediente funcional na indústria alimentícia.

**Palavras-chave:** proteínas, minerais, Sapindaceae, cor, análises microbiológicas, digestibilidade

### 1. Introduction

The family Sapindaceae comprises approximately 140 genera and 1,900 species, classified according to Radlkofer's system (1879, 1934, 1900). Most species occur in tropical and subtropical regions, with few representatives in temperate areas (Coelho et al., 2017). In Brazil, the family includes 28 genera and 418 species, distributed across most biomes, with greater representation in the Cerrado.

The genus *Matayba* Aubl. is one of the largest in the tribe Cupanieae, with approximately 50 species restricted to the Neotropical region, ranging from Mexico to northern Argentina (Acevedo-Rodríguez et al., 2011; Gonçalves et al., 2021; Silva-Neto et al., 2025). In Brazil, 30 *Matayba* species are recorded, 17 of which are endemic. Among them, *Matayba guianensis* Aubl. stands out, being widely distributed throughout the country, especially in the Cerrado and in riparian shrublands. The species has dispersed leaves, and its flowers occur throughout the year, measuring 5–7 mm in length (Coelho et al., 2017). Fruits (Figure 1A) appear mainly from October to February, with fruiting also possible between April and June. Seeds (Figure 1B) are ellipsoidal, measuring approximately  $0.5\text{--}1.1 \times 0.4\text{--}0.7$  cm, covered by a whitish aril extending to the seed apex, and contain an endogenous source of native starch.

Several plant species exhibit high starch content in their seeds. Starch is an abundant natural biopolymer, widely distributed in plants as a primary carbohydrate reserve, primarily used during germination, and is one of the most studied organic materials in food science and materials engineering. Structurally, starch is a polysaccharide composed of glucose units linked by  $\alpha$ -(1 $\rightarrow$ 4) and  $\alpha$ -(1 $\rightarrow$ 6) bonds, forming two main fractions: amylose (linear) and amylopectin (branched). The relative proportion of these fractions and the granular organization confer unique physicochemical properties, such as gelatinization, gel and paste formation, viscosity, and rheological behavior, which are essential for technological and agroindustrial applications (Rashwan et al., 2024; Jiménez-Sánchez et al., 2024).

In recent years, starch has received growing attention due to its renewability, biodegradability, low cost, and biocompatibility, qualifying it as a sustainable alternative to synthetic polymers derived from fossil fuels. Global starch production exceeds tens of millions of tons per year, mainly from corn, potato, rice, and cassava (Gurunathan et al., 2025; Ajala et al., 2023; Dhull et al., 2022).

The functionalization of starch and the development of starch-based materials are aligned with the principles of the circular economy and sustainable practices, as starch-based materials can reduce reliance on conventional plastics and lower the carbon footprint of industrial products (Souza et al., 2013; Roldán-San Antonio; Martín, 2025). However, limited knowledge exists regarding the physicochemical, structural, morphological, and rheological properties of native starches derived from plant species of the Brazilian Cerrado, which represent an alternative source for various industries, including the agroindustry. These characteristics make the study of native starch an interdisciplinary area of high relevance, both for food science and bio-based materials engineering (Carvalho et al., 2025; Wang; Tong, 2024).

The present study aimed to describe the physicochemical and structural properties of native starch from the seeds of *Matayba guianensis* Aubl., representing the first investigation focused on its potential application in the food and agroindustrial sectors.

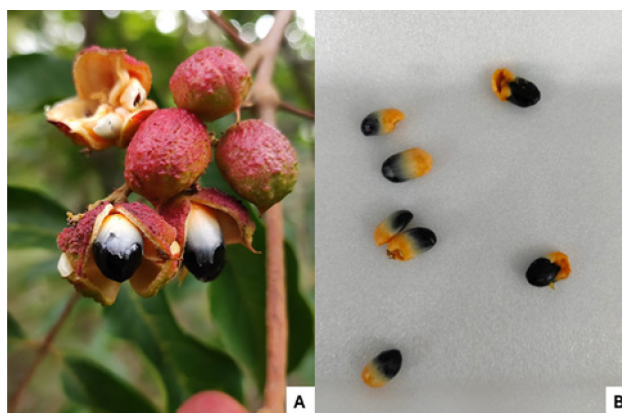


Figure 1. Fruits with exposed seeds (A) and isolated seeds (B) of *Matayba guianensis*. Source: Authors, 2026.

## 2. Materials and Methods

### 2.1 Identification and plant collection area

The species *M. guianensis* was identified based on vegetative and reproductive morphological characteristics using the dichotomous identification key proposed by Coelho et al. (2017). A voucher specimen was herbarized and deposited in the Herbarium of the Centro Universitário UniBRAS, Rio Verde, Goiás, Brazil, under accession number BRAS 15,901.

Approximately 5 kg of fruits were collected, and the seeds were manually extracted in the General Chemistry Laboratory. The collection area belongs to the Cerrado Domain, specifically the Cerrado sensu stricto vegetation type, located in the municipality of Rio Verde, Goiás State, Brazil (17°47'28.5" S and 50°57'48.7" W). The collection was carried out in January 2026. The region has a seasonal tropical climate with a well-defined dry season, an average altitude of approximately 786 m, and dystroferic red soil, characteristic of the Cerrado sensu stricto.

### 2.2 Starch extraction

Starch from *M. guianensis* seeds was extracted following the method described by Adebowale et al. (2002), with minor modifications. The seeds were thoroughly washed with distilled water and subsequently processed in a food processor for 5 min in the presence of distilled water to obtain a homogeneous slurry. The resulting suspension was passed through a 0.074 mm (200-mesh) sieve to separate the fibrous seed residue, which was discarded.

The filtrate containing the starch was allowed to stand undisturbed for 24 h to promote sedimentation, followed by centrifugation at 4,000 rpm (Kasvi, Mod. K14-4000, USA) for 15 min in 50 mL conical tubes with distilled water. The supernatant was carefully decanted and discarded. This washing–sedimentation–centrifugation cycle was repeated ten times to ensure the efficient removal of soluble and insoluble impurities, including saponins, which are commonly present in species of the Sapindaceae family.

The recovered starch was dried in a forced-air circulation oven at  $40 \pm 2.0$  °C for 24 h, ground to a fine powder, and sieved again through a 0.074 mm (200-mesh) sieve to obtain a uniform particle size. Finally, the starch was packed in food-grade plastic containers and stored under refrigeration at  $-10$  °C until further analyses.

### 2.3 Physicochemical analyses

To determine dry matter content, the seeds were previously dried in an oven at  $105 \pm 2.0$  °C (SolidSteel, Mod. SSD64L, Brazil) for 24 h until constant weight was achieved. Starch yield was determined according to the method described by Adebowale et al. (2005), based on the ratio between the mass of dry starch obtained after the extraction process and the initial dry mass of the seeds used. After starch extraction and drying, the masses were measured using an analytical balance, and the yield was expressed as a percentage (%). Moisture content was determined by measuring the mass loss of the sample after dehydration in a forced-air oven at  $100 \pm 2.0$  °C (SolidSteel, Mod. SSD64L, Brazil), following the methodology described by the Instituto Adolfo Lutz (IAL, 2008). The results were expressed as a percentage (%).

Titrateable acidity was determined according to the methodology described by the Instituto Adolfo Lutz (IAL, 2008), and the results were expressed as mL of 1 N NaOH per 100 g of sample-1. The amylose content was determined using the method proposed by Williams et al. (1970). A preweighed sample (0.1 g) was transferred to a 100 mL volumetric flask, to which 1 mL of 99% ethanol and 9 mL of 1.0 M sodium hydroxide were added. The suspension was thoroughly mixed and heated in a boiling water bath for 10 min to ensure complete gelatinization and solubilization. After cooling to room temperature (24 °C), the volume was adjusted to 100 mL with distilled water.

An aliquot of 5 mL from the resulting solution was transferred to a second 100 mL volumetric flask, followed by the addition of 1 mL of 1.0 M acetic acid and 2 mL of iodine solution. The volume was then made up to 100 mL with distilled water. Absorbance was measured at 620 nm using a UV-Vis spectrophotometer (Bell Engineering, Mod. UVM51, Italy), and the results were expressed as a percentage (%). The amylopectin content was estimated by difference, calculated as  $[100 - \text{amylose} (\%)]$ , following the approach described by Silva et al. (2019). Total sugar content was determined according to the official method described by the Association of Official Analytical Chemists (AOAC, 2016). The results were expressed as a percentage (%) relative to the dry mass of the sample.

pH was measured using a digital pH meter (Mylabor, Mod. PHB-550, Brazil), according to the procedures

described by IAL (2008). Protein content was determined after sample digestion using the Kjeldahl method, and the results were expressed as a percentage (%). Ash content was determined by incineration in a muffle furnace at  $500\text{ }^{\circ}\text{C} \pm 2.0\text{ }^{\circ}\text{C}$  (SolidSteel, Mod. SSFM 6,7L, Brazil) for 5 h, with results expressed as a percentage (%). Lipid content was determined using the Soxhlet extraction method, with *n*-hexane as the extraction solvent, and the results were expressed as a percentage (%) (IAL, 2008). Water activity ( $a_{\text{w}}$ ) was determined by direct reading using a water activity meter (AquaLab, Mod. 4TEV<sup>®</sup>, USA) at  $25\text{ }^{\circ}\text{C} \pm 1.0\text{ }^{\circ}\text{C}$ . Total dietary fiber was determined by acid–base digestion according to the Weende method (AOAC, 2006).

Starch color was determined by spectrophotometry (Konica Minolta, Mod. CM-5, Japan) using the CIELAB color coordinate system ( $L^*$ ,  $a^*$ , and  $b^*$ ).  $L^*$  values (lightness) range from 0 (black) to 100 (white), whereas  $a^*$  values range from green (–60) to red (+60), and  $b^*$  values range from blue (–60) to yellow (+60) (Nemțanu; Brașoveanu, 2026). Mineral content was determined by atomic absorption spectrometry (AAS) (Savantaa, Mod. GBC, Australia). The minerals evaluated were calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), phosphorus (P), iron (Fe), sulfur (S), boron (B), zinc (Zn), copper (Cu), manganese (Mn), and nickel (Ni). Potassium was determined by flame photometry. Macronutrient contents were expressed in  $\text{g kg}^{-1}$ , whereas micronutrient contents (Fe, Cu, Mn, and Zn) were expressed in  $\text{mg kg}^{-1}$  on a dry weight basis (Akhila et al., 2022).

Starch paste clarity was determined by transmittance (%T), as described by Akhila et al. (2022). A starch suspension ( $1\text{ g } 100\text{ mL}^{-1}$ ) was prepared, and 10 mL of the suspension was heated in a water bath (VDR, Mod. VD6S, Brazil) at  $100\text{ }^{\circ}\text{C} \pm 2.0\text{ }^{\circ}\text{C}$  for 30 min, with stirring for 30 s every 5 min. After heating, the sample was cooled under agitation to  $25\text{ }^{\circ}\text{C} \pm 2.0\text{ }^{\circ}\text{C}$ . Transmittance was then measured at 650 nm using a UV–Vis spectrophotometer (Bell Engineering, Mod. UVM51, Italy).

#### 2.4 In vitro digestibility

The starch digestibility of *M. guianensis* was determined according to the method proposed by Englyst et al. (1992) and cited by Akhila et al. (2022). Samples were then incubated at  $37\text{ }^{\circ}\text{C} \pm 2.0\text{ }^{\circ}\text{C}$  in a shaking water bath (VDR, Mod. VD6S, Brazil) with digestive enzymes. Based on digestion time, starch was classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). The fraction of starch digested within the first 20 min was considered RDS; the fraction digested between 20 and 120 min was classified as SDS; and the fraction not digested after 120 min was considered RS. The percentage of the resistant starch fraction (RS) was calculated using the following equation:

$$\text{RS (\%)} = \{ \text{Total starch} - (\text{SDS} + \text{RDS}) \} \times 100$$

#### 2.5 Bright-field microscopy, dark-field microscopy, and polarized light microscopy

The birefringence pattern and granular morphology of *M. guianensis* starch were analyzed using a bright-field optical microscope (Coleman, model XSZ N107D, Brazil), a dark-field microscope (Digilab, model DI-211T, Brazil), and a polarized light microscope (Global Optics, model NO229T, Brazil), following the procedure described by Sudheesh et al. (2019). The samples were prepared by mixing the starch with glycerol, and a drop of the resulting suspension was placed on a glass slide and covered with a coverslip. Observations were carried out under normal, oblique, and polarized light at magnifications of  $10\times$  and  $40\times$ . Images were captured using a digital camera (Digilab, Brazil) and processed with the ImageView software.

#### 2.7 In vitro cytotoxicity of starch

The cytotoxicity of *M. guianensis* starch was assessed according to the method described by Ahmed et al. (1994), using human fibroblasts (MRC-5, PD 30, Merck<sup>®</sup>, USA). An aqueous solution of Dimethyl sulfoxide (DMSO, Synth, Brazil) (0.01%) was used as the negative control, while doxorubicin (Merck, USA) ( $20\text{ }\mu\text{M}$  or  $34\text{ }\mu\text{g mL}^{-1}$ ) served as the positive control. Cell viability interpretation followed ISO 10993-5 criteria, as summarized in (Table 1).

Table 1. *In vitro* cytotoxicity of *Matayba guianensis* starch against human fibroblasts (MRC-5, PD 30).

Cell viability (%)	Interpretation
≥ 90%	Non-cytotoxic
70-89%	Low or no cytotoxicity
50-69%	Moderate cytotoxicity
< 50%	High cytotoxicity
< 30%	Severe cytotoxicity

Note: Ahmed et al. (1994).

### 2.8 Microbiological analysis of starch

Microbiological analyses of the starch were performed using *Petri* dishes containing sterilized specific media, following the manufacturer's instructions and food microbiological standards (RDC No. 12/2001; Brazil, 2001; Silva et al., 2010), with minor modifications. A sample of starch containing 10 g of dry mass was weighed. A 1:10 (10-1) suspension was prepared by adding the starch to 90 mL of sterile 0.85% saline solution. Serial dilutions were performed, homogenized, and inoculated onto *Petri* dishes containing differential media. For each dilution, 1,000 µL was used and spread on the plate using a *Drigalski* loop.

Thermotolerant coliforms at 45 °C ± 2.0 °C were assessed on VRBL Agar plates (Violet Red Bile Agar with Glucose, Merck®), incubated at 45 °C ± 2.0 °C (SolidSteel, Mod. SSBI21L, Brazil) for 24 h. *Bacillus cereus* was assessed on MYP Agar plates (Mannitol Egg Yolk Polymyxin, Merck®), incubated at 37 °C ± 2.0 °C for 24 h. *Salmonella* sp. was analyzed after enrichment, then plated on XLD Agar plates (Xylose Lysine Deoxycholate, Merck®) and incubated at 37 °C ± 2.0 °C for 24 h. The inoculated plates were incubated in a temperature-controlled microbiological chamber, following the specific conditions required for each microorganism. Results were expressed as Colony Forming Units (CFU g<sup>-1</sup>).

### 2.9 FTIR Fourier reflectance analysis

The native starch sample from *M. guianensis* seeds was analyzed in the infrared region using a PerkinElmer FTIR spectrometer operating in transmittance mode with Fourier transform, with 60 scans accumulated and a resolution of 4 cm<sup>-1</sup>, over the range of 500–4000 cm<sup>-1</sup>. The analysis was performed using a system equipped with a diamond attenuated total reflectance (ATR) accessory.

## 3. Results

### 3.1 Physicochemical parameters

Table 2 presents the results obtained from the physicochemical analyses of starch extracted from *M. guianensis* seeds. Expressive values were observed for starch content, with an extraction yield of 85%, amylose and amylopectin contents of 30% and 69%, respectively, a high protein content of 12%, and a notable paste clarity, with 66% light transmittance.

Table 2. Physicochemical composition of *Matayba guianensis* starch.

Parameters*	Mean ± SD
Starch yield (%)	85.51 ± 0.34
Moisture content (%)	13.18 ± 0.12
Titrate acidity (NaOH 100 g <sup>-1</sup> )	0.69 ± 0.07
Amylose content (%)	30.85 ± 0.37
Amylopectin content (%)	69.15 ± 0.37
Total sugars (%)	0.34 ± 0.21
pH (w/v)	5.75 ± 0.02

Protein content (%)	12.13 ± 0.05
Ash content (%)	0.64 ± 0.04
Lipid content (%)	0.39 ± 0.09
Water activity (a <sub>w</sub> )	0.299 ± 0.01
Paste clarity (%T)	66.74 ± 0.41

Note: All physicochemical properties were determined as percentages (%), and the results are expressed as mean ± standard deviation. Source: Authors, 2026.

### 3.2 Color, mineral content, and starch digestibility

The light transmittance rate was 70%, and the a\* and b\* chroma values indicated red and yellow hues, respectively. Expressive mineral contents were observed, with nitrogen as a macronutrient at 18 mg kg<sup>-1</sup> and the micronutrients iron, copper, and zinc at 72, 11, 9, and 20 mg kg<sup>-1</sup>, respectively. Digestibility values were 27% for RDS, 33% for SDS, and 39% for RS (Table 3).

Table 3. Color parameters, minerals (macro- and micronutrients), and *in vitro* digestibility of starch from *Matayba guianensis* seeds.

Parameters*	Mean ± SD
L*	70.90 ± 0.01
a*	0.12 ± 0.02
b*	9.91 ± 0.01
Ca (g kg <sup>-1</sup> )	0.21 ± 0.01
Mg (g kg <sup>-1</sup> )	0.34 ± 0.00
K (g kg <sup>-1</sup> )	2.01 ± 0.00
Na (g kg <sup>-1</sup> )	0.64 ± 0.01
S (g kg <sup>-1</sup> )	1.97 ± 0.01
P (g kg <sup>-1</sup> )	1.23 ± 0.00
N (g kg <sup>-1</sup> )	18.6 ± 0.01
Fe (mg kg <sup>-1</sup> )	72.8 ± 0.00
Cu (mg kg <sup>-1</sup> )	11.1 ± 0.01
Mn (mg kg <sup>-1</sup> )	9.45 ± 0.00
Zn (mg kg <sup>-1</sup> )	20.2 ± 0.00
Ni (mg kg <sup>-1</sup> )	0.03 ± 0.00
B (mg kg <sup>-1</sup> )	0.53 ± 0.00
RDS (%)	27.83 ± 0.41
SDS (%)	33.01 ± 0.29
RS (%)	39.16 ± 0.33

Note: \*Dry basis content. Values are expressed as means ± standard deviation. Source: Authors, 2026.

### 3.3 Morphological results

In Figure 2A, bright-field micrographs reveal the general morphology of the starch granules. Under polarized light (Figure 2B), the presence of the Maltese cross (Nicol cross) is clearly observed, indicating the semicrystalline nature of the starch granules and confirming the occurrence of birefringence. In dark-field microscopy (Figure 2C), enhanced contrast allows clearer visualization of the granule contours, providing improved definition of their edges and external structure.

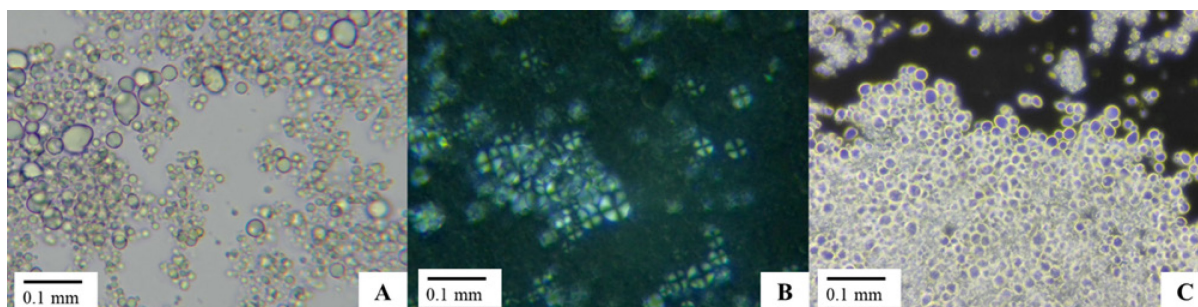


Figure 2. Optical micrographs under Bright Field (A), Polarized Light (B), and Dark Field (C), *Matayba guianensis* starch. Source: Authors, 2026.

### 3.4 Toxicity of starch

In the *in vitro* toxicity assay using MRC-5 fibroblasts (PD 30), starch extracted from *M. guianensis* seeds showed a cell viability of 94.56% at a concentration of  $100 \mu\text{g mL}^{-1}$  (Figure 3). The negative control (DMSO) exhibited 100% cell viability, whereas the positive control (doxorubicin) reduced cell viability to 45.12%.

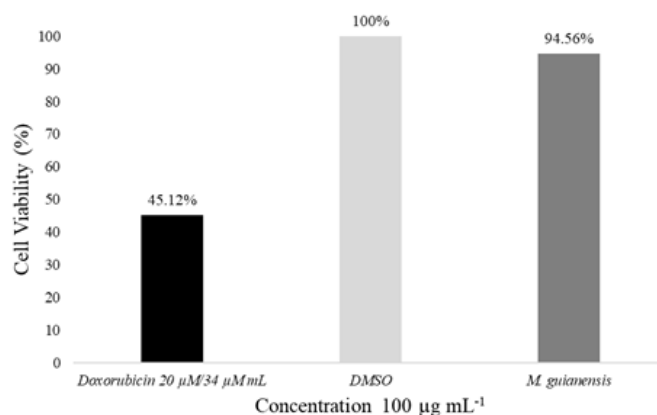


Figure 3. Results of the cytotoxicity assay of the extracts in human fibroblasts MRC-5 (PD 30) after 72 h of treatment: cell viability following treatment with doxorubicin (positive control, 20  $\mu\text{M}$  or 34  $\mu\text{g mL}^{-1}$ ), DMSO (negative control, 0.01%), and starch extract of *Matayba guianensis* at a concentration of  $100 \mu\text{g mL}^{-1}$ . Source: Authors, 2026.

### 3.5 Microbiological analysis of starch

In the microbiological analysis of starch extracted from *M. guianensis* seeds, no presence of coliforms at 45 °C, *B. cereus*, or *Salmonella* spp. was detected. According to Brazilian Resolution RDC No. 12 of January 2, 2001, the microbiological limits established for starch are up to  $3 \times 10^3$  CFU  $\text{g}^{-1}$  for *B. cereus*,  $1 \times 10^2$  CFU  $\text{g}^{-1}$  for coliforms at 45 °C, and the absence of *Salmonella* spp. in 25 g of sample.

### 3.6 FTIR Fourier reflectance

The infrared spectrum of native starch from *M. guianensis* seeds exhibited a broad band in the 3000–3500  $\text{cm}^{-1}$  region, attributed to O–H stretching, which is characteristic of the extensive hydrogen-bonding network present in the starch structure. The bands observed at 1164, 1155, 1146, 1135, and 1107  $\text{cm}^{-1}$  are mainly assigned to C–O and C–C stretching vibrations, with contributions from C–OH stretching, which are typical of polysaccharides.

The absorptions at 1088, 1062, 1047, 997, 984, and 930  $\text{cm}^{-1}$  are attributed to C–OH and CH<sub>2</sub> bending vibrations, related to the vibrational modes of hydroxyl and methylene groups in the glucose units. The C–O–C (ether) group, characteristic of the six-membered pyranose ring of the glucose monomer, shows absorption bands

in the 1150–1085  $\text{cm}^{-1}$  region (Figure 4).

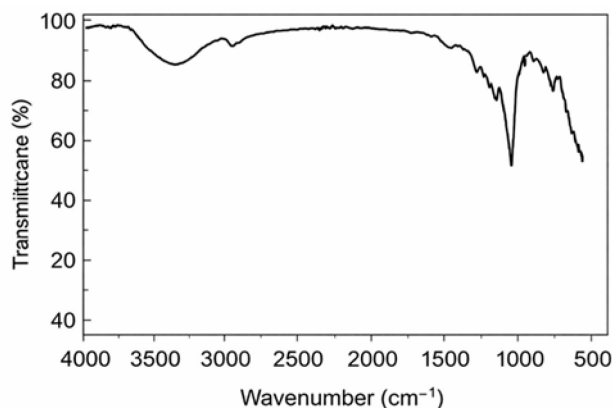


Figure 4. Fourier-transform infrared (FTIR) transmission spectrum of starch from seeds of *Matayba guianensis*. Source: Authors, 2026.

#### 4. Discussion

*Matayba guianensis* is a fruit-bearing species whose seeds have not yet been extensively studied for industrial food applications. However, the results obtained in the present study demonstrate that this species represents a promising alternative source for native starch extraction, exhibiting attractive physicochemical characteristics for potential technological applications.

Starch color is a highly relevant parameter in the food industry, as starches with lightness values above 70% are considered suitable for the production of light-colored foods. In this context, Santos et al. (2013) reported a starch content of 78.8% on a dry basis for jackfruit seeds, a value close to that observed in the present study. In contrast, Martins et al. (2022) reported a significantly lower yield (48.9% on a dry basis) for starch extracted from avocado seeds, highlighting the wide variability in starch yield among different plant species. Such differences may be attributed to the intrinsic characteristics of the raw material as well as to the extraction methods employed, emphasizing the importance of evaluating each starch source individually.

Moisture content is an important indicator of starch quality, since high moisture levels, associated with inadequate drying processes, may favor the growth of spoilage microorganisms. According to Brazilian standards for starch, the maximum allowable moisture content is 14%, and the value obtained in this study was below this limit, possibly due to the adoption of good manufacturing practices. Similar observations have been reported for starches extracted from tropical seeds and tubers, in which low moisture contents are associated with greater storage stability (Martins et al., 2022; Chacon et al., 2024).

The literature indicates that starch moisture content varies widely depending on its botanical origin and the technological processes applied. Rengsutthi & Charoenrein (2011) reported moisture contents of 9.59% for jackfruit seed starch and 11.74% for corn starch. Likewise et al. (2017) observed average moisture contents of 14.87% in tuber starches. These findings support the notion that variations in moisture content are influenced by both the plant species and the processing conditions employed during starch extraction (Wang et al., 2022).

The low titratable acidity and slightly acidic pH observed for *M. guianensis* starch are consistent with the typical profiles of native starches, indicating the absence of significant chemical degradation during the extraction process. These characteristics suggest a low content of free acids formed by hydrolysis or oxidation of the original starch components (Costa et al., 2023). For comparison, studies on starch extracted from avocado seeds reported a titratable acidity of 4.64%, a considerably higher value than that observed in the present study, reflecting intrinsic differences in chemical composition and metabolites of the source matrices that directly influence this parameter. These findings highlight the importance of considering the botanical source of starch when evaluating titratable acidity in physicochemical characterization studies (Martins et al., 2022).

Regarding pH, values within the range of approximately 5.0 to 6.5 are commonly reported for native starches extracted from seeds, roots, and tubers, without compromising important functional properties such as gelatinization, viscosity, and gel-forming capacity, as reported by Malumba et al. (2022). The results of this study indicate that the simple and alternative extraction process employed did not significantly alter the intrinsic

acidity of the material, preserving chemical characteristics comparable to those of other native starches described in the literature.

The amylose content above 20%, as observed in this study, can be classified as moderate to high, favoring retrogradation and the formation of firmer gels—properties that are desirable for applications requiring greater structural integrity, such as fillings, sauces, and thickening agents. Conversely, the high amylopectin content (69%) contributes to increased paste viscosity and stability, a behavior similar to that reported for starches with comparable compositional profiles (Martins et al., 2022). For comparison, starch extracted from avocado seeds showed amylose and amylopectin contents of 39.56% and 60.44%, respectively, highlighting differences in carbohydrate composition that directly influence functional properties (Martins et al., 2022).

Starch from *M. guianensis* seeds exhibited a low total sugar content, indicating high starch purity and a reduced likelihood of undesirable reactions, such as non-enzymatic browning during thermal processing. Similarly, the low levels of proteins, ash, and lipids fall within legal limits and are comparable to those reported for commercial and non-conventional starches studied recently, reflecting the efficiency of the extraction process in removing non-starch components (Martins et al., 2022; Chacon et al., 2024). The low water activity observed ( $< 0.300 a_w$ ) further reinforces the starch's characterization as an unfavorable environment for microbial growth, since values below 0.600  $a_w$  significantly limit microbial proliferation. In related studies, Lima et al. (2019) reported a water activity of 0.327  $a_w$  for arrowroot (*Maranta arundinacea*) starch, whereas Martins et al. (2022) found a much higher value (0.986  $a_w$ ) for starch extracted from avocado seeds, highlighting the wide variation of this parameter among different starch sources.

The high paste transparency ( $> 60\%$ ) observed for *M. guianensis* starch stands out when compared to several starches of plant origin and is directly associated with the low lipid and protein contents, components that typically reduce light transmittance. Recent studies indicate that starches with high paste transparency show great potential for applications in products that require visual clarity, such as jellies, edible coatings, and food encapsulation systems (Aaliya et al., 2021; Akhila et al., 2022; Navaf et al., 2020).

Regarding color parameters determined by the CIELAB system, *M. guianensis* starch showed  $L^*$  values above 50%, indicating high lightness—a desirable characteristic for food industry applications, as it is associated with a clear appearance and ease of incorporation into different matrices without significantly affecting the final product color. The  $a^*$  values close to zero indicate the absence of red or green tones, reflecting chromatic neutrality along this axis, while the positive  $b^*$  values suggest a slight yellowish tendency, a common feature in native starches and often associated with residual non-starch components or intrinsic characteristics of the botanical source. Overall, the color profile observed indicates a visually clear and lightly pigmented starch, consistent with good-quality raw materials and potentially suitable for food applications in which color is a relevant technological attribute. Akhila et al. (2022), when analyzing potato starch, reported  $L^*$ ,  $a^*$ , and  $b^*$  values of 94.84%, 0.24, and 3.93, respectively, indicating a lighter starch, although obtained through an extraction process different from that used in the present study.

The main macrominerals identified in the starch sample were potassium (K) and nitrogen (N), while the micronutrients included iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn), which are essential elements for human and animal nutrition. Similar results have been reported for starches from grains and seeds in the study by Vrancheva et al. (2019), who found substantial mineral contents in starches from quinoa, millet, oats, buckwheat, einkorn, amaranth, flaxseed, and chia. In contrast, Akhila et al. (2022) reported low levels of Mg, S, K, and Zn in potato starch, suggesting that lower mineral contents are generally associated with higher starch purity.

The contents of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) were above 20%, 30%, and close to 40%, respectively. According to Wolter et al. (2014), foods can be classified based on the estimated glycemic index (eGI) as low GI ( $GI \leq 55$ ), medium GI ( $56 \leq GI \leq 69$ ), and high GI ( $GI \geq 70$ ), following FAO/WHO criteria. Therefore, starch extracted from *M. guianensis* seeds can be classified as a low-glycemic-index ingredient.

Overall, *M. guianensis* starch, characterized by a high total starch content and a low proportion of rapidly digestible starch, represents a promising alternative for individuals with diabetes or those seeking glycemic and body weight control. In contrast, Peng et al. (2022) reported high GI values ( $> 70$ ) for modified starch samples from *Chenopodium quinoa*, indicating limited suitability for glycemic control diets, although these starches may be appropriate for specific applications, such as infant food formulations.

Figure 2 presents, for the first time, an overview of the crystallinity and morphology of *M. guianensis* starch granules at both macroscopic and microscopic scales. The structural description and identification of starch are fundamental for advancing investigative research and for understanding its functional properties in the food

industry. Microscopic techniques are widely used in starch granule characterization, as changes in microstructure can be readily detected through microscopic analysis. In this study, extraction carried out at 25 °C did not alter the morphological patterns of *M. guianensis* starch. The crystallinity pattern observed is similar to that reported by Vrancheva et al. (2019) for starches from various seed and grain sources. Under bright-field microscopy (Figure 2A), the general morphology of the starch granules is revealed. Under polarized light (Figure 2B), the presence of the Maltese cross (Nicol cross) is clearly observed, indicating the semicrystalline nature of the starch granules and confirming birefringence. In dark-field microscopy (Figure 2C), a pioneering technique applied in this study, enhanced contrast allows clearer visualization of granule contours, providing better definition of their edges and external structure.

The *in vitro* toxicity assay using MRC-5 fibroblasts (PD 30) demonstrated that starch extracted from *M. guianensis* seeds exhibited low cytotoxicity, maintaining high cell viability. This finding indicates that the extracted starch is biocompatible under the tested conditions, supporting its potential applicability in food formulations, pharmaceutical products, or biodegradable materials.

Alternative starches have shown satisfactory results in *in vitro* toxicity assays, demonstrating high biocompatibility. Similar findings were reported by Silva et al. (2019) for starches extracted from *Dioscorea alata* and *D. altissima* tubers, which exhibited cell viability values of 97.7% and 97.9%, respectively. Likewise, Bergel et al. (2022) reported low toxicity for starch-based materials applied in thermoplastics, further reinforcing the potential of these biopolymers for industrial applications and biomaterial development.

In the microbiological assays, *M. guianensis* starch met the “pass” criteria, as no significant presence of coliforms, *Bacillus cereus*, or *Salmonella* spp. was detected, in accordance with the Brazilian Resolution RDC No. 12 of January 2, 2001. Although each country has its own regulations and standards, this new native starch may be used within the framework of good manufacturing practices for food production in Brazil. The results obtained in this study indicate that *M. guianensis* starch was extracted in full compliance with the microbiological standards established by current Brazilian legislation.

Similar results have been reported for starches obtained from *D. alata* and *D. altissima* (Silva et al., 2019), as well as for starch extracted from *Phalaris canariensis* seeds (Batista et al., 2020), corroborating the microbiological safety of these starches when appropriate processing conditions are applied. The infrared spectrum of native starch extracted from *M. guianensis* seeds exhibited infrared bands typical of starches from seeds, grains, and tubers, as described by Silverstein et al. (1991) and Lima et al. (2012). The findings of this study are consistent with those reported by Lima et al. (2012) and Kushwaha et al. (2023), who evaluated native starches and identified absorption bands in regions between approximately 4000 and 500  $\text{cm}^{-1}$ , which are characteristic of functional groups present in starch, such as  $-\text{OH}$ ,  $\text{C}-\text{O}$ ,  $\text{C}-\text{C}$ ,  $\text{C}-\text{H}$ , and  $\text{C}-\text{O}-\text{C}$ , associated with polysaccharide structures (amylose and amylopectin) and bound water.

## 5. Conclusions

The starch extracted from *Matayba guianensis* seeds demonstrated, in these pioneering results, to be a promising raw material for industrial applications, particularly in the food sector. The starch exhibited a high starch yield, physicochemical properties consistent with high-quality starch sources, and relevant levels of amylose and amylopectin, in addition to favorable color attributes, mineral composition, and low cytotoxicity, which may be associated with its botanical origin within the Sapindaceae family. Overall, these findings highlight the technological potential of *Matayba guianensis* seed starch as an alternative and sustainable starch source. However, further studies are required, particularly focusing on its rheological, thermal, and functional properties, to better elucidate its performance and applicability in food industry formulations.

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## 7. Authors' Contributions

*João Batista Casemiro*: conceptualization, investigation, data curation, formal analysis, writing – original draft,

and corresponding author. *Júlio Cesar Fernandes dos Santos*: investigation, data curation, formal analysis, writing – original draft. *Aurélio Ferreira Melo*: funding acquisition, resources, project administration. *Hellen Regina Fernandes Batista Ventura*: funding acquisition, resources, project administration. *Oswaldo Resende*: resources, methodology, supervision, validation. *Antonio Carlos Pereira de Menezes Filho*: conceptualization, methodology, supervision, project administration, formal analysis, writing – review & editing, and validation.

## 8. Conflicts of Interest

No conflicts of interest.

## 9. Ethics Approval

Not applicable.

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