horticulturae

MDPI

Article

Banana and Plantain Starches: Exploring Differences and Potential Applications

Jaciene Lopes de Jesus Assis [1,*], Magali Leonel [2,*], Eliseth de Souza Viana [1], Edson Perito Amorim [1], Ronielli Cardoso Reis [1], Carlos Wanderlei Piler de Carvalho [3], Palmira de Jesus Neta [4] and Sarita Leonel [2,5]

1 Embrapa Cassava & Fruits, Cruz das Almas 44380-000, BA, Brazil; eliseth.viana@embrapa.br (E.d.S.V.); edson.amorim@embrapa.br (E.P.A.); ronielli.reis@embrapa.br (R.C.R.)
2 Center for Tropical Roots and Starches, São Paulo State University (UNESP), Botucatu 18610-307, SP, Brazil; sarita.leonel@unesp.br
3 Embrapa Food Technology, Rio de Janeiro 23020-470, RJ, Brazil; carlos.piler@embrapa.br
4 Center for Agricultural, Environmental and Biological Sciences, Federal University of Bahia Recôncavo (UFRB), Cruz das Almas 44380-000, BA, Brazil; palmiraneta@aluno.ufrb.edu.br
5 School of Agriculture (FCA), São Paulo State University (UNESP), Botucatu 18610-034, SP, Brazil
* Correspondence: jaciene.jesus@embrapa.br (J.L.d.J.A.); magali.leonel@unesp.br (M.L.)

Abstract

The diversification of cultivars and sustainable production in banana and plantain cultivation, with a view to reducing losses and differentiating derivative products, are of great importance for productive advances linked to sustainable development. In this study, the morphological, physicochemical, and functional characteristics of starches isolated from four dessert cultivars of Prata subgroup (BRS Platina, Gorutuba Biocell, Prata-Anã, BRS Gerais) and plantain cultivars (Tipo Velhaca, Mongolo, and BRS Terra-Anã) were evaluated. All starches exhibited a B-type crystalline pattern, with variations in granule shape and in amylose and resistant starch contents, which particularly differentiated plantains. Differences in viscosity and gelatinization properties highlighted the potential of certain cultivars for specific industrial applications. Multivariate analysis emphasized the diversity among starches, reinforcing their importance as versatile and sustainable raw materials for industry, with the potential to add value and reduce losses in the production chain.

Keywords: *Musa* spp.; green fruit; physicochemical characteristics

check for updates

Academic Editors: Dasha Mihaylova and Maria Dolores Muy-Rangel

Received: 28 August 2025
Revised: 27 September 2025
Accepted: 2 October 2025
Published: 9 October 2025

Citation: Assis, J.L.d.J.; Leonel, M.; Viana, E.d.S.; Amorim, E.P.; Reis, R.C.; Carvalho, C.W.P.d.; Neta, P.d.J.; Leonel, S. Banana and Plantain Starches: Exploring Differences and Potential Applications. *Horticulturae* **2025**, *11*, 1214. https://doi.org/10.3390/horticulturae11101214

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Starch is one of the main natural sources of carbohydrates and is an essential component in various food and non-food products. Traditionally, it is obtained from corn, potatoes, rice, cassava, and wheat. Starch is widely used as a thickening agent, binder, encapsulant, stabilizer, and gelling agent in various food products [1–5].

With the growing demand from the food industry for clean label starches, i.e., those that are not chemically modified, efforts to find non-traditional sources of starch have intensified [5]. Green bananas stand out as a promising alternative source due to their high starch content and wide availability in tropical and subtropical regions. In 2023, bananas and plantains were grown in more than 115 countries, with global production reaching 139.2 million tons, with Brazil being the sixth largest producer [6–10].

The potential of different banana genotypes as a starchy raw material is based on the importance of cultivar diversification for the sustainable development of banana cultivation, as well as the fact that the characteristics of banana starch vary significantly between

cultivars, reflecting morphological and functional differences related to genetic factors, agronomic conditions, geographic location, and harvest season.

Studies conducted in different countries highlight this variability. Starches isolated from banana cultivars grown in Tanzania, Indonesia, India, China, Mexico, Thailand, Colombia, and Brazil showed significant differences in the physical, chemical, thermal, and functional properties of the starch [2,10–20].

In Brazil, the Prata Anã (AAB) cultivar is the most widely grown banana in the Prata subgroup, known for its high yield and ability to adapt to the country's soil and climate conditions. It is cold-tolerant but susceptible to yellow Sigatoka and black Sigatoka. The BRS Platina (AAAB) cultivar is the result of crossing 'Prata-Anã' (AAB—female parent) with the diploid M53 (AA—male parent). This cultivar differs from 'Prata Anã' in that it is resistant to Panama disease, yellow Sigatoka, and is particularly resistant to the most severe form of *Fusarium* wilt, Tropical Race 4 (TR4). The Gorutuba Biocell (AAB) cultivar is a clone of the Prata-Anã cultivar resulting from spontaneous mutation and has shown advances in planted areas, mainly in the states of Minas Gerais and São Paulo, as it shows empirical evidence of resistance to Panama disease. The BRS Gerais cultivar (AAB), developed through crossbreeding, is resistant not only to race 1 but also to subtropical race 4 of the *Fusarium*, yellow Sigatoka, and black Sigatoka, proving to be a robust alternative for sustainable banana production [21].

Plantains, grown mainly in the North and Northeast regions of Brazil, represent an important source of income for small farmers. Genotypes from the plantain germplasm collection maintained by the Brazilian Agricultural Research Corporation (Embrapa) are being selected and developed for resistance to important diseases such as yellow Sigatoka, black Sigatoka, and *Fusarium* wilt. One example is the BRS Terra-Anã cultivar, which is resistant to yellow Sigatoka and Fusarium wilt but remains susceptible to black Sigatoka [21].

In response to the growing demand for alternative starch sources, this study aimed to isolate and characterize the physicochemical properties of starches isolated from four dessert banana cultivars and three plantain cultivars, with the goal of contributing to the sustainable development of banana cultivation in Brazil, expanding knowledge about variations between genotypes, and evaluating the potential of these starches as raw materials for innovative industrial applications.

2. Materials and Methods

2.1. Banana Cultivars and Location of the Orchard

Four dessert banana cultivars from the Prata subgroup (*Musa* spp.) were selected for the trial: BRS Platina (AAAB), Gorutuba Biocell (AAB), Prata-Anã (AAB), BRS Gerais (AAB) and three plantain cultivars (*Musa paradisiaca*, AAB): Tipo Velhaca (AAB), Mongolo (AAB), and BRS Terra-Anã (AAB).

The banana orchard is located in the city of Jaíba, in the state of Minas Gerais, Brazil (15°23′08″ S 43°45′43″ W and 491 m a.s.l.), with average rainfall indices of 858.9 mm. The soil in the experimental area was classified as Dystrophic red latosol [22].

The bunches were harvested from April to May 2024. Eight bunches of each cultivar were harvested at stage 1 of ripeness (fully green peel) [23]. The second and third hands of each bunch were used for starch extraction.

Figure 1 shows an overview of the experiment.

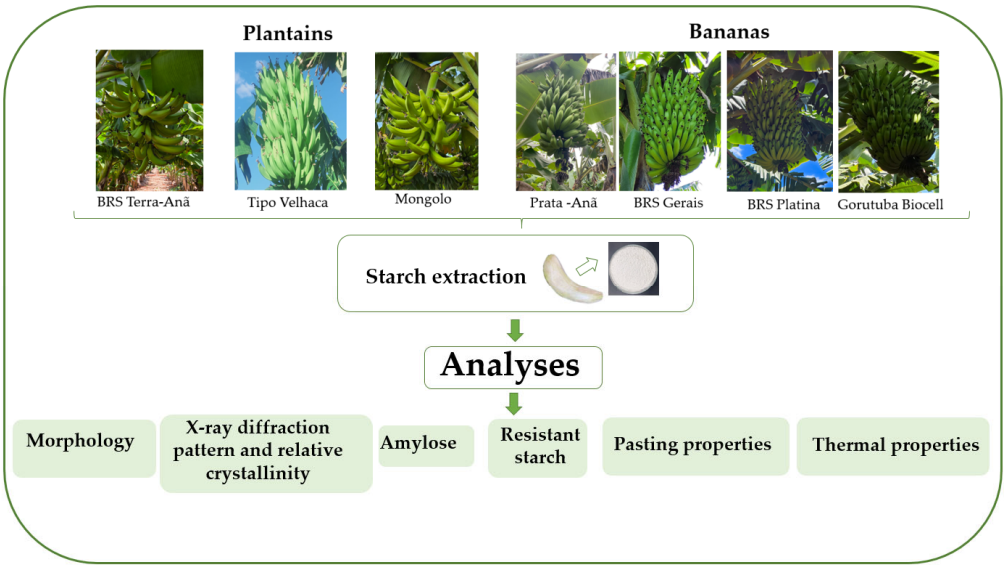


Figure 1. Flowchart of the extraction and characterization of banana and plantain starches.

2.2. Extraction of Starches

The starches were isolated from the green fruits using the method described by Chiang et al. [24], with modifications. The fruits were washed to remove impurities, peeled, and the pulp was sliced into 2–3 cm thick slices and immediately immersed in a sodium metabisulfite solution (1.0%, pH 4.5) in a 1:1 ratio (100 g of fruit to 100 mL of solution), where they remained until the end of slicing. The mixture was ground in a Skymsen industrial blender, model LS-06MB-N (Metalúrgica Skymsen, Brusque, Brazil) for 10 min, and the resulting mass was stirred at 300 rpm in a water bath with constant agitation for 4 h at a temperature of 20 °C. Successive washings and filtrations of the mass were performed using screens with 290 µm and 37 µm, respectively, until the wash water was clear. Starches recovered were dried by convection in an air-circulating oven (40 °C, air speed of 1.5 m/s, 24 h). The starch was then macerated using a mortar and pestle and stored at room temperature.

2.3. Morphology of Starch Granules

Prior to analysis, the starches were kept in a desiccator with silica gel until the moisture content reached 4 ± 0.5%. In the analysis, small samples of starch were collected with spatulas and carefully deposited on double-sided carbon adhesive tape, fixed to an aluminum stub and covered with a thin layer of gold in a Sputter Coater (BAL-TEC, modelo SCD-050, Balzers, Liechtenstein) for 220 s. The morphology of the starch granules was analyzed using a scanning electron microscope, model TM 3000 (Hitachi, Tokyo, Japan) (acceleration voltage of 15 kV and scale of 50 µm) [5].

2.4. X-Ray Diffraction Pattern

The diffraction patterns of the starch samples were measured using an X-ray diffractometer D2 Phaser (Bruker AXS, Rheinfelden, Germany) operating with Cu-Kα radiation (λ = 0.154 nm), at 30 kV and 10 mA. Starches samples were preconditioned in a desiccators for 10 days with a saturated solution of $BaCl_2$ (Barium chloride, 25 °C, aw = 0.9). The samples were placed in acrylic sample holders and analyzed in the range of 2° to 32° (2θ), with a scan rate of 1.73°/min, a step of 0.01°, a fixed divergent slit of 1 mm, a scattering slit width of 0.5 mm, and using a Lynxeye 1D detector. The relative crystallinity (RC) was calculated considering the ratio of the crystalline area to the total area using the Diffrac.

Suite Eva version 3 software (Bruker AXS, Rheinfelden, Germany) [25]. The analysis was conducted using the Origin Pro software (version 9.8.5.212).

2.5. Amylose

Amylose content was determined using the iodine colorimetric method [26], which is based on light transmission through a colored complex formed by the reaction between amylose and iodine. Starch samples were dispersed with ethanol and gelatinized with sodium hydroxide. An aliquot was acidified and, after reaction with iodine, the blue-colored complex formed was quantified by spectrophotometry at 620 nm. The standard curve was prepared with diluted and serial solutions of standard amylose (CAS: 9005-82-7, Sigma-Aldrich, St. Louis, MO, USA) and amylopectin (waxy starch extracted from waxy cassava root donated by the International Center for Tropical Agriculture—CIAT, Cali, Colombia).

2.6. Resistant Starch

The resistant starch (RS) content of the samples was determined using the method described by Goñi et al. [27]. Samples (100 mg) were incubated with a 0.2 M KCl-HCl buffer solution, pH 1.5, and a 40 mg mL^{-1} pepsin solution (Sigma No. P7125) at 40 °C for 60 min. After this time, 0.1 M tris-maleate solution, pH 6.9, and 40 mg mL^{-1} pancreatic α-amylase solution (Sigma No. A3176-2) were added, and the samples were incubated at 37 °C for 16 h. The samples were then centrifuged, and the residues were solubilized and incubated with amyloglucosidase (Prozyn No. STARMAX GA 300 L) at 60 °C for 45 min. The RS content in the samples was obtained by determining the glucose concentration in the sample (glucose oxidase method) using a correction factor of 0.9, which accounts for the molecular weight ratio between anhydroglucose units (162 $g{\cdot}mol^{-1}$) and free glucose (180 $g{\cdot}mol^{-1}$).

2.7. Pasting Properties

Pasting properties were analyzed using a rapid viscosity analyzer (RVA), model 4500, (PerkinElmer, Stockholm, Sweden), using Standard 1 programming of the Thermocline for Windows software, version 3.0 [5]. For the analysis, 3 g of each sample was weighed according to their respective moistures, adding approximately 25 g of water to reach a concentration of 10% starch, and were placed in the sample holder of the equipment. The sample was heated to 50 °C and stirred at 960 rpm for 10 s for complete dispersion and maintained at 160 rpm during the test. The paste was kept at 50 °C for up to 1 min, then heated to 95 °C for 3.7 min and kept at 95 °C for 2.5 min. Cooling then took place (50 °C for 3.8 min) and maintenance at 50 °C for 2 min. The parameters analyzed were: paste temperature (°C), peak viscosity (cp), peak time (min), minimum viscosity (cp), breakdown (cp), final viscosity (cp), and tendency to retrogradation or setback (cp).

2.8. Thermal Properties

The thermal properties of the starches were determined using a differential exploration calorimeter Q200 (TA Instruments, New Castle, DE, USA), according to Bernardo et al. [25], with modifications. The samples (~2.5 mg d.w.) were weighed in hermetically sealed aluminum pans and deionized water was added in a water:starch ratio of 2:1. The pans were then sealed in a universal press (Perkin Elmer, Norwalk, CT, USA) and left to stand for 18 h at room temperature before analysis. The scan was performed from 5 to 120 °C at a rate of 10 °C/min. An empty pan was used as a reference. The equipment was calibrated with indium. The onset (To), peak (Tp), and final (Tf) gelatinization temperatures, as well as the gelatinization enthalpy (ΔH), were determined from the thermograms using Thermal Advantage software version 5.5.20 (TA Instruments, New Castle, DE, USA).

2.9. Data Analysis

Data were submitted to analysis of variance (ANOVA) and compared by using Tukey's test at 5% significance level, with SISVAR software. Principal component analysis (PCA) was performed in R software (v.4.5.1) with the packages of the R computer program. The data were standardized and submitted to PCA for dimensionality reduction and identification of patterns among cultivars. Eigenvalues, eigenvectors, scores, and factor loadings were extracted to interpret the explained variance and the contribution of the variables. For the cluster analysis, Euclidean distance was used as a measure of dissimilarity. Hierarchical clusters were obtained using the UPGMA method—Unweighted Pair Group Method with Arithmetic Mean [28–31]. The validation of the clusters was determined by the cofenetic correlation coefficient according to Sokal and Rohlf [32]. The significance of the cofenetic correlation coefficients was calculated using the Mantel test with 10,000 permutations. The criterion for defining the number of groups was performed using the pseudo-t method, using the NbClust package, belonging to the R computer program [28–35].

3. Results and Discussion

3.1. Morphology

The shapes of the starch granules of the seven banana cultivars observed by scanning electron microscopy are shown in Figure 2. The starch granules were elongated, ovoid, and spheroid in shape, flattened, with smooth surfaces and different sizes. No damage was observed on the surface of the granules, indicating that the extraction technique used was adequate.

The morphology of starch granules in banana and plantain cultivars (Figure 2) is consistent with observations reported in previous studies, which also reported variations in the shape of banana starch granules from different cultivars. Leonel et al. [16] observed smooth-surfaced, oval, spherical, or irregular granules in five Brazilian genotypes (Nanica, Figo Cinza, Terra, Prata Anã, and Prata Graúda). Yang et al. [10], analyzing five Tanzanian cultivars (Mshale, Mzuzu, Mshigudu, Bukoba, and Zanzibar), identified smooth-surfaced granules of irregular shape (spherical, ellipsoidal, or polygonal). Similarly, Paramasivam et al. [2] reported oval and polygonal granules with smooth surfaces in Indian cultivars Saba and Monthan. These findings highlight the close relationship between the shape of starch granules and the cultivar.

The size distribution of starch granules from different cultivars of same botanical source has been shown to change during the development of plant storage organs. Variations in the structure of amylose and amylopectin, as well as in the relative proportions of the glucose polymers that compose the granule, play a key role in determining starch granule size and shape. Additionally, differences in the activity of enzymes such as granule-bound starch synthase during plant growth have been reported to influence granule morphology. Therefore, variations in starch granule morphology can be attributed to the plant's biological origin and physiology, as well as to the biochemical processes occurring within the amyloplast [36].

The shape and size of starch granules plays a crucial role in their functional properties. Larger granules, for example, tend to have higher water retention capacity, which promotes swelling and contributes to more efficient gelatinization. These characteristics make this type of starch particularly suitable for applications that require high viscosity and a high degree of swelling. On the other hand, smaller granules generally have a denser and more compact crystalline structure, giving them higher thermal stability. Thus, they are more suitable for products or formulations that require a lower degree of gelatinization but demand greater resistance to freezing and thawing cycles [37].

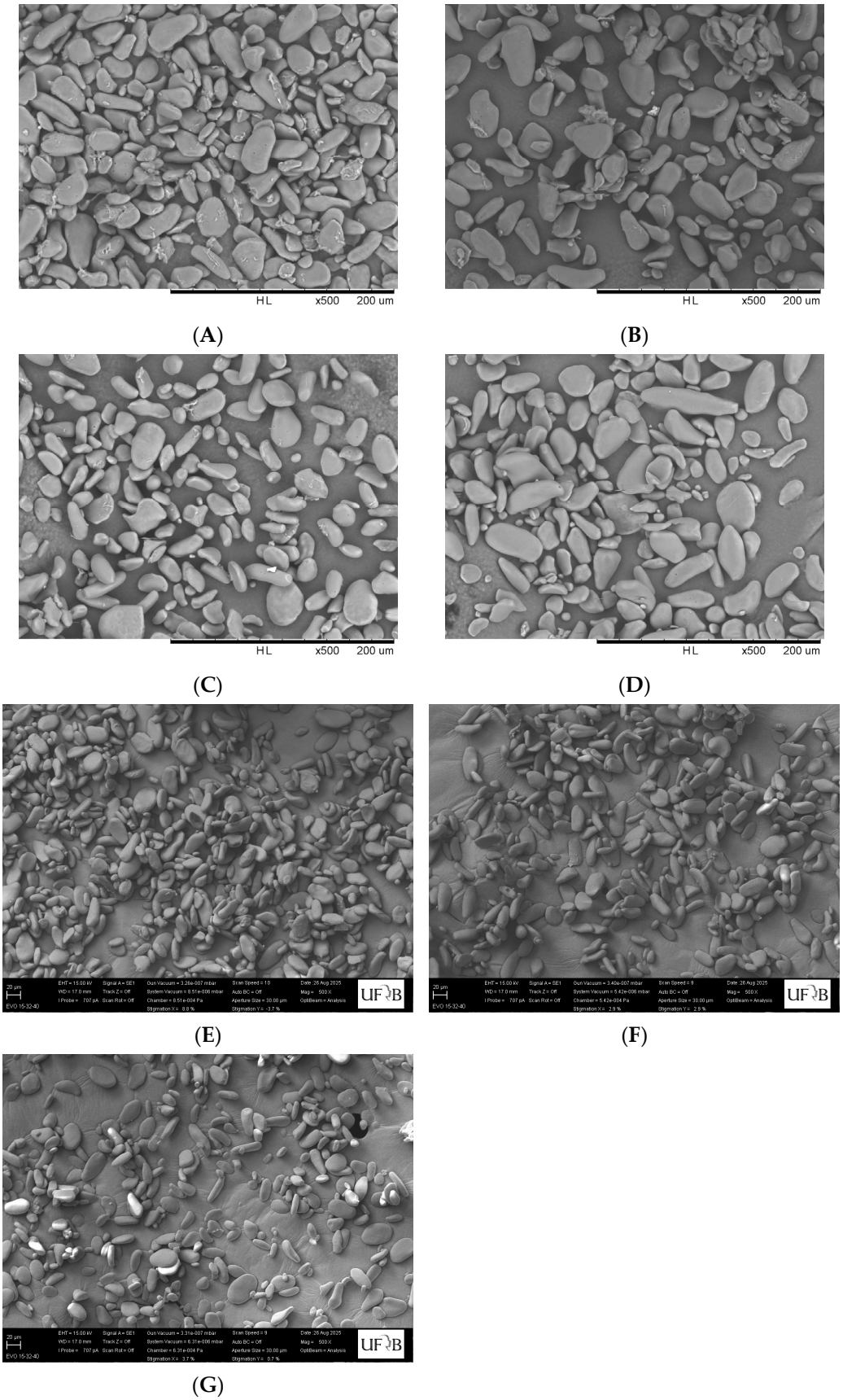


Figure 2. Scanning electron micrographs of banana and plantain starches. BRS Gerais (**A**), BRS Platina (**B**); Gorutuba Biocell (**C**), Prata-Anã (**D**); BRS Terra-Anã (**E**); Tipo Velhaca (**F**) e Mongolo (**G**).

3.2. X-Ray Diffraction Pattern and Relative Crystallinities

Starches isolated of seven cultivars showed a B-type diffraction pattern, with the most intense peaks occurring at 5, 15, 17, 22, and 24° 2θ. The crystalline regions of starches are formed by short outer segments of amylopectin. Relative crystallinity of starches ranged from 19.31 to 26.86% (Figure 3).

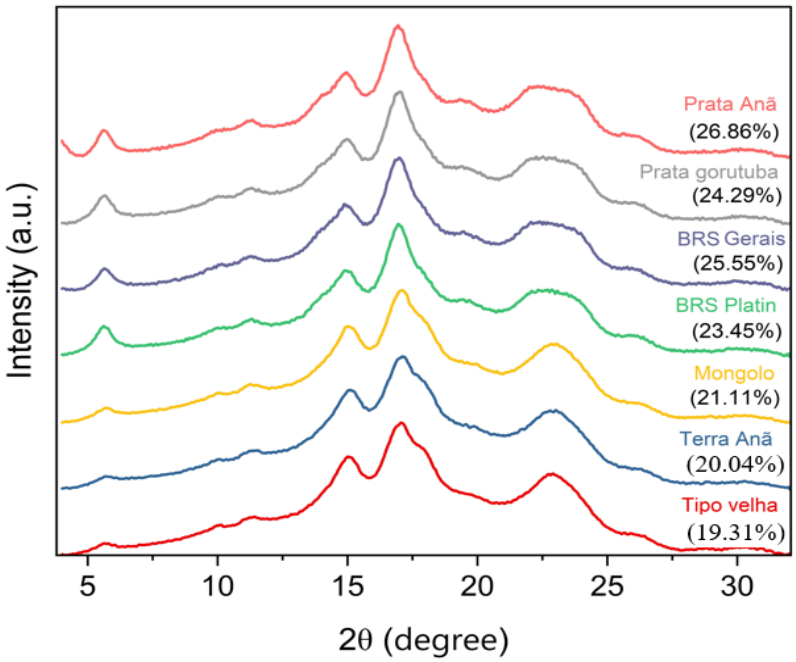


Figure 3. X-ray diffraction patterns and relative crystallinities of starches.

X-ray diffraction patterns are classified based on variations in water content and the packing form of the amylopectin double helix. The starches from the Prata subgroup cultivars and those isolated from plantains cultivars had a B-type X-ray diffraction pattern (Figure 3). Type B starches are more resistant to enzymatic and acid hydrolysis and are more sensitive to hydrothermal treatment, characteristics that are relevant for applications in processed foods [38–40].

Starch crystallinity influences the physical, mechanical, and technological properties of various starchy products, making it a key factor in product development, quality assurance, and process control. In this study, cultivars from the Prata subgroup showed higher crystallinity than plantains (Figure 3). However, the crystallinity levels were lower than those reported for Indonesian varieties (33.20–38.64%) by Marta et al. [17], as well as for the cooking cultivars Saba (52.02%), Popoulu (48.10%), and Monthan (35.11%) described by Paramasivam et al. [2], likely due to genetic differences among genotypes.

Such variations in starch crystallinity can influence key functional properties like viscosity, gelation, and matrix formation, ultimately affecting the texture, stability, quality, and digestibility of food products.

3.3. Amylose and Resistant Starch

The amylose and resistant starch (RS) contents varied significantly among the banana cultivars studied, with amylose contents ranging from 31.4 to 43% and RS contents ranging from 66.6 to 74.8% (Table 1).

Table 1. Amylose and resistant starch contents of starches isolated from different cultivars of bananas and plantains.

Cultivars	Amylose (%)	Resistant Starch (%)
BRS Terra-Anã	43.0 ± 0.2 [a]	74.4 ± 1.0 [a]
Tipo Velhaca	42.5 ± 0.3 [a]	74.8 ± 1.5 [a]
Mongolo	42.9 ± 1.2 [a]	74.0 ± 1.7 [a]
BRS Gerais	31.4 ± 0.2 [c]	68.5 ± 0.1 [b]
BRS Platina	35.3 ± 0.2 [b]	68.8 ± 1.2 [b]
Gorutuba Biocell	34.8 ± 1.3 [b]	68.1 ± 0.4 [b]
Prata-Anã	32.7 ± 0.3 [c]	66.6 ± 1.3 [b]

Means followed by the different lowercase letters in the columns differ significantly from each other according to Tukey's test ($p < 0.05$) (n = 4).

Terra Anã (43.0%), Mongolo (42.9%), and Tipo Velhaca (42.5%) had the highest amylose content (Table 1). Amylose influences several properties, such as crystallinity, gelatinization, retrogradation, and susceptibility to enzymatic digestion. Starches with high amylose content form denser and firmer gels and are therefore used in the production of edible packaging, emulsions, and formulations that require greater thermal stability and firm texture [2].

The cultivars BRS Gerais (31.4%) and Prata-Anã (32.7%) had the lowest amylose contents, characterizing starches with a predominantly branched structure. This characteristic favors the formation of more viscous pastes (Table 1). These characteristics are desirable in products that require short-term gel stability or lower syneresis.

Resistant starch is the portion of total starch that resists digestion and absorption in the small intestine and instead ferments in the large intestine. It is recognized as a functional food that promotes benefits to health [4,11]. RS contents varied among cultivars, with Tipo Velhaca, Terra Anã, and Mongolo standing out with contents of 74.8, 74.4, and 74%, respectively (Table 1). This result is consistent with the high amylose content observed in these same cultivars, reinforcing the direct relationship between amylose and RS. According to Bi et al. [41], plantain starch tends to have higher RS content, higher gelatinization temperatures, and higher enthalpy than dessert banana starches.

Prata-Anã, Prata Gorutuba, BRS Platina, and BRS Gerais cultivars had significantly lower RS contents (68%) but were still considered high compared to other commercial starchy sources such as corn and potatoes [42], data that corroborate the results found by Leonel et al. [16] when evaluating the cultivars BRS Platina (74.55%) and BRS Conquista (69.84%).

These results indicate the functional potential of these starches for use as prebiotic ingredients in food products or in low glycemic index diets. In addition, RS contributes to improving the absorption of minerals such as iron and calcium and helps regulate blood glucose and cholesterol levels, reinforcing the potential of banana starches as functional ingredients. The relationship between high amylose content and higher RS content confirms the role of molecular structure in starch digestibility and highlights the functional potential of the cultivars evaluated.

3.4. Pasting and Thermal Properties

Viscoamylographic profiles of starches isolated from banana and plantain cultivars are shown in Figure 4. Significant differences were observed in the pasting properties between the cultivars studied (Table 2).

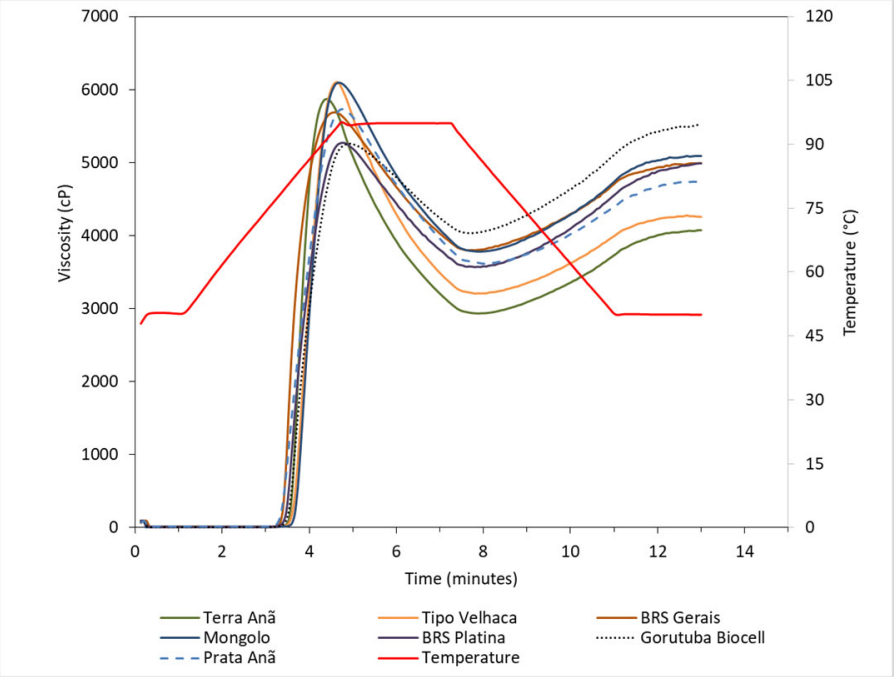


Figure 4. Viscoamylogram of starches analyzed in Rapid Visco Analyzer.

Table 2. Pasting properties of starches isolated from banana and plantain cultivars.

Cultivars	TP	PT	PV	MV	BD	FV	SB
	(°C)	(min.)	cP				
BRS Terra-Anã	4.4 ± 0.0 ^{d}	79.5 ± 0.1 ^{b}	5877 ± 264 ^{ab}	2931 ± 148 ^{c}	2945 ± 279 ^{a}	4074 ± 158 ^{d}	1142 ± 36 ^{abc}
Tipo Velhaca	4.6 ± 0.0 ^{c}	80.4 ± 0.4 ^{a}	6127 ± 143 ^{a}	3202 ± 116 ^{c}	2925 ± 26 ^{a}	4257 ± 118 ^{cd}	1055 ± 18 ^{c}
Mongolo	4.7 ± 0.0 ^{b}	81.0 ± 0.4 ^{a}	6104 ± 200 ^{a}	3778 ± 122 ^{ab}	2327 ± 263 ^{b}	5091 ± 142 ^{ab}	1313 ± 25 ^{abc}
BRS Gerais	4.6 ± 0.0 ^{c}	77.3 ± 0.3 ^{de}	5693 ± 108 ^{c}	3787 ± 117 ^{ab}	1906 ± 143 ^{bc}	4992 ± 179 ^{b}	1205 ± 80 ^{abc}
BRS Platina	4.8 ± 0.1 ^{b}	78.0 ± 0.2 ^{cd}	5291 ± 299 ^{cd}	3560 ± 222 ^{b}	1732 ± 398 ^{c}	4988 ± 449 ^{b}	1429 ± 410 ^{ab}
Gorutuba Biocell	4.9 ± 0.1 ^{a}	78.4 ± 0.6 ^{c}	5273 ± 161 ^{d}	4047 ± 135 ^{a}	1226 ± 37 ^{d}	5517 ± 335 ^{a}	1471 ± 210 ^{a}
Prata-Anã	4.7 ± 0.1 ^{b}	76.8 ± 0.5 ^{e}	5752 ± 139 ^{ab}	3611 ± 179 ^{b}	2141 ± 259 ^{bc}	4739 ± 235 ^{bc}	1127 ± 58 ^{bc}

PT = pasting temperature, TP = time of peak, PV = peak viscosity, MV = minimal viscosity, BD = breakdown, FV = final viscosity, SB = setback. Means followed by the different lowercase letters in the columns differ significantly from each other according to Tukey's test ($p < 0.05$) (n = 4).

The cultivars Tipo Velhaca, Mongolo, Terra Anã, and Prata Anã showed higher peak viscosities, indicating greater swelling power (Figure 4, Table 2). Higher peak viscosities may be associated with larger granules and a less dense structure, which facilitates swelling [2]. Starches with higher PV finds application as thickener in food applications.

The Gorutuba Biocell, BRS Gerais, and Mongolo cultivars showed higher minimum viscosity (4047, 3787, and 3778 cP, respectively), demonstrating greater resistance to thermal rupture (Table 2). This stability can be attributed to the presence of longer amylopectin chains and a higher degree of crystallinity, as observed by Bi et al. [40] in plantain starches with high thermal stability.

The cultivars Terra Anã and Tipo Velhaca presented the highest viscosity breakdowns (2925 and 2946 cP, respectively), indicating higher sensitivity to thermal breakdown. This characteristic may limit their use in formulations requiring high heat and shear resistance. In contrast, the cultivar Gorutuba presented the lowest viscosity breakdown (1226 cP), suggesting higher thermal stability of the starch granules [41,42]. This result indicates the efficiency of this starch in withstanding heat and thermal stress, which is a characteristic of

cross-linked starches, suggesting a possible use of starch from this cultivar as a substitute for chemically cross-linked starch.

The final viscosity, an important parameter for evaluating gel formation after cooling, was higher for the starches from the Gorutuba (5518 cP) and Mongolo (5091 cP) cultivars, indicating a higher capacity for molecular re-alignment, especially of amylose, during cooling. This pattern is linked to the formation of firmer textures, as discussed by Castañeda-Niño et al. [43] when they studied starches from plantain with high amylose content and high melting enthalpy.

The setback, expressed by the difference between the final and minimum viscosity, was higher in the starches of the Gorutuba (1471 cP), BRS Platina (1429 cP), Mongolo (1313 cP), BRS Gerais (1205 cP), and Terra anã (1142 cP), suggesting a greater tendency for the reassociation of amylose or linear chain fragments and the formation of retrograded gel (Figure 1). As reported by Marta et al. [11], retrogradation is higher in starches with higher amylose content, due to the ability of linear chains to reassociate through hydrogen bonds, forming more organized crystalline structures.

The peak time ranged from 4.4 to 4.9 min. The shortest time was observed for starch isolated from the 'BRS Terra Anã' (4.4 min), indicating lower thermal resistance of the granules and higher gelatinization speed, which may be related to a less compact granular structure or higher porosity. Higher values, such as those for starch from the Gorutuba cultivar (4.9 min), indicate more thermal stability, which is usually linked to a more organized structure of the granules and less porosity, as reported by Kaur et al. [44].

Pasting temperature ranged from 76.8 to 81.0 °C. Starches isolated from the Mongolo and Tipo Velhaca cultivars had the highest temperatures, 81 and 80.4 °C, respectively, suggesting greater internal organization and the possible presence of complexing compounds such as lipids [10]. The starch from 'Prata-Anã', with a lower gelatinization temperature (76.8 °C), tends to gelatinize more quickly, a desirable characteristic for quick-cooking products [2,12,45].

The differences observed in the pasting properties of banana starches can be attributed to differences in composition (amylose, lipid, and protein content), granule structure (morphology, size distribution), and type of crystallinity.

The thermal gelatinization properties of starches differed among the banana and plantain cultivars studied. The initial gelatinization temperature (To) ranged from 67.9 to 71.8 °C, the peak temperature (Tp) from 72.7 to 76.6 °C, the final temperature (Tf) from 83.4 to 95.5 °C, with the temperature range (ΔT) varying from 15.5 to 26.6 °C and the gelatinization enthalpy (ΔH_{gel}) from 17.6 to 21.1 J/g (Table 3).

Table 3. Thermal properties of gelatinization of starches.

Cultivars	To (°C)	Tp (°C)	Tf (°C)	ΔT (°C)	ΔH_{gel} (J g^{-1})
BRS Terra-Anã	70.1 ± 0.2 ^{b}	74.9 ± 0.1 ^{b}	92.7 ± 1.8 ^{a}	22.6 ± 2.0 ^{ab}	17.6 ± 0.7 ^{c}
Tipo Velhaca	70.1 ± 0.2 ^{b}	74.9 ± 0.1 ^{b}	92.7 ± 1.8 ^{a}	22.6 ± 2.0 ^{ab}	17.6 ± 0.7 ^{c}
Mongolo	71.8 ± 0.2 ^{a}	76.6 ± 0.3 ^{a}	95.5 ± 2.5 ^{a}	23.6 ± 2.6 ^{a}	18.0 ± 1.6 ^{ab}
BRS Gerais	70.5 ± 0.3 ^{b}	74.4 ± 0.6 ^{b}	87.8 ± 0.1 ^{bc}	17.3 ± 0.4 ^{c}	21.1 ± 2.4 ^{a}
BRS Platina	69.5 ± 1.3 ^{b}	74.8 ± 1.3 ^{b}	89.2 ± 3.8 ^{b}	19.7 ± 3.0 ^{bc}	17.6 ± 1.7 ^{c}
Gorutuba Biocell	69.9 ± 1.3 ^{b}	74.6 ± 1.1 ^{b}	87.7 ± 1.7 ^{bc}	17.8 ± 1.0 ^{bc}	19.2 ± 1.6 ^{ab}
Prata-Anã	67.9 ± 0.3 ^{c}	72.7± 0.1 ^{c}	83.4 ± 4.4 ^{c}	15.5 ± 4.2 ^{c}	19.6 ± 1.3 ^{ab}

Means followed by the different lowercase letters in the columns differ significantly from each other according to Tukey's test ($p < 0.05$) (n = 4).

The endothermic transition of starch is related to interactions between its components, such as amylose, amylopectin, amylose-lipid complexes, and amylose-amylose associations. The physicochemical characteristics, initial temperature (To), peak temperature (Tp), final temperature (Tf), and enthalpy change (ΔH) are mainly determined by the molecular organization of the crystalline fraction. Thermal property results for different starches showed differences in gelatinization temperature and enthalpy values among the cultivars analyzed (Table 3).

The onset temperature of gelatinization (To) represents the moment when the crystalline regions of the granules begin to disorganize with heating, with the starch isolated from the Mongolo cultivar differing from the others due to its higher initial temperature, and that isolated from the Prata Anã cultivar due to its lower To (Table 3).

The peak temperature (Tp) represents the maximum point of disorganization of the crystalline regions of the starch granules. The different characteristics of the Mongolo and Prata Anã cultivars remained the same, with the highest and lowest Tp, respectively. This suggests that the starch from the 'Mongolo' plantain is more thermally stable, while the starch from the 'Prata Anã' banana is less resistant.

According to Chang et al. [46], higher peak temperatures are typical of starches with longer and well-organized amylopectin chains, while lower values reflect structures with weak or disorganized crystallinity.

The final temperature indicates the point at which the entire crystal structure has been disrupted. The starches isolated from plantains differed in terms of higher Tf. Starches from dessert banana cultivars differed in terms of lower Tf, with the starch isolated from the Prata Anã cultivar having the lowest Tf.

The gelatinization temperature range reflects the uniformity of the crystalline structure. Narrower ranges indicate more homogeneous granules in terms of crystallinity, which was observed for the starches of the Prata-Anã, BRS Gerais, Gorutuba Biocell, and Platina cultivars. A lower organization of the crystalline structure may explain the higher ΔT values of the isolated starches from the Mongolo, Terra Anã, and Tipo Velhaca cultivars.

3.5. Clustering and Principal Component Analyses

Hierarchical clustering based on Euclidean distance and the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) algorithm revealed two primary genotype clusters (Figure 5). Group G1 included plantain starches from the Mongolo, BRS Terra-Anã, and Tipo Velhaca cultivars, with the starches isolated from BRS Terra-Anã and Tipo Velhaca showing the greatest similarity. Group G2 consisted of banana cultivars from the Prata, Prata Anã, BRS Gerais, BRS Platina, and Gorutuba Biocell subgroup.

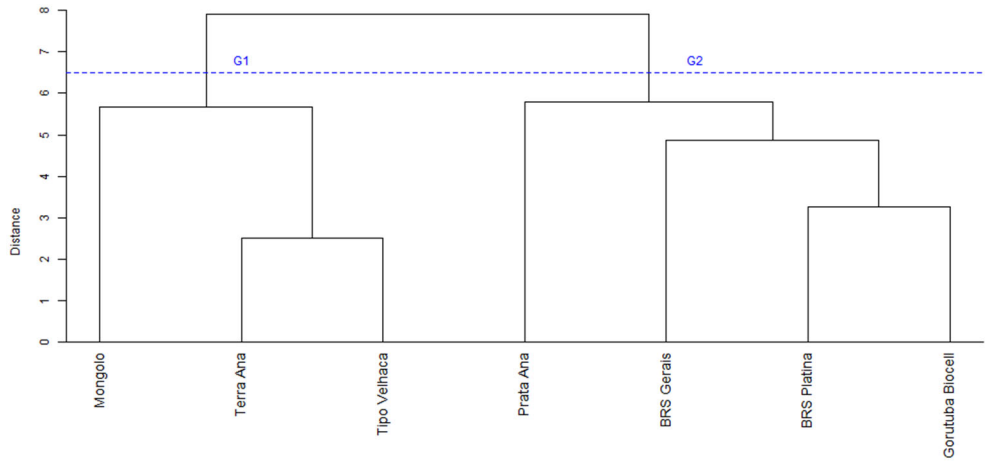
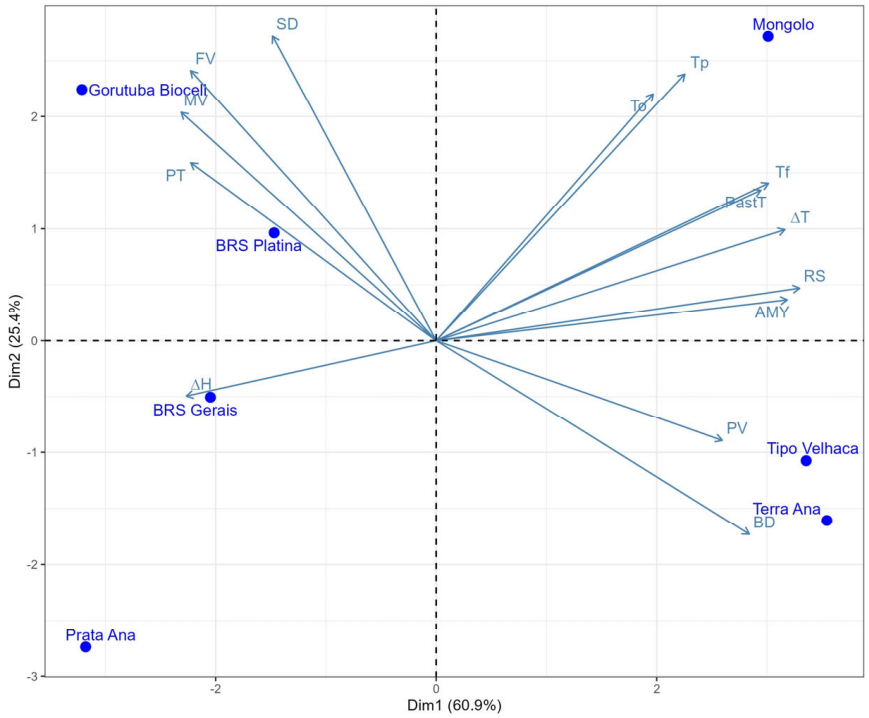


Figure 5. Cluster analysis showing the correlations between starches isolated from different cultivars.

Hierarchical cluster analysis showed the formation of two groups that clearly separate the starches isolated from plantains from those isolated from the cultivars of the Prata subgroup. Variables such as resistant starch, amylose, and final gelatinization temperature had a strong influence on group formation (Figure 5).

Principal component analysis was applied to establish a descriptive model of genotype grouping according to their viscoamylographic parameters, thermal properties, amylose content, and resistant starch content. The results showed that only the first two components (PC1 and PC2) explained 86.21% of the total variability (Figure 6).



AMY= amylose - RS= resistant starch - PT= peak time - PastT= pasting temperature - PV=peak viscosity
MV= minimal viscosity - BD=breakdown - FV= final viscosity - SD= setback - To= onset temperature
Tp= peak temperature - Tf= final temperature - ΔT= temperature range - ΔH = enthalpy of gelatinization

Figure 6. Biplot of principal component analysis.

The analysis of the principal components showed that the first component (PC1) was mainly influenced by the resistant starch (11.42%), amylose (10.66%), gelatinization temperature range (10.51%), final temperature (9.55%), paste temperature (9.12%), breakdown (8.48%), peak viscosity (7.08%), and enthalpy (Figure 6). The Mongolo cultivar, located in the positive region of PC1, was characterized by high amylose and resistant starch contents. The Terra Anã and Tipo Velhaca cultivars, positioned close to the peak viscosity and breakdown vectors, indicate greater thermal stability and resistance to mechanical agitation. In contrast, the BRS Gerais cultivar, located in the negative region of PC1, suggests higher gelatinization enthalpy, i.e., it requires more energy to rupture the crystalline structure of the starch. These starches may have the potential to complement common industrial starches, such as corn, potato, and cassava starches, for different applications.

The second principal component (PC2) was defined by the tendency to retrogradation (18.71%), final viscosity (14.69%), peak gelatinization temperature (14.32%), initial gelatinization temperature (12.25%), minimum viscosity (10.49%), and peak time (6.37%). The Prata Gorutuba and BRS Platina cultivars are associated with final viscosity, minimum viscosity, and peak temperature vectors, which are related to paste behavior. The Prata-Anã cultivar, located at the lower left extreme, far from most vectors, indicates low values in

almost all evaluated characteristics. Mesquita et al. [39] reported that the starch of the 'Prata-Anã' had a lower homogeneity of its crystalline area compared to other cultivars.

Banana and plantain starches may have the potential to complement common industrial starches, such as corn, potato, and cassava starches, for different applications. In order to increase the applications of these starches, the structure-function-utilization relationships of individual starch are essential.

The banana production chain involves significant losses, which can be reduced by using unripe, disqualified fruits as starch sources. The differences and similarities observed in banana and plantain starches contribute to the introduction of different banana and plantain cultivars in various regions of Brazil, expanding the viability of a circular economy in the production chain, and aligning with the goals of sustainable development, with the reduction in losses and waste and technological development. However, it is important to highlight the impacts of environmental factors and agricultural practices on starch biosynthesis in plants. Therefore, studies analyzing the properties of the starches of these cultivars under different conditions are necessary.

4. Conclusions

The results showed that plantain cultivars, which have a more limited market for fresh sales, can be explored as sources of starches with higher amylose and resistant starch contents, as well as greater thermal stability. The characteristics of starches isolated from Prata subgroup bananas contribute to promoting varietal diversification, considering the different potential uses of their starches.

The demand for native starches with properties that meet the diverse market for industrial uses in clean-label foods highlights the importance of the results obtained in this study. Starch blends emerge as a technology to overcome the restrictions of chemically modified starches in food products. Thus, this study reveals important dissimilarities between the starches studied, which can be explored in blends of banana and plantain starches, as well as in blends with traditional starches.

Author Contributions: Conceptualization, J.L.d.J.A., M.L., E.P.A., and S.L.; methodology, validation, formal analysis, and investigation, J.L.d.J.A., E.d.S.V., R.C.R., C.W.P.d.C., P.d.J.N.; data curation, writing—original draft preparation, writing—review and editing, J.L.d.J.A., E.P.A., C.W.P.d.C., M.L., and S.L.; supervision, project administration, funding acquisition, J.L.d.J.A., E.P.A., M.L. All authors have read and agreed to the published version of the manuscript.

Funding: The authors acknowledge the financial support of the Embrapa Mandioca e Fruticultura and National Council for Scientific and Technological Development (CNPq) (Grant numbers 302848/2021-5, 302611/2021-5).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: Authors Jaciene Lopes de Jesus Assis, Eliseth de Souza Viana, Edson Perito Amorim and Ronielli Cardoso Reis were employed by the company Embrapa Cassava & Fruits. Author Carlos Wanderlei Piler de Carvalho was employed by the company Embrapa Food Technology. The remaining authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Kumari, B.; Sit, N. Comprehensive review on single and dual modification of starch: Methods, properties and applications. *Int. J. Biol. Macromol.* **2023**, *253*, 126952. [CrossRef]
2. Paramasivam, S.K.; Saravanan, A.; Narayanan, S.; Shiva, K.N.; Ravi, I.; Mayilvaganan, M.; Pushpa, R.; Uma, S. Exploring differences in the physicochemical, functional, structural, and pasting properties of banana starches from dessert, cooking, and plantain cultivars (*Musa* spp.). *Int. J. Biol. Macromol.* **2021**, *191*, 1056–1067. [CrossRef]
3. Leonel, M.; Bolfarini, A.C.B.; Rodrigues Da Silva, M.J.; Souza, J.M.A.; Leonel, S. Banana fruits with high content of resistant starch: Effect of genotypes and phosphorus fertilization. *Int. J. Biol. Macromol.* **2020**, *150*, 1020–1026. [CrossRef]
4. Shrivastava, A.; Gupta, R.K.; Srivastav, P.P. Exploring novel frontiers of advancements in purple yam (*Dioscorea alata* L.) starch extraction, modification, characterization, applications in food and other industries. *Meas. Food* **2024**, *15*, 100196. [CrossRef]
5. Lossolli, N.A.B.; Leonel, M.; Leonel, S.; Izidoro, M.; de Paula, G.V.; Santos, T.P.R.; de Oliveira, L.A. Cultivars and fruit part as differentiating factors of physicochemical characteristics of mango starches. *Horticulturae* **2023**, *9*, 69. [CrossRef]
6. FAO—Food and Agriculture Organization of the United Nations. Faostat. Available online: http://www.fao.org/faostat/en/#rankings/countries_by_commodity (accessed on 8 June 2025).
7. Kumar, P.S.; Saravanan, A.; Sheeba, N.; Uma, S. Structural, functional characterization and physicochemical properties of green banana flour from dessert and plantain bananas (*Musa* spp.). *LWT* **2019**, *116*, 108524. [CrossRef]
8. Jiang, H.; Zhang, Y.; Hong, Y.; Bi, Y.; Gu, Z.; Cheng, L.; Li, Z.; Li, C. Digestibility and changes to structural characteristics of green banana starch during in vitro digestion. *Food Hydrocoll.* **2015**, *49*, 192–199. [CrossRef]
9. Salazar, D.; Arancibia, M.; Lalaleo, D.; Rodríguez-Maecker, R.; López-Caballero, M.E.; Montero, M.P. Physico-chemical properties and filmogenic aptitude for edible packaging of ecuadorian discard green banana flours (*Musa acuminanta* AAA). *Food Hydrocoll.* **2022**, *122*, 107048. [CrossRef]
10. Yang, M.; Chang, L.; Jiang, F.; Zhao, N.; Zheng, P.; Simbo, J.; Yu, X.; Du, S. Structural, physicochemical and rheological properties of starches isolated from banana varieties (*Musa* spp.). *Food Chem. X* **2022**, *16*, 100473. [CrossRef]
11. Marta, H.; Cahyana, Y.; Djali, M.; Pramafisi, G. The properties, modification, and application of banana starch. *Polymers* **2022**, *14*, 3092. [CrossRef]
12. Quintero-Castaño, V.D.; Castellanos-Galeano, F.J.; Álvarez-Barreto, C.I.; Lucas-Aguirre, J.C.; Bello-Pérez, L.A.; Rodríguez-Garcia, M.E. Starch from two unripe plantains and esterified with octenyl succinic anhydride (osa): Partial characterization. *Food Chem.* **2020**, *315*, 126241. [CrossRef]
13. De La Torre-Gutiérrez, L.; Chel-Guerrero, L.A.; Betancur-Ancona, D. Functional properties of square banana (*Musa balbisiana*) starch. *Food Chem.* **2008**, *106*, 1138–1144. [CrossRef]
14. Zhu, F. Underutilized and unconventional starches: Why should we care? *Trends Food Sci. Technol.* **2020**, *100*, 363–373. [CrossRef]
15. Tagliapietra, B.L.; Felisberto, M.H.F.; Sanches, E.A.; Campelo, P.H.; Clerici, M.T.P.S. Non-conventional starch sources. *Curr. Opin. Food Sci.* **2021**, *39*, 93–102. [CrossRef]
16. Leonel, M.; Leonel, S.; Santos, T.P.R.D.; Souza, J.M.A.; Martins, R.C.; Silva, M.S.C.D. Agronomic yield and starch properties of banana cultivars. *Pesqui. Agropecuária Bras.* **2021**, *56*, e02491. [CrossRef]
17. Marta, H.; Cahyana, Y.; Djali, M.; Arcot, J.; Tensiska, T. A Comparative study on the physicochemical and pasting properties of starch and flour from different banana (*Musa* spp.) cultivars grown in Indonesia. *Int. J. Food Prop.* **2019**, *22*, 1562–1575. [CrossRef]
18. Wu, T.-Y.; Tsai, S.-J.; Sun, N.-N.; Dai, F.-J.; Yu, P.-H.; Chen, Y.-C.; Chau, C.-F. Enhanced thermal stability of green banana starch by heat-moisture treatment and its ability to reduce body fat accumulation and modulate gut microbiota. *Int. J. Biol. Macromol.* **2020**, *160*, 915–924. [CrossRef]
19. Torres-Vargas, O.L.; Gaytan-Martinez, M.; Fernanda, C.-C.; Millán-Malo, B.M.; Rodriguez-Garcia, M.E. Changes in the physicochemical properties of isolated starch and plantain (*Musa* AAB Simmonds) flours for early maturity stage. *Heliyon* **2023**, *9*, e18939. [CrossRef]
20. Jaiturong, P.; Laosirisathian, N.; Sirithunyalug, B.; Eitssayeam, S.; Sirilun, S.; Chaiyana, W.; Sirithunyalug, J. Physicochemical and prebiotic properties of resistant starch from *Musa sapientum* Linn., ABB Group, cv. Kluai Namwa Luang. *Heliyon* **2020**, *6*, e05789. [CrossRef]
21. Pereira, L.V.; Narita, N.; Suguino, E.; Takata, W.H.S. Productive behavior of cultivars and banana genotype originating from Prata anã', irrigated and non-irrigated in the State of Minas Gerais, Brazil. *Int. J. Environ. Agric. Res.* **2019**, *5*, 1–7.
22. Santos, H.G.; Jacomine, P.K.T.; Anjos, L.H.C.; Oliveira, V.A.; Lumbreras, J.F.; Coelho, M.R.; Almeida, J.A.; Araújo Filho, J.C.; Oliveira, J.B.; Cunha, T.J.F. *Sistema Brasileiro de Classificação de Solos*, 5th ed.; Embrapa: Brasília, Brazil, 2018; p. 356.
23. PBMH & PIF—Programa Brasileiro Para a Modernização da Horticultura & Produção Integrada de Frutas. 2006. Available online: https://ceagesp.gov.br/wp-content/uploads/2015/07/banana.pdf (accessed on 7 July 2025).
24. Chiang, B.H.; Chu, W.C.; Chu, C.L. A pilot scale study for banana starch production. *Starch-Stärke* **1987**, *39*, 5–8. [CrossRef]
25. Bernardo, C.O.; Ascheri, J.L.R.; Chávez, D.W.H.; Carvalho, C.W.P. Ultrasound assisted extraction of yam (*Dioscorea bulbífera*) starch: Effect on morphology and functional properties. *Starch-Stärke* **2018**, *70*, 1700185. [CrossRef]

26. *ISO 6647:1987*; Norme Internationale: Riz-Détermination de la Teneur em Amylose. ISO: Geneva, Switzerland, 1987. Available online: https://www.iso.org/standard/13073.html (accessed on 18 June 2025).
27. Goñi, I.; García-Diz, L.; Mañas, E.; Saura-Calixto, F. Analysis of resistant starch: A method for foods and food products. *Food Chem.* **1996**, *56*, 445–449. [CrossRef]
28. Kassambara, A.; Mundt, F. Factoextra: Extract and Visualize the Results of Multivariate Data Analyses. 2020. Available online: https://CRAN.R-project.org/package=factoextra (accessed on 15 July 2025).
29. Lê, S.; Josse, J.; Husson, F. FactoMineR: An *R* package for multivariate analysis. *J. Stat. Soft.* **2008**, *25*, 1–18. [CrossRef]
30. R Core Team. R: A Language and Environment for Statistical Computing. 2024. Available online: https://www.R-project.org/ (accessed on 15 July 2025).
31. Sneath, P.H.A.; Sokal, R.R. *Numerical Taxonomy: The Principles and Practice of Numerical Classification*; A Series of Books in Biology; W. H. Freeman: San Francisco, CA, USA, 1973.
32. Sokal, R.R.; Rohlf, F.J. The Comparison of dendrograms by objective methods. *TAXON* **1962**, *11*, 33–40. [CrossRef]
33. Mantel, N. The Detection of Disease Clustering and a Generalized Regression Approach. *Cancer Res.* **1967**, *27*, 209–220.
34. Mingoti, S.A. *Análise de Dados Através de Métodos de Estatística Multivariada: Uma Abordagem Aplicada*; Editora UFMG: Belo Horizonte, Brazil, 2005.
35. Charrad, M.; Ghazzali, N.; Boiteau, V.; Niknafs, A. NbClust: An *R* package for determining the relevant number of clusters in a data set. *J. Stat. Soft.* **2014**, *61*, 1–36. [CrossRef]
36. Kaur, L.; Singh, J.; McCarthy, O.J.; Singh, H. Physico-chemical, rheological and structural properties of fractionated potato starches. *J. Food Eng.* **2007**, *80*, 383–394. [CrossRef]
37. Yuan, D.; Zhang, Y.; Chen, X.; Xu, F.; Zhu, K.; Wang, J.; Zhang, Y. Physicochemical, structural, and digestive properties of green banana starch from five Chinese mutant banana species. *Foods* **2025**, *14*, 706. [CrossRef]
38. Huang, J.; Zhao, L.; Man, J.; Wang, J.; Zhou, W.; Huai, H.; We, C. Comparison of physicochemical properties of B-type nontraditional starches from different sources. *Int. J. Biol. Macromol.* **2015**, *78*, 165–172. [CrossRef]
39. Mesquita, C.B.; Leonel, M.; Franco, C.M.L.; Leonel, S.; Garcia, E.L.; Santos, T.P.R. Characterization of banana starches obtained from cultivars grown in Brazil. *Int. J. Biol. Macromol.* **2016**, *89*, 632–639. [CrossRef] [PubMed]
40. Bi, Y.; Zhang, Y.; Gu, Z.; Cheng, L.; Li, Z.; Li, C.; Hong, Y. Effect of ripening on in vitro digestibility and structural characteristics of plantain (*Musa* ABB) starch. *Food Hydrocoll.* **2019**, *93*, 235–241. [CrossRef]
41. Viana, E.B.M.; Oliveira, N.L.; Ribeiro, J.S.; Almeida, M.F.; Souza, C.C.E.; Resende, J.V.; Santos, L.S.; Veloso, C.M. Development of starch-based bioplastics of green plantain banana (*Musa paradisiaca* L.) modified with heat-moisture treatment (HMT). *Food Packag. Shelf Life* **2022**, *31*, 100776. [CrossRef]
42. Alimi, B.A.; Workneh, T.S.; Oke, M.O. Effect of hydrothermal modifications on the functional, pasting and morphological properties of south african cooking banana and plantain. *CyTA-J. Food* **2016**, *14*, 489–495. [CrossRef]
43. Castañeda-Niño, J.P.; Mina-Hernández, J.H.; Solanilla-Duque, J.F. Extraction and characterization of starches from the pulp and peel of native plantain (*Musa* AAB Simmonds) from two Colombian departments. *Polysaccharides* **2025**, *6*, 34. [CrossRef]
44. Kaur, L.; Dhull, S.B.; Kumar, P.; Singh, A. Banana starch: Properties, description, and modified variations—A review. *Int. J. Biol. Macromol.* **2020**, *165*, 2096–2102. [CrossRef]
45. Xu, B.; Zhang, C.; Liu, Z.; Xu, H.; Wei, B.; Wang, B.; Sun, Q.; Zhou, C.; Ma, H. Starches modification with rose polyphenols under multi-frequency power ultrasonic fields: Effect on physicochemical properties and digestion behavior. *Ultrason. Sonochemistry* **2023**, *98*, 106515. [CrossRef]
46. Chang, L.; Yang, M.; Zhao, N.; Xie, F.; Zheng, P.; Simbo, J.; Yu, X.; Du, S. Structural, physicochemical, antioxidant and in vitro digestibility properties of banana flours from different banana varieties (*Musa* spp.). *Food Biosci.* **2022**, *47*, 101624. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.