



Physico-chemical and nutritional properties of breadfruit pulp and peel flours: Insights into starch molecular characteristics and their impact on starch digestibility

Caleb S. Calix-Rivera^{a,b,d}, Grazielle Náthia-Neves^{a,c}, Raúl Ricardo Mauro^{a,b},
Marina Villanueva^{a,b}, Felicidad Ronda^{a,b,*}

^a Department of Agriculture and Forestry Engineering, Food Technology, College of Agricultural and Forestry Engineering, University of Valladolid, 34004 Palencia, Spain

^b Bioeconomy Research Institute - BioEcoUva, PROCEREALtech Group, University of Valladolid, 47005 Valladolid, Spain

^c Research group for Bioactives – Analysis and Application, Technical University of Denmark, National Food Institute, Kgs. Lyngby DK-2800, Denmark

^d Department of Agroindustrial Engineering, Regional University Center of the Pacific Littoral, National Autonomous University of Honduras (UNAH), 51101 Choluteca, Honduras

ARTICLE INFO

Keywords:

Breadfruit flour
Flow field-flow fractionation
MALS detection
Thermal properties
In vitro starch digestibility

ABSTRACT

Breadfruit is an underutilized crop with significant nutritional potential as a gluten-free starch-rich food ingredient. This study evaluated the chemical, molecular, structural, and nutritional properties of breadfruit (BF) flours derived from both pulp and peel, along with banana flour as a reference. Starch digestibility, estimated *in vitro*, was linked to these properties. Both BF flours showed high starch and fiber contents, with low amylose levels. Flow Field-Flow Fractionation-MALS-dRI analysis revealed similar amylopectin molecular weights ($M_w = 1.04\text{--}1.15 \cdot 10^8 \text{ g/mol}$) and root mean square radius ($r_{rms} = 172\text{--}174 \text{ nm}$) in both BF flours, which were lower than those of banana flour ($M_w = 1.73 \cdot 10^8 \text{ g/mol}$; $r_{rms} = 187 \text{ nm}$). Scanning electron micrographs revealed that BF starch granules were smaller ($3\text{--}15 \mu\text{m}$) and rougher compared to those found in the banana sample ($15\text{--}50 \mu\text{m}$). X-ray diffraction showed a B-type crystalline pattern in BF samples. Fourier-transform infrared spectroscopy showed a higher ordered crystallinity of starch and a significantly higher amount of disordered structures in the Amide I region in BF flours compared to banana. BF flours also exhibited higher gelatinization temperatures with a narrower range, indicating increased granular thermostability and amylopectin crystallite homogeneity. Peel flour contained high levels of polyphenols and minerals. The lower amylopectin molecular weight and size, smaller starch granules and lower amylose content of BF flours compared to banana flour could explain the higher starch-digestion-rate-index (SDRI) of their uncooked samples. Cooked BF flours, however, showed an SDRI 10 % lower than banana, suggesting a reduced glycemic index after gelatinization. This study provides valuable insights into BF flours composition, molecular and structural properties, and their relationship with digestibility. These findings are relevant for developing novel gluten-free foods. Further research is needed to assess the starch digestibility of real food products made with BF flours and to investigate their functional properties and technological performance.

1. Introduction

Breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) belongs to the Moraceae family and is cultivated in over 90 countries [1]. Although widely grown in tropical regions, it is often regarded as an underutilized crop [2]. The value of the breadfruit (BF) as a food crop lies in its high productivity, which can reach approximately 50 t/ha/year [3], making it a potentially attractive agronomic, nutritional and socio-economic

alternative in regions where it is cultivated [2]. However, in developing countries, the use of BF is limited due to its poor storage properties, which lead to the deterioration of fresh fruits within 3–5 days after harvest [1]. The conversion of fresh BF fruit into flour has been investigated as an alternative to obtain a product with extended shelf life that is suitable for use in various food applications [4]. Some BF cultivars are recognized for their high antioxidant levels and pro-vitamin A carotenoids [1]. Additionally, BF is notable for its high energy content

* Corresponding author at: Av. Madrid, s/n, 34004, Palencia, Spain.

E-mail address: mfronda@uva.es (F. Ronda).

<https://doi.org/10.1016/j.ijbiomac.2025.141224>

Received 25 July 2024; Received in revised form 14 February 2025; Accepted 16 February 2025

Available online 17 February 2025

0141-8130/© 2025 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

(3870 kcal/kg), low crude fat content (3.94 g/100 g), rich dietary fiber, and high-quality proteins that contain essential amino acids such as lysine and leucine [3]. These attributes make it a valuable nutritional source compared to other crops such as cassava, sweet potato, and banana. In addition, it is considered an easily digestible fruit, as it does not contain antinutrients or substances that could cause intolerance [2]. Nutritionists have highlighted BF as one of the top 25 superfoods [5] for managing prevalent diet-related diseases in the Caribbean, including diabetes and hypertension [3]. This versatile and nutritious fruit can be prepared and consumed at all stages of ripeness, offering a variety of culinary options, including roasting, baking, boiling, drying, pickling, fermenting, freezing, dehydrating, and processing into flour [6]. In light of these findings, this underutilized fruit offers unique advantages in terms of sustainability, food security and diversity, as well as low allergenicity, making it a promising candidate for the development of novel food products [2]. Flour production has been identified as an effective way to incorporate BF into a variety of food products [7]. Several studies have shown that the inclusion of BF flour in food products does not negatively affect their sensory acceptability [8–11]. As a novel ingredient with a high starch content [12], BF offers significant potential as a complement to common starch sources such as corn, potato, wheat, and cassava. Moreover, this gluten-free fruit provides a valuable nutritional alternative flour for gluten-sensitive individuals. Given that BF is a highly productive crop, flour production generates significant residues, mainly BF peel, which is often discarded into the environment without treatment, leading to both environmental and economic issues [11]. Some researchers have shown that BF peel contains valuable compounds and nutrients, making it a promising resource for both food and non-food industries [13]. However, most studies to date have focused on producing flour exclusively from the fruit pulp, overlooking the potential of the peel [14]. The inclusion or exclusion of the peel can alter the chemical and physical properties of resulting flour, somewhat analogous to whole flours *versus* refined flours [6].

Recent studies have documented certain physical and structural characteristics of BF flour [7,12], while others have examined these attributes in isolated starch [15]. However, research on its molecular and structural properties and their relationship to digestibility remains scarce. Considering the various intrinsic and extrinsic factors that could affect starch digestion [16], further investigations are required to fully understand these relationships in BF flour.

This study hypothesizes that breadfruit flours derived from pulp and peel exhibit significant differences in their physical, molecular, and structural properties, which are expected to subsequently influence their *in vitro* digestibility. It is anticipated that the inclusion of peel in flour production will yield a product with unique attributes, potentially offering superior nutritional and functional advantages compared to pulp-only flour.

Therefore, the aim of this study was to investigate the physical parameters, molecular and structural properties of flours made from both BF pulp and peel, and to relate these properties to their *in vitro* digestibility. Additionally, the study aimed to compare them with banana flour, a well-known reference among starchy tropical fruits. By examining these two BF fractions, this study sought to promote the full utilization of BF, addressing environmental concerns related to peel waste while enhancing the overall value of BF processing. An accurate characterization of the digestive performance, molecular structure and their interactions in BF flours will provide valuable information on the full applicability of BF, contributing to economic development, food security, and the development of healthy food products aligned with current health trends.

2. Materials and methods

2.1. Flour preparation

The breadfruits, from var. Otea (white heart), were collected from

local growers in *El Progreso*, northern Honduras. Fig. 1 illustrates the process used to obtain the flour from both the peel and the pulp of the seedless BF. Briefly, the BFs were washed with potable water, peeled, and the pulp was cut into pieces. To prevent enzymatic browning, the pulp pieces and peels were submerged in 0.5 % (w/v) citric acid solution for 10 min, followed by drying in an oven at 60 °C for 7 h. The dried pulp and peels were then ground using a hammer mill (Corona, Colombia) and sieved through a 600 µm mesh to achieve a uniform particle size. The resulting flours were stored in airtight plastic bags at 4 °C for further assays. Commercial banana (*Musa sp.*) flour of unknown variety was obtained from Products Goya Nativo S.L. (Ecuador).

2.2. Proximate composition

Moisture, ash content, and total dietary fiber were determined using the official AACC methods 44–19, 08–12 and 32–05 [17], respectively. The crude fat content was measured by the Soxhlet method at 85 °C for 6 h, using n-hexane as the extraction solvent [15]. Protein content was determined by Dumas method using an Elemental Analyzer EA Flash 2000 (Thermo Fisher Scientific, Waltham, MA, US), applying a multiplication factor of 6.25 to the measured nitrogen [17]. Total starch content was determined by the Englyst method [18], with slight modifications described in Section 2.10. Amylose content was determined by the Concanavalin A method, using the Amylose/Amylopectin determination kit from Megazyme (Megazyme Bray, Ireland), with absorbance readings at 510 nm [19]. Each sample was analyzed at least in duplicate.

2.3. Molecular features by asymmetric flow field-flow fractionation (AF4-MALS-dRI)

Asymmetrical flow field-flow fractionation (AF4-MALS-dRI) was employed to determine molecular features such as weight-average molar mass (M_w), root mean square radius (r_{rms}) and polydispersity (M_w/M_n) from studied samples. Prior to AF4 analysis, samples were prepared following the procedure described by Syahariza et al. (2010) [20], with slight modifications. Briefly, 50 mg of starch equivalent of each flour (estimated based on total starch content) was suspended in 1 mL of a protease solution (0.2 U/mg starch) in tricin buffer (250 mM, pH 7.5) and incubated at 37 °C for 30 min. After centrifugation and removal of the supernatant, the precipitate was suspended in a sodium bisulfite solution (0.45 % w/w), incubated, and centrifuged again, discarding the supernatant. The precipitate was then resuspended in 1.5 mL of dimethyl sulfoxide (DMSO, HPLC grade, 99.9 %) and incubated in a thermal shaker (ISTHBLCTS, Ohaus, Parsippany, NJ, USA) at 80 °C for 24 h. The suspension was centrifuged at 4000 ×g for 10 min, and the supernatant was collected in centrifuge tubes (15 mL). The sample was precipitated twice with 10 mL of absolute ethanol (99.5 %) to isolate starch, which was then transferred to an 11 mL glass container, freeze-dried, and stored in a desiccator. The isolated starch samples were solubilized by adding 3 mL of DMSO (ThermoFisher GmbH, Kandel, Germany) and stirring for 1 h at 100 °C. Subsequently, the sample was diluted with the carrier liquid to a final concentration of 1 mg/mL and stirred for 5 min at 100 °C. The carrier liquid was composed of 10 mM NaNO₃ (Acros Organics, Geel, Belgium) and 200 ppm NaN₃ (Acros Organics, Geel, Belgium) dissolved in Milli-Q water and filtered through a 0.1 µm cellulose nitrate membrane filter of 47 mm diameter (Whatman, Merck KGaA, Darmstadt, Alemania). An isocratic pump (Agilent 1260 Infinity II, Agilent Technologies, Waldbronn, Germany) with a vacuum degasser delivered the carrier flow.

The extracted starch samples were fractionated and characterized using AF4 (Eclipse WEC-04, Wyatt Technology, Santa Barbara, CA, USA) connected to online MALS (Dawn WD3–04, Wyatt Technology) and dRI detection (Optilab WOP1–03, Wyatt Technology), both operating at 658 nm wavelength. All separations were carried out under the same experimental conditions, with a sample injection volume of 120 µL at 0.3 mL/min. The analysis was performed using a channel flow rate of

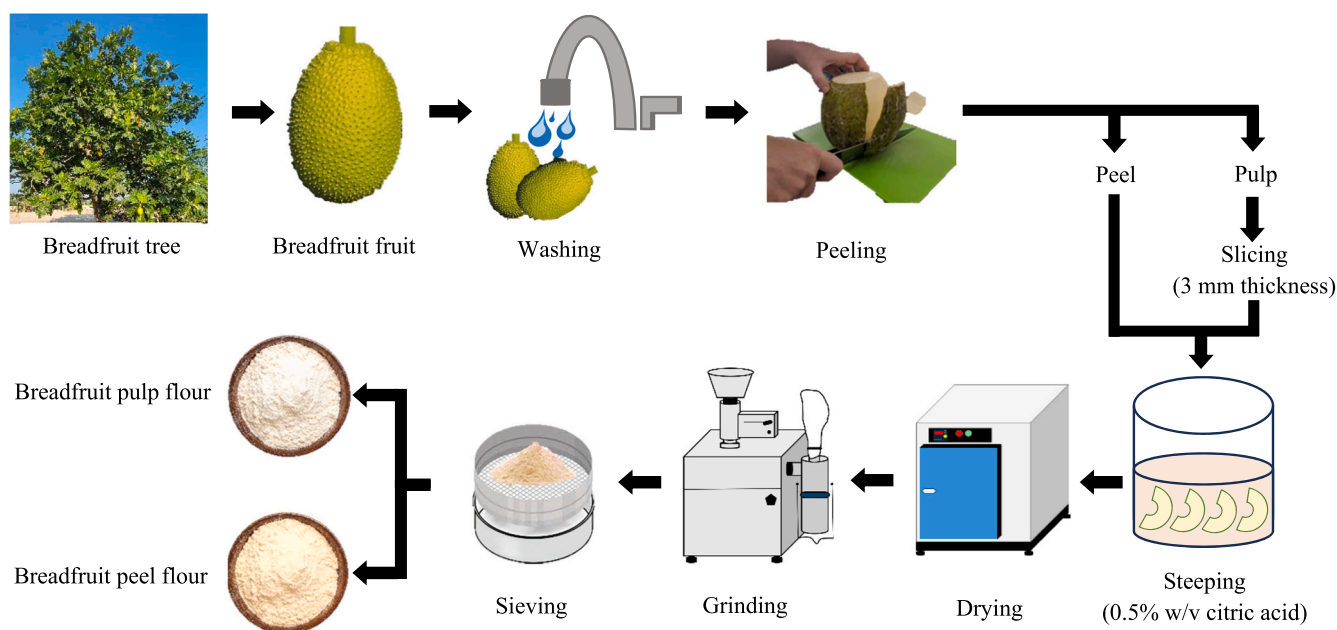


Fig. 1. Schematic representation of the processing of breadfruit fruits into pulp and peel flours.

0.6 mL/min and a detector flow rate of 0.3 mL/min. After injection, a focusing/relaxation was performed at a constant cross-flow of 3.5 mL/min for 5 min. The cross-flow rate was programmed to decay linearly from 3.5 mL/min to 0 mL/min over 25 min. The total duration of each run was 60 min. The scattering data obtained from MALS and DRI detectors after the AF4 separation were processed using the ASTRA software (v. 8.1.2.1, Wyatt Technology, Santa Barbara, CA, USA). The weight-average molar mass (M_w), the number-average molar mass (M_n), and the root-mean-square radius (r_{rms}) distributions were calculated using the Berry model with a fit degree of 1 [21]. The second virial coefficient (A_2) was neglected, and a specific refractive index increment (dn/dc) of 0.151 mL/g was used [22].

2.4. Scanning electron microscopy (SEM)

A Scanning Electron Microscope (SEM) model Quanta 200FEG (FEI, Hillsboro, OR, USA), equipped with a backscattered electron detector (BSED), was used to examine the flours. Samples were prepared following the method described by Calix-Rivera et al. (2023) [23] and analyzed in low-vacuum setting, with an accelerating voltage ranging from 4 to 5 keV. Photomicrographs were captured at magnifications of $\times 500$, $\times 1000$, and $\times 3000$ to illustrate the surface microstructure of the samples. The size of the starch granules was determined by image analysis of SEM micrographs using the ImageJ software (National Institutes of Health, USA).

2.5. X-ray diffraction (XRD)

Prior to measurement, the samples were equilibrated to a 15 % moisture content. The crystallinity characteristics of the samples were tested using a Bruker-D8-Discover-A25 diffractometer (Bruker AXS, Rheinfelden, Germany) with a Cu-K α radiation ($\lambda = 0.154$ nm), operating at 40 kV and 40 mA. X-ray diffraction (XRD) patterns of the flours were recorded with a diffraction angle (2θ) spanning from 5° to 40° and a scanning step of 0.02° . The receiving slit width was 0.02 nm, the scatter slit width was 2.92° , the divergence slit width was 1° and the scanning rate was $1.2^\circ/\text{min}$. The crystallinity of each flour was analyzed by DiffracEVA software with PDF2-2004 and Crystallography Open Database. The degree of crystallinity was determined by calculating the ratio of the diminished peak area attributed to the crystalline portion to

the total area [24].

2.6. Fourier-transform infrared (FT-IR) spectroscopy

FT-IR spectra of flours were measured by a FT-IR Nicolet iS50 spectrophotometer (Thermo Fisher Scientific, USA) utilizing an ATR sampling accessory with a diamond crystal. Prior to analysis, the samples were conditioned to a 15 % moisture level using a Memmert ICP260 saturated incubator (Schwabach, Germany). Spectra were collected over the range of 4000 cm^{-1} to 400 cm^{-1} with a resolution of 4 cm^{-1} and an accumulation of 64 scans. The short-range molecular ordering of starch and the secondary structure of proteins in the amide I region were analyzed following the procedure described by Calix-Rivera et al. (2023) [23]. Measurements were performed at least in triplicate.

2.7. Thermal analysis by differential scanning calorimetry (DSC)

The gelatinization properties of the studied flours were analyzed using a Differential Scanning Calorimeter (DSC3, STARe-System, Mettler-Toledo, Switzerland) according to the methodology described by Calix-Rivera et al. (2023) [23]. Briefly, approximately 6 mg of each sample was prepared at a ratio of 30:70 (flour:water, w/w) in 40 μL aluminum pans. The temperature scan ranged from 0 to 110°C at a heating rate of $5^\circ\text{C}/\text{min}$, with an empty sealed pan used as the reference. The enthalpy of gelatinization (ΔH) (J/g of starch) and the gelatinization temperatures (onset (T_O), peak (T_P), endset (T_E)) were recorded. Each measurement was carried out in duplicate.

2.8. Mineral content

Minerals contents (K, P, Mg, Ca, Fe, Cu, Zn, Mn, Na) were determined following the methodology established by Ronda et al. (2015) [25]. Briefly, 0.7 g of fruit flours was digested with 8 mL of high-purity 65 % HNO_3 and 2 mL of 30 % H_2O_2 using microwave digester (ETHOS SEL, Milestone, Italy). The mineral content was determined using a Varian 725-ES ICP-OES spectrophotometer (Agilent Technologies, Santa Clara, CA, USA), and each sample was analyzed in duplicate.

2.9. Total phenolic content

Total phenolic compounds (TPC) were extracted according to the method described by Li et al. (2011) [26]. One gram of each sample was mixed with 10 mL of a solvent composed of 80 % methanol and 1 N HCl (in 85:15 v/v ratio). This mixture was agitated at 30 °C for 4 h in a rotary shaker set at 300 rpm, and then centrifuged at 4060 ×g at 5 °C for 20 min. The supernatant was collected, and two additional extractions of the residue were conducted under the same conditions. The three supernatants were combined, and 40 µL were taken for TPC analysis using the Folin–Ciocalteu method, with a total final volume of 3.6 mL [27], and absorbance measured at 725 nm. Gallic acid standards ranging from 20 to 2000 µg·mL⁻¹ were used to generate a standard curve ($R^2 = 0.997$). All analyses were carried out in triplicate, and the results were expressed as mg of gallic acid equivalents (GAE) per 100 g of sample on a dry matter basis (mg GAE /100 g dry basis, db).

2.10. In vitro starch digestibility of samples

In vitro starch digestibility was evaluated following the method described by Englyst et al. (2006) [18], with modifications from Abebe et al. (2015) [28]. Both uncooked and cooked flours were assessed. For the uncooked sample, 0.8 g of the raw flour were used. The cooked sample was obtained by boiling 2 g of flour in 20 mL water for 30 min in centrifuge tubes (50 mL), with vortexing every 5 min. After cooling to room temperature, the entire gelatinized sample was frozen, freeze-dried, disaggregated, and sieved through a 600 µm mesh size prior to analysis. The hydrolyzed glucose at 20 min (G_{20}), 120 min (G_{120}), and the total glucose (TG) were measured. Free glucose + glucose from sucrose content (FSG) were determined using the rapid method described by Englyst et al. (2006) [18]. From these results, rapidly digested starch (RDS) = $0.9 \cdot (G_{20} - \text{FSG})$, slowly digestible starch (SDS) = $0.9 \cdot (G_{120} - G_{20})$, resistant starch (RS) = $0.9 \cdot (TG - G_{120})$, and total starch (TS) = $0.9 \cdot (TG - \text{FSG})$ were calculated. The starch digestibility rate index (SDRI) was computed as the percentage of RDS in TS in the flours. All tests were performed at least in triplicate.

2.11. Statistical analysis

Experimental data were evaluated through one-way analysis of variance (ANOVA) using Statgraphics Centurion XIX software (Statgraphics Technologies, Inc., Virginia, U.S.A.). Differences between means were considered significant at $p < 0.05$, as determined by Fisher's least significant difference (LSD) test.

3. Results and discussion

3.1. Proximate composition

The proximate composition of BF pulp, BF peel, and banana flours is shown in Table 1. The composition of the BF flour obtained from the pulp differed from that of the peel in all measured parameters, except for amylose content. BF flours showed a moisture content of 8.01–8.77 g/100 g, significantly lower than that of banana flour (11.33 g/100 g). However, all samples exhibited moisture contents below 15 g/100 g, ensuring safe storage and stability. Differences in the moisture content

of the matrices could be attributed to variations in the composition of their dry matter, as well as differences in processing methods and storage conditions. Commercial banana flour was produced through an industrial process, while breadfruit flours were prepared at a laboratory scale and analyzed immediately after production. Dietary fiber was an important fraction in both BF flours (16.6 and 20.9 g/100 g in the pulp and peel, respectively), which was more than double the fiber content of banana flour. The fiber content of BF samples was also higher than that of conventional flours, such as rice (0.9–4.3 g/100 g), maize (7.3 g/100 g) and oat (6.3 g/100 g) [29]. The high dietary fiber content found in BF flours is consistent with previous studies [1,7]. Due to their elevated fiber content, BF flours can serve as partial substitutes for fat or be integrated into products to reduce calorie content, increase complex carbohydrate content, and enhance binding properties in food products. The highest ash content was found in the peel, which was 58 % higher than in the pulp and 117 % higher than in banana flour. This is likely due to the higher fiber content in the peel, suggesting that it is richer in minerals than both the pulp [13,15] and banana flour. BF flours were also richer in fat and proteins compared to banana flour, with higher concentrations found in the peel than in the pulp. Similar results were reported by Graham and Bravo. (1981) [14], who attributed the higher protein content in the peel to the natural latex adhesion from the BF, which contains nitrogen compounds. The protein content of BF flour samples was lower than that of wheat (12.7 g/100 g) and rice (7.8 g/100 g) flours [28], but comparable to roots and tubers like sweet potato (3.4 g/100 g) and cassava (5.0 g/100 g) [30]. Since BF lacks gluten, its flours could serve as an alternative to wheat flour in plant-based substitutes for individuals with celiac disease [2]. Starch was the main component in both BF flours, with 61 g/100 g in the pulp and 50 g/100 g in the peel. However, the starch content in the BF pulp flour was 30 % lower than that in banana flour. The starch content of BF was also lower than that of cereals (e.g., wheat with 79 g/100 g and rice 88 g/100 g) [28], tubers (potato (85 g/100 g)) and roots (cassava (89 g/100 g)) [29,31]. However, it was higher than that of certain legumes, such as chickpea (26 g/100 g) and lentil (49 g/100 g) [31]. Therefore, BF could be considered a possible substitute or complement to conventional starchy raw materials. Amylose content was significantly lower in BF flours compared to banana flour. The amylose content in BF flours in this study was lower than previously reported in the literature, which ranged from 16.6 to 52.7 g/100 g of starch, depending on factors such as fruit maturity, origin, and growing conditions [2]. This confirms that amylose content in starch granules is influenced by the botanical source and is affected by climatic conditions and soil type during growth [30]. The amylose content is of technological importance and plays a crucial role in starch digestibility, thereby impacting its nutritional value [31]. BF shows great potential as an ingredient for enhancing the nutritional profile of a wide range of food products, such as snacks, breakfast cereals, and noodles. Notably, the high nutritional value of BF peel makes it a particularly valuable by-product for these applications.

3.2. Molecular conformation of starch samples by AF4-MALS-dRI analysis

Asymmetric flow field-flow fractionation (AF4) has proven to be a useful technique for the separation and characterization of a wide range of macromolecules and colloidal particles [21]. When coupled with

Table 1
Proximate chemical composition of breadfruit pulp, breadfruit peel and banana flours.

Sample	Moisture (g/100 g)	Protein (g/100 g)	Crude fat (g/100 g)	Dietary fiber (g/100 g)	Ash (g/100 g)	Total starch (g/100 g)	AC (g/100 g of starch)
Breadfruit pulp	8.77 ± 0.07 b	3.35 ± 0.09 b	1.7 ± 0.1 b	16.6 ± 1.6 b	3.65 ± 0.07 b	61.2 ± 2.2 b	10.9 ± 0.6 a
Breadfruit peel	8.01 ± 0.09 a	3.64 ± 0.02 c	3.0 ± 0.1 c	20.9 ± 2.1 b	5.78 ± 0.06 c	50.1 ± 2.5 a	12.2 ± 0.3 a
Banana	11.33 ± 0.01 c	2.09 ± 0.06 a	0.8 ± 0.1 a	8.0 ± 1.6 a	2.66 ± 0.08 a	78.8 ± 0.9 c	17.7 ± 0.3 b

AC: amylose content. All values are referred in dry basis. Results are the mean ± standard deviation. Values in the same column with different letters are significantly different ($p < 0.05$).

multi-angle light scattering (MALS) and differential refractive index (dRI) detectors, AF4 is particularly suitable for starch characterization, enabling the determination of parameters such as molar mass (M_W), radius of gyration or root mean-square radius (r_{rms}), and molecular conformation across the size distribution [21]. The M_W , r_{rms} and molecular dispersity or polydispersity (M_W/M_n) of the starch samples are presented in Table 2. The determination of these molecular features can be used to infer characteristics such as digestion susceptibility, resistance to crystallinity loss upon cooking, and molecular changes that impact the nutritional functionality of food products [16,32]. Fractograms illustrating molecular mass distributions and the root-mean-square radius are depicted in Supplementary Fig. 1, with distinct profiles for light scattering (LS) at 90° scattering angle and differential refractive index (dRI). The fractograms clearly showed two distinct groups, differentiated by molecular mass and elution time. The fraction with lower molecular mass and shorter elution time is mainly composed of simpler molecules like amylose and malto-oligosaccharide fragments, while the higher molecular mass fraction with longer elution times includes more complex molecules such as amylopectin.

The analyzed starch in BF samples exhibited a M_W of 1.5 and $3.1 \cdot 10^6$ g/mol for amylose and 1.04 and $1.15 \cdot 10^8$ g/mol for amylopectin, in pulp and peel, respectively (Table 2), which is consistent with findings in the literature for different starch sources [33,34], where amylopectin typically showed higher M_W values than amylose. Additionally, the r_{rms} values (31.4 and 52.7 nm for amylose and 174.2 nm and 172.3 nm for amylopectin in pulp and peel, respectively) closely matches those observed in previous studies (in the range of 10 – 60 nm for amylose and ~ 200 nm for amylopectin) [34]. The M_W of banana amylopectin fell within the range reported in earlier research (10^7 – 10^8 g/mol) [35]. For amylopectin molecules in BF, the M_W , r_{rms} , and polydispersity were similar across both fractions studied. Since these samples were derived from the same fruit, it is expected that their molecular characteristics would show high similarity [35]. BF amylopectin showed lower M_W and r_{rms} and higher polydispersity than banana amylopectin, regardless of whether the pulp or peel fractions were compared. The difference in r_{rms} was less pronounced than that observed in M_W , as evidenced by the lower M_W/r_{rms} ratio in BF flours (597 and 667 kDa·nm^{−1} for the pulp and the peel, respectively), compared to banana (927 kDa·nm^{−1}). This implies that BF amylopectin has a less compact structure compared to banana amylopectin which may make it more accessible to digestive enzymes for hydrolysis. The amylose molecules in BF flours varied significantly ($p < 0.05$) between pulp and peel (see Table 2). These results are in agreement with Tetlow and Bertoft (2020), who reported differences in amylose molecular weight between different parts of the plant [35].

3.3. Scanning electron microscopy (SEM)

The scanning electron micrographs of the flours, shown in Fig. 2,

Table 2

The AF4-MALS-dRI results for breadfruit pulp, breadfruit peel and banana samples.

Parameters	Breadfruit pulp	Breadfruit peel	Banana
		Amylose	
M_W (10^6 g·mol ^{−1})	1.5 ± 0.1 a	3.1 ± 0.2 c	2.3 ± 0.4 b
r_{rms} (nm)	31.4 ± 2.0 a	52.7 ± 2.5 c	41.3 ± 4.2 b
M_W/M_n	1.22 ± 0.01 ab	1.16 ± 0.01 a	1.24 ± 0.05 b
		Amylopectin	
M_W (10^8 g·mol ^{−1})	1.04 ± 0.06 a	1.15 ± 0.07 a	1.73 ± 0.02 b
r_{rms} (nm)	174.2 ± 0.6 a	172.3 ± 0.3 a	186.7 ± 1.4 b
M_W/M_n	2.0 ± 0.1 b	1.8 ± 0.1 b	1.4 ± 0.1 a

M_W : weight-average molar mass; r_{rms} : root mean square radius; M_W/M_n : polydispersity. Data are the mean \pm standard deviation. Similar letters in the same row indicate no statistically significant differences ($p > 0.05$).

revealed that starch granules were the predominant particles. The morphology and size of these granules vary according to their botanical source, serving as taxonomic criteria for plant species identification and influencing their suitability for specific food applications [30]. In all analyzed flours, starch granules were surrounded by irregular substances (pectin, cellulose, and a small amount of protein), along with integument coatings that contribute to their rough surface appearances. This association with larger structures like fiber, as indicated in the compositional analysis (Section 3.1), was particularly evident in BF flours, especially in the peel flour, which had a higher fiber content. The results indicated that BF starch granules were smaller than those of banana starch granules, ranging from 3 to 15 μ m in size and exhibiting irregular shapes (spherical, elliptical, polyhedral) in both pulp and peel samples, consistent with previous studies on BF [4,15]. Likewise, the size of BF starch granules was comparable to those of rice, buckwheat, and oat starch granules, and larger than those of amaranth and quinoa [36]. In contrast, banana starch granules were larger (15 to 50 μ m) and displayed varied shapes, including ellipsoidal flattened, irregular oval, slender rod, and cone shapes, corroborating earlier reports [37]. This indicates that BF flours had a relatively more uniform granule size than banana flour. These morphological features of small granule size observed in BF starch may influence its *in vitro* digestibility, as reported by Lindeboom et al. (2004) [36] (see Section 3.9). Differences in granule morphology are influenced by biological origin, amyloplast biochemistry, and plant physiology [38]. Starch granule size plays a crucial role in determining starch's physicochemical properties, affecting characteristics such as gelatinization and pasting behavior, enzyme susceptibility, crystallinity, and solubility [15]. The smaller granular sizes observed in BF flours may be advantageous for food products that require a smooth texture [30].

3.4. Crystalline structure of flours (XRD patterns)

The XRD patterns of the studied samples are presented in Fig. 3. BF flours exhibited diffraction peaks at 5.4° , 15.3° , 17.1° , 22.3° , 24.7° (2θ), indicative of a typical B-type pattern, as previously reported by Marta et al. (2019) [4]. The XRD patterns of the BF samples were similar to those reported for potato and white yam starches [15]. In contrast, banana flour showed a C-type pattern ($2\theta = 5.6^\circ$, 15° , 17.2° , 23.2°), reflecting a mixture of A-type and B-type crystalline arrangements, which is in agreement with findings from other studies [24]. However, some studies also reported a B-type pattern for banana flour [37,39], illustrating variability influenced by factors such as banana cultivar, growth conditions, isolation techniques, and other environmental variables [39]. The relative crystallinity, calculated based on diffraction intensity, was 50.2% for BF pulp and 49.7% for BF peel, both higher than the value observed in banana (48.8%). Some studies have indicated an inverse relationship between amylose levels and crystallinity, as amylose can disrupt the crystalline packing of amylopectin, potentially accounting for the relatively higher crystallinity observed in BF flours compared to banana flour [15,30,40]. Other studies have reported lower crystallinity values for BF starch [4] and banana starch [24,39] compared to those observed in this study. Factors such as amylose content, non-starch components, amylopectin chain length distribution, crystal size, and the alignment of double helices and their interactions can affect crystallinity [37].

3.5. FT-IR spectral analysis of samples

The results from FTIR analysis are shown in Table 3 and the corresponding spectra in Fig. 4. Infrared spectroscopy was used to characterize the ordered structure of starch and the secondary structure of proteins [23]. The starch band at 900 – 1100 cm^{−1} is known to be sensitive to starch structure [24], particularly the bands at 995 , 1022 , and 1047 cm^{−1}. The absorption peaks at 995 and 1047 cm^{−1} are indicative of the crystalline region, while the absorption peak at 1022 cm^{−1} is

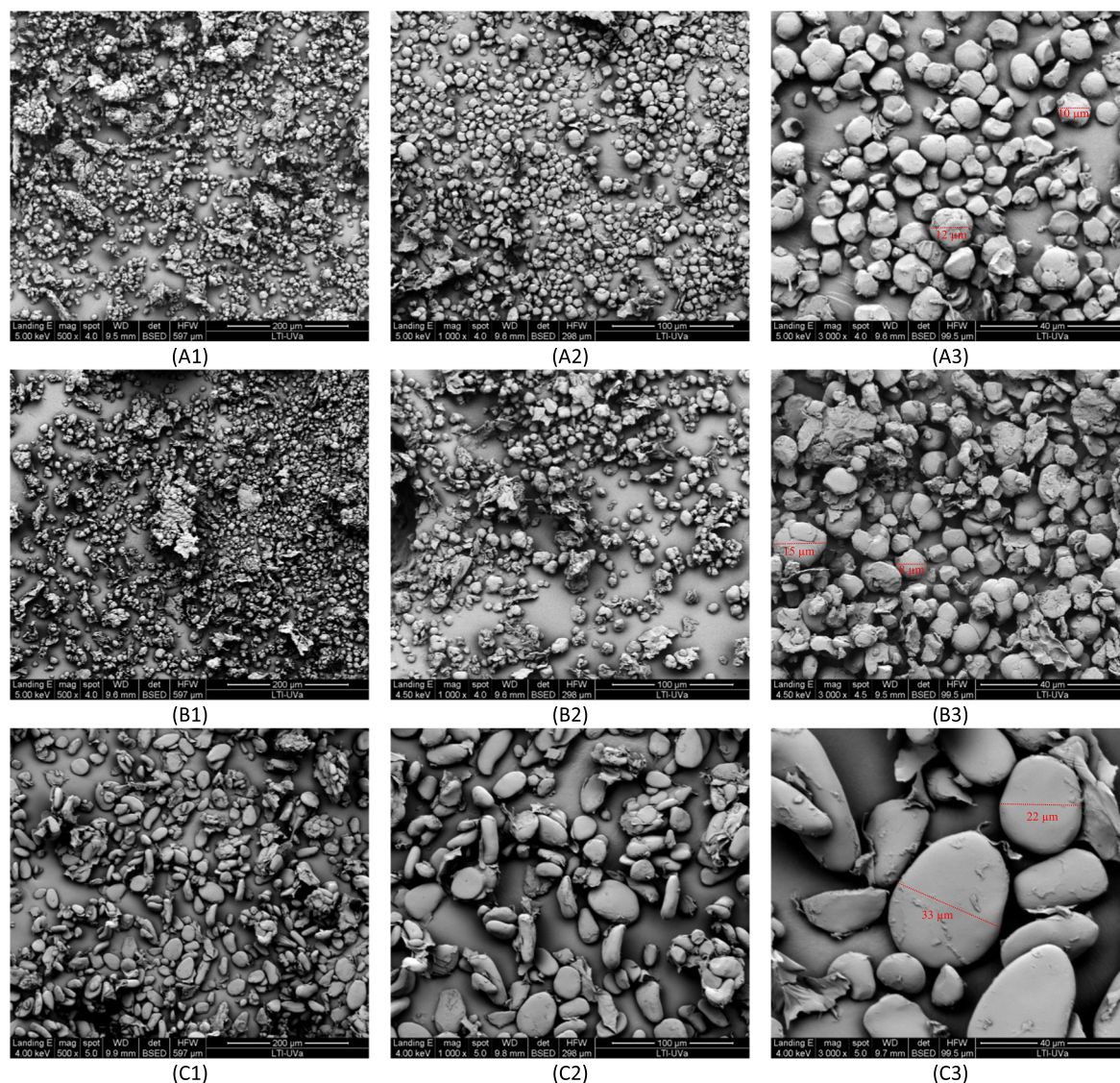


Fig. 2. Scanning electron microscopy (SEM) photomicrographs of studied flours: breadfruit pulp (A), breadfruit peel (B) and banana (C). Magnifications: $\times 500$ (1), $\times 1000$ (2) and $\times 3000$ (3).

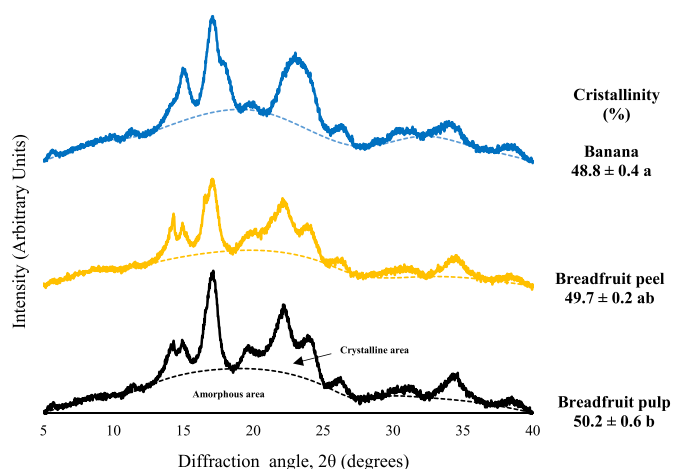


Fig. 3. X-ray diffraction patterns of breadfruit pulp, breadfruit peel, and banana. Intensity of crystalline peaks and percentage of relative crystallinity of individual samples are shown. Data have been offset for clarity.

associated with the amorphous region [41]. The 1047/1022 ratio is commonly used to measure short-range order in starch, while the 1022/995 ratio is employed to determine the ratio of amorphous to ordered starch structures [23]. Significant differences ($p < 0.05$) in the measured short-range ordered structures were observed among the samples. The 1047/1022 ratio ranged from 0.741 (banana) to 0.765 (BF peel), and the 1022/995 ratio varied from 0.790 (banana) to 0.868 (BF peel). The values of 1047/1022 ratio for BF samples were higher than those reported for BF starch by Li et al. (2022) (0.55–0.59) [42]; these differences may be ascribed to the variations in variety and starch composition [43]. BF peel flour exhibited higher values for both ratios compared to BF pulp and banana flour, indicating a higher degree of short-range order in the starch. This observation aligns with the XRD results (Section 3.4), which also showed higher crystallinity in BF flours. This suggests that the crystalline regions of BF starches are more ordered than those of banana starch. Understanding the distribution of ordered and amorphous regions within starch is crucial for predicting its behavior during processing, e.g. heat treatments, and for predicting the characteristics of starchy products during storage [44]. The higher degree of short-range order observed in BF starch compared to banana starch may partly be attributed to its lower amylose content.

Table 3
Results derived from FTIR analysis and gelatinization thermal properties.

	Parameters	Breadfruit pulp	Breadfruit peel	Banana
FTIR	Intensity ratio of bands associated with starch			
	IR 1047/ 1022 cm^{-1}	0.753 ± 0.001 b	0.765 ± 0.001 c	0.741 ± 0.001 a
	IR 1022/ 995 cm^{-1}	0.811 ± 0.001 b	0.868 ± 0.002 c	0.790 ± 0.002 a
	Secondary structures of protein in amide I Region			
	LF β -sheet (%)	42.8 ± 0.5 b	38.9 ± 0.2 a	44.8 ± 0.3 c
	Random coil (%)	11.9 ± 0.3 b	15.7 ± 0.4 c	11.1 ± 0.2 a
	α -helix (%)	23.2 ± 0.3 b	24.3 ± 0.2 c	22.5 ± 0.1 a
	β -turn (%)	20.5 ± 0.7 a	19.8 ± 0.2 a	20.2 ± 0.2 a
	HF β -sheet (%)	1.45 ± 0.09 b	1.28 ± 0.07 a	1.43 ± 0.07 ab
	$T_{O\text{-gel}}$ ($^{\circ}\text{C}$)	75.5 ± 0.1 b	76.3 ± 0.1 c	68.4 ± 0.1 a
DSC	$T_{p\text{-gel}}$ ($^{\circ}\text{C}$)	78.4 ± 0.1 b	79.2 ± 0.2 c	73.9 ± 0.1 a
	$T_{E\text{-gel}}$ ($^{\circ}\text{C}$)	81.3 ± 0.1 a	81.9 ± 0.1 ab	82.2 ± 0.3 b
	ΔT ($^{\circ}\text{C}$)	5.8 ± 0.1 a	5.7 ± 0.1 a	13.8 ± 0.3 b
	ΔH_{gel} (J/g of starch)	20.3 ± 0.1 a	18.3 ± 0.5 a	19.1 ± 1.1 a

FTIR: Fourier Transform Infrared Spectroscopy; DSC: Differential Scanning Calorimetry. LF: Low frequency; HF: High frequency. H_{gel} : Enthalpy of gelatinization. $T_{O\text{-gel}}$, $T_{p\text{-gel}}$, $T_{E\text{-gel}}$: Onset, peak and endset temperatures of gelatinization. ΔT : ($T_{E\text{-gel}}$ - $T_{O\text{-gel}}$). Data are the mean \pm standard deviation. Different letters in the same row indicate statistically significant differences ($p < 0.05$) between means.

Additionally, molecular features such as the M_w and r_{rms} of amylopectin may also affect the ordered short-range molecular structure. Consequently, the FTIR results align with the AF4 analysis (Section 3.2), since smaller r_{rms} of BF starches indicate a more compact structure [45], better helical organization, and more ordered crystals [42]. The secondary structure of proteins was analyzed using the Amide I band (1700–1600 cm^{-1}), including β -sheet (Low Frequency) (1615–1640 cm^{-1}), random coil (1640–1650 cm^{-1}), α -helix (1650–1665 cm^{-1}), β -turn (1665–1690 cm^{-1}), and β -sheet (High Frequency) (1690–1700 cm^{-1}) [46], Table 3. The Amide I band is commonly employed in infrared spectroscopy to investigate protein folding, unfolding, and aggregation due to its strong protein signal and minimal interference from side chains [23,46]. LF β -sheet was the main secondary structure observed in all flours, representing over 38 % of the proteins. Significant differences ($p < 0.05$) in LF β -sheet content were found among the BF flours, ranging from 38.9 % to 42.8 %, with the lowest value observed in BF peel and the highest in banana flour. Additionally, BF flours showed higher values of random coil and α -helix structures compared to banana flour, with these structures being more prevalent in BF peel than in pulp. These findings indicate a higher degree of disorder in the protein structures of BF flours, particularly in peel samples, which may contribute to increased protein digestibility and functional properties [23]. No statistically significant differences ($p > 0.05$) were observed in β -Turn and β -sheet structures (High Frequency) among the samples. Notably, banana flour exhibited the highest LF β -sheets and lowest random coil and α -helix absorbance. The botanical origin has been

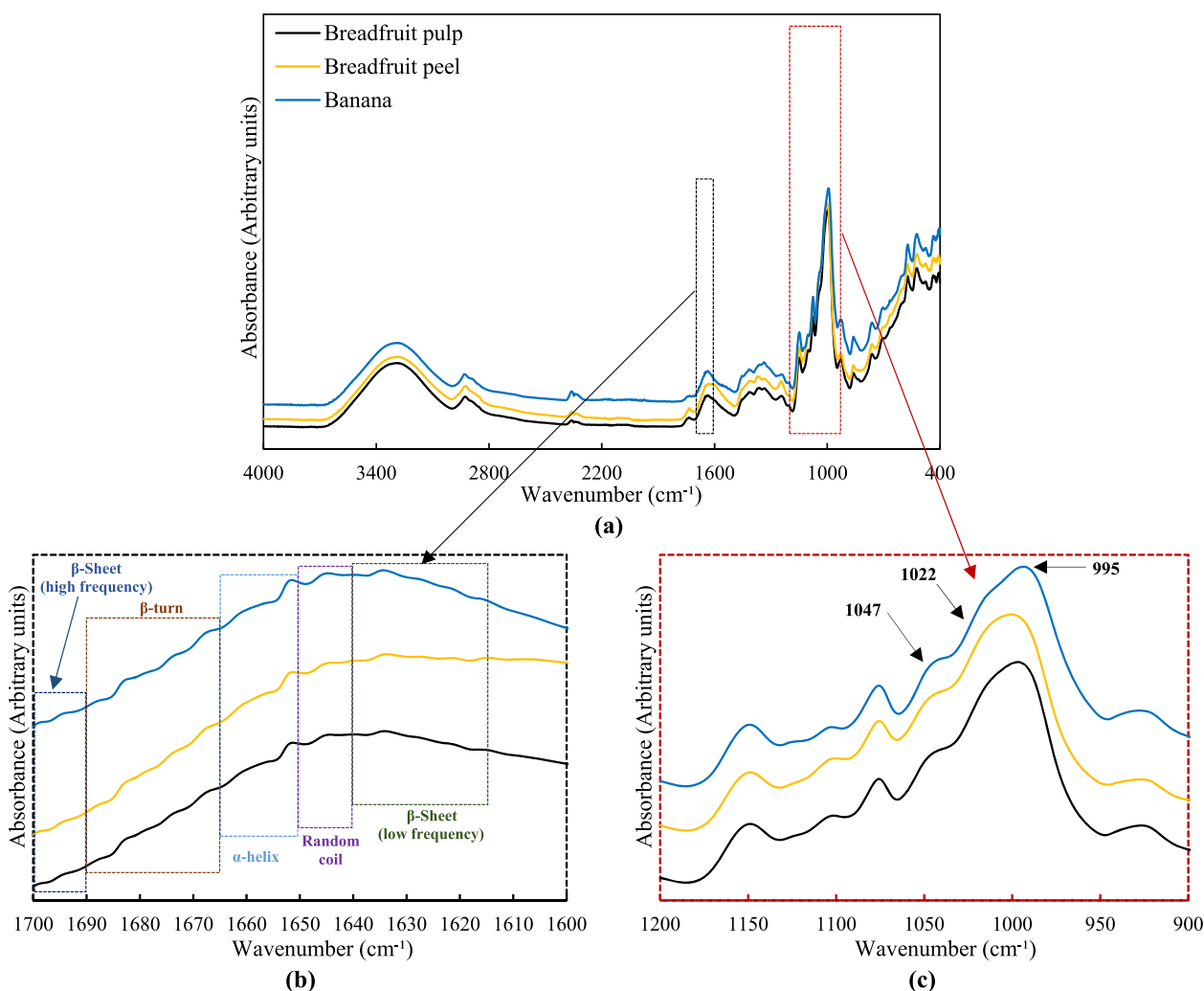


Fig. 4. FTIR spectra of the studied samples. Overall spectra (a), Amide I region (b), and Starch fingerprint region around 1000 cm^{-1} (c).

reported to influence the secondary structure of proteins [43]. Understanding the secondary structure of proteins in BF flours provides valuable information for optimizing their functional properties in food applications, enhancing both processing efficiency and product quality [47].

3.6. Gelatinization thermal properties

The gelatinization properties of the studied flours, including enthalpy of gelatinization (ΔH_{gel}), onset (T_O), peak (T_P) and endset (T_E) temperatures, as well as the gelatinization temperature range ($\Delta T = T_E - T_O$) measured by DSC, are summarized in Table 3, with the corresponding DSC thermograms shown in Supplementary Fig. 2. The flours from BF exhibited significantly ($p < 0.05$) higher T_O and T_P values compared to banana flour, with average increases of 7.5 °C and 4.9 °C, respectively. This denotes that the starch in BF requires a higher temperature to begin gelatinization, suggesting a more stable starch structure that is more resistant to thermal disruption. The gelatinization temperature has been positively correlated with starch structure and degree of crystallinity in previous studies [30]. The lower amylose content, smaller granule size and higher crystallinity of the starch in BF flours compared to banana flour can explain their higher gelatinization temperatures [7,48]. Within BF flours, peel samples exhibited higher T_O and T_P values compared to pulp samples, while ΔT remained similar between the parts of the fruit. The higher fiber and non-starch contents in the peel could potentially compete with starch for water, thereby increasing T_O and T_P values [49].

Despite the differences in T_O and T_P , the temperature at which gelatinization is completed, T_E , was similar for all three flours. Consequently, the ΔT values were significantly lower in BF flours, indicating a more homogeneous gelatinization behavior compared to banana flour. This observation is consistent with the SEM micrographs (see Section 3.3), which revealed differences in granule size uniformity between the BF and banana samples.

The ΔH_{gel} value reflects the melting of starch crystallites during gelatinization and provides insights into the degree of starch crystallinity [39]. No significant differences in gelatinization ΔH_{gel} values were observed between BF flour and banana flours, nor between the pulp and peel of the fruit when the enthalpy was referred to starch content. This suggests similarities in the double helices present in both crystalline and non-crystalline regions of starch granules across the samples. As shown above, the amylopectin mass/radius ratios obtained by A4F in BF flours were lower than those of banana. Therefore, it is reasonable to hypothesize that, once the competition for water is overcome and the temperature required to initiate gelatinization is reached, the process should be more rapid in BF flours, as reflected by the lower ΔT , in agreement with the experimental observations. All samples showed an absence of the amylose-lipid complex dissociation peak, in agreement with previous reports [7]. This absence may be attributed to the types of lipids present in these flours, which influence the thermal transitions of the amylose-lipid complex, depending on factors such as lipid chain length, polar head, water content, and starch type [50].

3.7. Mineral elements

The mineral composition of BF pulp and peel flours, as well as banana flour is presented in Table 4. Minerals are essential for human nutrition, acting as cofactors for many physiological and metabolic processes [29]. The mineral content in flours is influenced by both genetic and environmental factors [25]. BF flours were found to be rich in K, with concentration similar to those of banana flour, a well-known source of this essential mineral [19]. BF peel flour contained 2350 mg/100 g of K, which was 46 % higher than the concentration found in banana flour. Potassium plays a crucial role in maintaining electrolyte balance and is important in alleviating hypertension. In general, BF and banana flours contained low sodium levels compared to other starch

Table 4

Mineral composition, total phenolic content and starch digestibility parameters of breadfruit pulp, breadfruit peel and banana flours.

Parameters	Breadfruit pulp	Breadfruit peel	Banana
Mineral composition			
K (g/100 g)	1.51 ± 0.04 a	2.35 ± 0.04 b	1.61 ± 0.04 a
P (g/100 g)	0.13 ± 0.01 a	0.18 ± 0.01 c	0.16 ± 0.01 b
Mg (g/100 g)	0.08 ± 0.01 a	0.13 ± 0.01 b	0.16 ± 0.01 c
Ca (g/100 g)	0.07 ± 0.01 b	0.09 ± 0.01 c	0.02 ± 0.01 a
Fe (mg/100 g)	1.62 ± 0.20 a	11.56 ± 0.20 c	2.43 ± 0.20 b
Cu (mg/100 g)	0.34 ± 0.01 a	0.55 ± 0.01 c	0.42 ± 0.01 b
Zn (mg/100 g)	0.32 ± 0.03 a	0.48 ± 0.03 b	1.01 ± 0.03 c
Mn (mg/100 g)	0.33 ± 0.01 a	0.68 ± 0.01 b	0.78 ± 0.01 c
Na (mg/100 g)	4.57 ± 1.10 a	7.11 ± 1.10 a	12.8 ± 1.10 b
Total phenolic content			
TPC (mg GAE/100 g)	209 ± 3 a	829 ± 8 c	325 ± 1 b
Starch digestibility parameters			
FSG (g/100 g)	4.6 ± 0.1 c	4.0 ± 0.1 b	1.3 ± 0.5 a
Uncooked flours			
RDS (g/100 g)	8.7 ± 1.8 a	9.4 ± 1.7 a	10.0 ± 2.0 a
SDS (g/100 g)	5.1 ± 0.5 a	4.9 ± 1.8 a	8.9 ± 0.8 b
RS (g/100 g)	47.0 ± 2.2 b	36.4 ± 2.9 a	59.2 ± 2.1 c
SDRI (%)	14.3 ± 2.8 ab	18.5 ± 3.3 b	12.8 ± 2.5a
Cooked flours			
RDS (g/100 g)	53.8 ± 1.5 b	44.8 ± 0.4 a	78.0 ± 0.9 c
SDS (g/100 g)	7.7 ± 0.8 b	6.4 ± 1.4 b	0.8 ± 0.3 a
RS (g/100 g)	0.2 ± 0.3 a	0.5 ± 0.5 a	1.7 ± 1.2 a
SDRI (%)	87.1 ± 1.8 a	86.8 ± 1.5 a	97.0 ± 1.9 b

All results, except SDRI, are referred to dry basis. FSG: Free sugar glucose; RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch; TS: total starch. SDRI: starch digestion rate index (g RDS/100 g starch). Results are the mean ± standard deviation. Values in the same row with different letters are significantly different ($p < 0.05$).

sources such as tef (16 mg/100 g db) [29]. BF flours exhibited low Na concentrations, up to 60 % lower than banana flour, which aligns with dietary recommendations to reduce cardiovascular risks by balancing high K and low Na intake [29]. Phosphorous and Mg contents in BF flours ranged from 130 to 180 mg/100 g and 83 to 131 mg/100 g, respectively. These values are higher than those found in common gluten-free flours like rice and maize, highlighting their nutritional significance for bone health and nerve function [29,51]. In addition, BF flours exhibited higher Ca and Fe levels compared to rice and maize, which are commonly used for gluten-free products development [29]. Calcium is essential for bone metabolism, while Fe plays a key role in hemoglobin production, combating anemia [51]. The relatively high Ca content in BF flours likely contributed to their higher ash content, as reported in Section 3.1. On the other hand, BF flours were deficient in other microelements such as Cu, Zn and Mn (<1 mg/100 g in each mineral) in comparison to other gluten-free sources. For example, rice and tef are notable for Zn (2.3 and 2.8 mg/100 g, respectively) and Mn (6.0 and 6.7 mg/100 g, respectively) contents. Tef is also known for its high Cu content (0.63–0.68 mg/100 g) [25,29,52]. The reference daily intakes (RDIs) established by Food and Drug Administration (FDA) are as follows: K (3500 mg), P (1000 mg), Mg (400 mg), Ca (1000 mg), Fe (18 mg), Cu (2 mg), Zn (15 mg), Mn (2 mg) and Na (2400 mg). The BF flours contain adequate minerals amounts to support a balanced diet. The mineral profiles observed in this study align with previous findings [6,14], with some variability likely due to differences in soil mineral composition [19]. Overall, incorporating both BF pulp and peel, into gluten-free products could significantly enhance their mineral content and nutritional value, contributing to balanced diets and improved health benefits.

3.8. Determination of the total phenolic content (TPC)

The total phenolic content (TPC) measured in the studied flours is

presented in Table 4. Phenolic compounds are essential secondary metabolites produced by plants, known for their antioxidant properties and health benefits, including potential roles in disease prevention such as tumors, diabetes, obesity, and cardiovascular disease [19]. The TPC of BF flours showed significant variations ($p < 0.05$) between them, ranging from 209 ± 3 to 829 ± 8 mg GAE/100 g db. The highest TPC value was observed in BF peel sample, with a concentration four times higher than that of the BF pulp flour and 2.5 times higher than that of banana flour. Although no literature was found reporting the TPC of BF, the TPC of banana flour aligns with previous reports [19]. The TPC of BF samples was higher than conventional flours such as rice (0.1 mg GAE/g), sorghum (0.5 mg GAE/g), and oat (0.9 mg GAE/g) [31]. The observed differences in TPC among the flours may be attributed to factors such as the geographic region of production, plant variety, fresh weight, harvest season, genetic factors, agricultural practices, post-harvest handling, processing methods, storage conditions, cultivation techniques, climatic conditions, ripening stages, and extraction conditions [37]. The high TPC found in the BF peel flour can be attributed to the central role of phenolic compounds in a plant's defense and resistance mechanisms to environmental stressors. These compounds act as protective agents, and their distribution varies across different plant organs. The peel, being the most exposed part of the fruit, faces greater challenges from environmental factors, leading to a higher production or accumulation of these secondary metabolites [53]. Similar trends have been observed by Vieira et al. (2022) [53], who reported higher TPC values in peel flours compared to pulp flours across various cactus species in Brazil. The presence of certain phenolic compounds can inhibit α -amylase activity, thereby affecting starch digestibility [31].

In conclusion, incorporating these flour samples into food products could enhance their antioxidant properties, potentially extending shelf life and preserving or enhancing their intrinsic quality characteristics.

3.9. *In vitro* starch digestibility

The free sugar glucose (FSG), rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS), and the starch digestibility rate index (SDRI) for both uncooked and cooked flours of BF pulp, peel, and banana are shown in Table 4. The samples were measured both in their raw form and after gelatinization. Although flour is not typically consumed raw, enzymatic hydrolysis of ungelatinized starches helps assess the granules' susceptibility to enzyme attack, which depends on the molecular structure of starch and the degree of its internal damage. In gelatinized samples, this *in vitro* test allows for predicting the glycaemic response of the flours when cooked under standard conditions [18]. The FSG of both BF flours was high, 4.0 g/100 g in the peel and 4.6 g/100 g in the pulp; more than three times the value found in banana flour. These FSG contents were also higher than those in other cereal flours known for their sweetness, such as tef flour [28]. The higher FSG in BF flours may be the remnants of starch not yet used for the accumulation of starch in the fruit [13], potentially providing a slight sweet taste to products using them as ingredients. For uncooked flours, the RDS content ranged from 8.7 to 10.0 g/100 g with no significant differences ($p > 0.05$) between samples. BF flours had significantly lower SDS and RS values than banana flour, decreasing by an average of 44 % and 30 %, respectively. Lower RS content of BF flours compared to banana flour may be attributed to its lower amylose content (Table 1) [38]. The differences observed in the RS values between the pulp and peel of BF could be related to the differences in the M_w and r_{rms} of the amylose molecular structure (section 3.2). The lower RDS and higher RS content in BF flour compared to wheat and rice, as reported by Abebe et al. (2015) [28], makes BF particularly interesting for patients suffering from diabetes [18]. The SDRI values of uncooked BF flours were 14.3 % for pulp and 18.5 % for peel, both higher than that of banana flour (12.8 %), with significant differences only observed between the peel and banana flours. This suggests that uncooked BF flours may be hydrolyzed more rapidly. The differences in *in vitro* digestibility among the samples

could be attributed to various factors, including the different sizes and surface areas of starch granules (see Fig. 2). The relationship between surface area and starch volume affects enzyme contact, and the smaller granules in BF compared to banana increase enzyme-substrate contact. Additionally, the rougher granules of BF may facilitate digestive enzymes' access to the interior of starch granules through surface pores [36,37]. The lower amylose/amylopectin ratio in BF flours may also explain their higher digestibility (SDRI values), as amylopectin is more susceptible to hydrolysis than amylose [31]. The higher amylose content in banana flour may also account for its higher SDS content, as amylose is more resistant to enzymatic digestion. These findings are in agreement with the positive correlation between SDS and amylose content reported by Kaur et al. (2010) [40]. The smaller M_w and r_{rms} of amylopectin in BF and in particular, the lower M_w/r_{rms} ratio (see Section 3.2) may also contribute to its higher SDRI, in agreement with findings from other studies that reported a negative correlation between M_w and r_{rms} with digestibility [16,32]. The lower crystallinity of banana flour did not result in a lower RS content, as might be expected [16]. The starch granular and molecular structures likely plays a more crucial role in the accessibility of digestive enzymes than differences in crystallinity, especially when these are small. Additionally, the presence of specific compounds, such as phenolic compounds, may influence the activity of hydrolytic enzymes [31].

As expected, the RDS contents of cooked flours were significantly higher than those of raw flours. In contrast, RS contents decreased dramatically after cooking (gelatinization), decreasing by up to 99 % due to the conversion of most RS into RDS and SDS (Table 4) [16]. These results could be attributed to the fact that raw flours, with intact starch granules, have a concentrically arranged starch structure in both amorphous and crystalline regions, which makes them highly resistant to digestion. However, cooking enlarges the voids around the swollen starch granules, disrupting the continuity of intervening proteins. This structural change facilitates the action of α -amylase on the starch polymers, leading to significantly increased digestion rates [16,38]. The SDS content varied among the samples: it increased in cooked BF flours but decreased in banana flour compared to their uncooked counterparts. Cooked BF flours had lower RDS values (−31 % and −43 % for pulp and peel, respectively) and higher SDS contents (6–7 times higher) compared to banana flour. These results are significant because low RDS values and high levels of SDS may help avoid hyperglycemia [38]. Additionally, RS content showed no significant differences ($p > 0.05$) between cooked samples, while SDRI was up to 10 % lower in BF flours compared to banana flour, showing an opposite trend to that observed by their uncooked counterparts, indicating slower digestion. The statistically lower SDRI values observed in cooked BF flours indicated that BF starch hydrolyzed more slowly than cooked banana starch.

Based on these results, incorporating raw BF flours into foods could help prevent rapid increases in the glycemic index and support stable postprandial blood sugar levels due to their SDS and RS contents. However, if technological considerations require the use of cooked flours, BF flours, especially peel flour, still have a significantly lower glycemic impact than other starchy ingredients.

4. Conclusions

This study demonstrated that BF flours, obtained from both the pulp and the peel of the fruit, represent a new starchy ingredient with high intrinsic nutritional value. Their high fiber, mineral and polyphenol contents confirm this statement. The findings of this work demonstrated that the lower amylose content (10.9–12.2 vs 17.7 g/100 g starch), the smaller starch granules (3–15 vs 15–50 μ m) and the lower molecular mass and size of amylopectin and, in particular of their M_w/r_{rms} ratio (597–667 vs 927 kDa/nm), of BF flours compared to banana flour, explain the higher *in vitro* starch digestibility of uncooked BF samples. Additionally, the low rapidly digestible starch (33.8–44.8 vs 78 g/100 g dry flour) and high slowly digestible starch (7.7–6.4 vs 0.8 g/100 g dry

flour) of cooked BF flours compared to banana flour, suggest a low glycemic index and prolonged satiety when added to final/cooked products. Utilizing the entire fruit will be crucial to minimize economic losses, increase its value, and promote sustainable processing. Furthermore, despite its lower starch content, BF peel flour is richer in fiber, mineral and polyphenols compared to BF pulp flour, making it an attractive option for the nutritional enhancement of food products. Further research is needed to explore the techno-functional properties of BF flours and their potential for developing new, high-quality, healthier gluten-free products. Additionally, starch digestibility assessments in food products made with these flours are essential to determine their actual glycemic impact.

CRediT authorship contribution statement

Caleb S. Calix-Rivera: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Grazielle Náthia-Neves:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Raúl Ricardo Mauro:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Marina Villanueva:** Writing – review & editing, Validation, Supervision, Methodology, Investigation. **Felicidad Ronda:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Acknowledgments

The authors thank the financial support of the Ministerio de Ciencia e Innovación (PID2019-110809RB-I00) and (EQC2021-006985-P), the Ministerio de Ciencia, Innovación y Universidades (PID2023-153330OB-I00) and the Junta de Castilla y León/FEDER (VA195P20). This work was supported by the Regional Government of Castilla y León and the EU-FEDER program (CLU 2019-04 – BIOECOUVA Unit of Excellence of the University of Valladolid). Authors thank Ana Benitez for providing the breadfruit fruits and Octavio Rivera-Flores, Karla Martinez and Genesis Nohal Limas for processing the flours analyzed. Raúl R. Mauro thanks the Ministry of Science and Innovation for the predoctoral contract. Caleb S. Calix-Rivera thanks the University of Valladolid for the doctorate grant.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijbiomac.2025.141224>.

Data availability

Data will be made available on request.

References

- [1] D. Ragone, Breadfruit—*Artocarpus altilis* (Parkinson) Fosberg, in: *Exot. Fruits*, Elsevier, 2018, pp. 53–60, <https://doi.org/10.1016/B978-0-12-803138-4.00009-5>.
- [2] C.E. Turi, Y. Liu, D. Ragone, S.J. Murch, Breadfruit (*Artocarpus altilis* and hybrids): a traditional crop with the potential to prevent hunger and mitigate diabetes in Oceania, *Trends Food Sci. Technol.* 45 (2015) 264–272, <https://doi.org/10.1016/j.tifs.2015.07.014>.
- [3] N. Badrie, J. Broomes, Beneficial Uses of Breadfruit (*Artocarpus altilis*): Nutritional, Medicinal and Other Uses, First Edit, Elsevier Inc., 2010, <https://doi.org/10.1016/B978-0-12-374628-3.00033-5>.
- [4] H. Marta, Y. Cahyana, H.R. Arifin, L. Khairani, Comparing the effect of four different thermal modifications on physicochemical and pasting properties of breadfruit (*Artocarpus altilis*) starch, *Int. Food Res. J.* 26 (2019) 269–276.
- [5] H. Magnus, The top 25 superfoods, in: *Cajanus*, Kingston, University of the West Indies, 1997, pp. 13–33, https://doi.org/10.1007/978-3-322-94076-6_1.
- [6] A.M.P. Jones, D. Ragone, K. Aiona, W.A. Lane, S.J. Murch, Nutritional and morphological diversity of breadfruit (*Artocarpus*, Moraceae): identification of elite cultivars for food security, *J. Food Compos. Anal.* 24 (2011) 1091–1102, <https://doi.org/10.1016/j.jfca.2011.04.002>.
- [7] S. Huang, M.M. Martinez, B.M. Bohrer, The compositional and functional attributes of commercial flours from tropical fruits (breadfruit and banana), *Foods* 8 (2019) 586, <https://doi.org/10.3390/foods8110586>.
- [8] C.L. Nochera, D. Ragone, Development of a breadfruit flour pasta product, *Foods* 8 (2019) 110, <https://doi.org/10.3390/foods8030110>.
- [9] T.O. Omobuwajo, Compositional characteristics and sensory quality of biscuits, prawn crackers and fried chips produced from breadfruit, *Innov. Food Sci. Emerg. Technol.* 4 (2003) 219–225, [https://doi.org/10.1016/S1466-8564\(03\)00006-7](https://doi.org/10.1016/S1466-8564(03)00006-7).
- [10] K.A. Mehta, Y.C.R. Quek, C.J. Henry, Breadfruit (*Artocarpus altilis*): processing, nutritional quality, and food applications, *Front. Nutr.* 10 (2023) 433, <https://doi.org/10.3389/fnut.2023.1156155>.
- [11] M.M. Kemsli, A. Cottonaro, E. Vittadini, Y. Vodovotz, Development of gluten-free muffins made from breadfruit and unripe plantain flours, *Int. J. Food Sci. Technol.* 57 (2022) 2980–2991, <https://doi.org/10.1111/ijfs.15619>.
- [12] S. Huang, B.M. Bohrer, The effect of tropical flours (breadfruit and banana) on structural and technological properties of beef emulsion modeling systems, *Meat Sci.* 163 (2020) 108082, <https://doi.org/10.1016/j.meatsci.2020.108082>.
- [13] H.A. Bakare, O.F. Osundahunsi, M.O. Adegunwa, Composition and pasting properties of breadfruit (*Artocarpus communis* Forst) from south-west states of Nigeria, *Niger. Food J.* 30 (2012) 11–17, [https://doi.org/10.1016/S0189-7241\(15\)30008-4](https://doi.org/10.1016/S0189-7241(15)30008-4).
- [14] H.D. Graham, E.N. Bravo, Composition of the breadfruit, *J. Food Sci.* 46 (1981) 535–539, <https://doi.org/10.1111/j.1365-2621.1981.tb04904.x>.
- [15] L.M. Nwokocha, P.A. Williams, Comparative study of physicochemical properties of breadfruit (*Artocarpus altilis*) and white yam starches, *Carbohydr. Polym.* 85 (2011) 294–302, <https://doi.org/10.1016/j.carbpol.2011.01.050>.
- [16] Y. Bi, Y. Zhang, H. Jiang, Y. Hong, Z. Gu, L. Cheng, Z. Li, C. Li, Molecular structure and digestibility of banana flour and starch, *Food Hydrocoll.* 72 (2017) 219–227, <https://doi.org/10.1016/j.foodhyd.2017.06.003>.
- [17] AACC, Approved Methods of the American Association of Cereal Chemists, AACC, St. Paul, MN, USA, 2000. <https://books.google.es/books?id=xJwQAQAAMAAJ>.
- [18] K.N. Englyst, G.J. Hudson, H.N. Englyst, Starch Analysis in Food, in: *Encycl. Anal. Chem.*, Wiley, 2006, pp. 1–17, <https://doi.org/10.1002/9780470027318.a1029>.
- [19] M. Khoza, E. Kayitesi, B.C. Dlamini, Physicochemical characteristics, microstructure and health promoting properties of green banana flour, *Foods* 10 (2021) 1–15, <https://doi.org/10.3390/foods10122894>.
- [20] Z.A. Syahariza, E. Li, J. Hasjim, Extraction and dissolution of starch from rice and sorghum grains for accurate structural analysis, *Carbohydr. Polym.* 82 (2010) 14–20, <https://doi.org/10.1016/j.carbpol.2010.04.014>.
- [21] C. Fuentes, R. Castañeda, F. Rengel, J.M. Peñarrieta, L. Nilsson, Characterization of molecular properties of wheat starch from three different types of breads using asymmetric flow field-flow fractionation (AF4), *Food Chem.* 298 (2019), <https://doi.org/10.1016/j.foodchem.2019.125090>.
- [22] S. Chakraborty, B. Sahoo, I. Teraoka, R.A. Gross, Solution properties of starch nanoparticles in water and DMSO as studied by dynamic light scattering, *Carbohydr. Polym.* 60 (2005) 475–481, <https://doi.org/10.1016/j.carbpol.2005.03.011>.
- [23] C.S. Calix-Rivera, M. Villanueva, G. Náthia-Neves, F. Ronda, Changes on techno-functional, thermal, rheological, and microstructural properties of Tef flours induced by microwave radiation—development of new improved gluten-free ingredients, *Foods* 12 (2023) 1345, <https://doi.org/10.3390/foods12061345>.
- [24] B. Olawoye, O.F. Fagbohun, O.O. Popoola, S.O. Gbadamosi, C.T. Akanbi, Understanding how different modification processes affect the physicochemical, functional, thermal, morphological structures and digestibility of cardaba banana starch, *Int. J. Biol. Macromol.* 201 (2022) 158–172, <https://doi.org/10.1016/j.ijbiomac.2021.12.134>.
- [25] F. Ronda, W. Abebe, S. Pérez-Quirce, C. Collar, Suitability of tef varieties in mixed wheat flour bread matrices: a physico-chemical and nutritional approach, *J. Cereal Sci.* 64 (2015) 139–146, <https://doi.org/10.1016/j.jcs.2015.05.009>.
- [26] W. Li, Y. Qiu, C.A. Patterson, T. Beta, The analysis of phenolic constituents in glabrous canaryseed groats, *Food Chem.* 127 (2011) 10–20, <https://doi.org/10.1016/j.foodchem.2010.12.033>.
- [27] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, [14] analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, in: *Academy Press (Ed.), Methods Enzymol.*, Academy Press, 1999, pp. 152–178, [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1).
- [28] W. Abebe, C. Collar, F. Ronda, Impact of variety type and particle size distribution on starch enzymatic hydrolysis and functional properties of tef flours, *Carbohydr. Polym.* 115 (2015) 260–268, <https://doi.org/10.1016/j.carbpol.2014.08.080>.
- [29] A. Culet, I.E. Susman, D.E. Duta, N. Belc, Nutritional and functional properties of gluten-free flours, *Appl. Sci.* 11 (2021) 6283, <https://doi.org/10.3390/app11146283>.
- [30] S. Wonglek, C. Jaikaew, Y. Ogawa, J. Singh, Characterization of flours from some underutilized carbohydrate sources of Thailand for potential food applications, *LWT* 199 (2024) 116079, <https://doi.org/10.1016/j.lwt.2024.116079>.
- [31] M. Di Cairano, N. Condelli, M.C. Caruso, A. Marti, N. Cela, F. Galgano, Functional properties and predicted glycemic index of gluten free cereal, pseudocereal and

- legume flours, LWT 133 (2020) 109860, <https://doi.org/10.1016/j.lwt.2020.109860>.
- [32] K.S. Sandhu, S.T. Lim, Digestibility of legume starches as influenced by their physical and structural properties, Carbohydr. Polym. 71 (2008) 245–252, <https://doi.org/10.1016/j.carbpol.2007.05.036>.
- [33] P. Guo, Y. Li, J. An, S. Shen, H. Dou, Study on structure-function of starch by asymmetrical flow field-flow fractionation coupled with multiple detectors: a review, Carbohydr. Polym. 226 (2019), <https://doi.org/10.1016/j.carbpol.2019.115330>.
- [34] A. Rolland-Sabaté, P. Colonna, M.G. Mendez-Montealvo, V. Planchot, Branching features of amylopectins and glycogen determined by asymmetrical flow field flow fractionation coupled with multiangle laser light scattering, Biomacromolecules 8 (2007) 2520–2532, <https://doi.org/10.1021/bm070024z>.
- [35] L.J. Tetlow, E. Bertoft, A review of starch biosynthesis in relation to the building block-backbone model, Int. J. Mol. Sci. 21 (2020) 1–37, <https://doi.org/10.3390/ijms21197011>.
- [36] N. Lindeboom, P.R. Chang, R.T. Tyler, Analytical, biochemical and physicochemical aspects of starch granule size, with emphasis on small granule starches: a review, Starch/Stärke 56 (2004) 89–99, <https://doi.org/10.1002/star.200300218>.
- [37] L. Chang, M. Yang, N. Zhao, F. Xie, P. Zheng, J. Simbo, X. Yu, S. kui Du, Structural, physicochemical, antioxidant and in vitro digestibility properties of banana flours from different banana varieties (*Musa* spp.), Food Biosci. 47 (2022) 101624, <https://doi.org/10.1016/j.fbio.2022.101624>.
- [38] M. Ma, Y. Wang, M. Wang, J. lin Jane, S. kui Du, Physicochemical properties and in vitro digestibility of legume starches, Food Hydrocoll. 63 (2017) 249–255, <https://doi.org/10.1016/j.foodhyd.2016.09.004>.
- [39] H. Marta, Y. Cahyana, M. Djali, G. Pramañisi, The properties, modification, and application of Banana starch, Polymers (Basel) 14 (2022) 1–20, <https://doi.org/10.3390/polym14153092>.
- [40] M. Kaur, K.S. Sandhu, S.T. Lim, Microstructure, physicochemical properties and in vitro digestibility of starches from different Indian lentil (*Lens culinaris*) cultivars, Carbohydr. Polym. 79 (2010) 349–355, <https://doi.org/10.1016/j.carbpol.2009.08.017>.
- [41] S. Wang, C. Li, L. Copeland, Q. Niu, S. Wang, Starch Retrogradation: a comprehensive review, Compr. Rev. Food Sci. Food Saf. 14 (2015) 568–585, <https://doi.org/10.1111/1541-4337.12143>.
- [42] B. Li, Y. Wang, L. Zhu, C. Huang, Y. Zhang, Y. Zhao, G. Wu, L. Tan, Starch characterizations of two kinds of seedless *Artocarpus altilis* (Parkinson) Fosberg originated from China, Food Hydrocoll. 123 (2022) 107145, <https://doi.org/10.1016/j.foodhyd.2021.107145>.
- [43] X. Wang, R. Zhao, W. Yuan, Composition and secondary structure of proteins isolated from six different quinoa varieties from China, J. Cereal Sci. 95 (2020) 103036, <https://doi.org/10.1016/j.jcs.2020.103036>.
- [44] S. Pragati, G.I. K. Ravish, Comparative study of ripe and unripe Banana flour during storage, J. Food Process. Technol. 5 (2014), <https://doi.org/10.4172/2157-7110.1000384>.
- [45] F.F. Velásquez-Barreto, H.A. Miñano, J. Alvarez-Ramírez, L.A. Bello-Pérez, Structural, functional, and chemical properties of small starch granules: Andean quinoa and kiwicha, Food Hydrocoll. 120 (2021), <https://doi.org/10.1016/j.foodhyd.2021.106883>.
- [46] M. Fevzioglu, O.K. Ozturk, B.R. Hamaker, O.H. Campanella, Quantitative approach to study secondary structure of proteins by FT-IR spectroscopy, using a model wheat gluten system, Int. J. Biol. Macromol. 164 (2020) 2753–2760, <https://doi.org/10.1016/j.ijbiomac.2020.07.299>.
- [47] H.Y. Zeng, L.H. Cai, X.L. Cai, Y.J. Wang, Y.Q. Li, Structure characterization of protein fractions from lotus (*Nelumbo nucifera*) seed, J. Mol. Struct. 1001 (2011) 139–144, <https://doi.org/10.1016/j.molstruc.2011.06.031>.
- [48] A. Xu, L. Lin, K. Guo, T. Liu, Z. Yin, C. Wei, Physicochemical properties of starches from vitreous and floury endosperms from the same maize kernels, Food Chem. 291 (2019) 149–156, <https://doi.org/10.1016/j.foodchem.2019.04.024>.
- [49] A.C. Bertolini, L.A. Bello-Pérez, G. Méndez-Montealvo, C.A.S. Almeida, F. Lajolo, Rheological and functional properties of flours from banana pulp and peel, Starch/Stärke 62 (2010) 277–284, <https://doi.org/10.1002/star.200900216>.
- [50] C.G. Biliaderis, Structural Transitions and Related Physical Properties of Starch, in: R.W. James BeMiller (Ed.), Starch (Third Ed., 3rd ed., Academic Press, 2009: pp. 293–372. doi:<https://doi.org/10.1016/B978-0-12-746275-2.00008-2>.
- [51] F. Appiah, I. Oduro, W.O. Ellis, Nutritional Composition of Breadfruits (*Artocarpus* Spp. and *Treculia Africana*) in Ghana, in: Acta Hort., International Society for Horticultural Science, 2016, pp. 15–20, <https://doi.org/10.17660/ActaHortic.2016.1128.3>.
- [52] J.A. R.C. Delcours, Hoseney, Principles of Cereal Science and Technology Third Edition, Am. Assoc. Cereal Chem. 3 (2010) (1986) 1–327.
- [53] É. de A. Vieira, J.G.S. Coelho, C.V.B. Grisi, B.S. dos Santos, J.C. da Silva Júnior, M. A. Alcântara, B.R.L. de A. Meireles, N.A. dos Santos, A.M.T. de M. Cordeiro, Correlation and influence of antioxidant compounds of peels and pulps of different species of cacti from Brazilian Caatinga biome using principal component analysis, South African J. Bot. 147 (2022) 434–442. doi:<https://doi.org/10.1016/j.sajb.2022.02.005>.