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Functional properties and *in vitro* starch digestibility of infrared-treated (micronized) green banana flour

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Abstract

BACKGROUND: The consumption of green banana flour (GBF) products has been linked to reduced glycemic index (GI) and low risk of type 2 diabetes and obesity. The purpose of this study was to investigate the effect of micronization (high-intensity infrared heating method) on the molecular, microstructure and *in vitro* starch digestibility of five GBF cultivars grown in South Africa. The GBF was micronized at three surface temperatures (90, 120 and 150 °C for 30 min) and the *in vitro* starch digestibility was determined with Megazyme kits.

RESULTS: Micronization at the highest temperature (150 °C) increased the swelling power by 6.00% in all five GBF cultivars when compared to control (unmicronized GBF). Micronization slightly reduced the resistant starch (RS) of the GBF cultivars by up to 8.63%. The FHIA-01 cultivar showed the highest RS (86.50%), whereas Grande Naine – 150 °C cultivar had the lowest RS (76.00%). Both micronized and control GBF exhibited similar X-ray diffraction patterns with all cultivars and at all micronization temperatures. Similarly, the functional properties of the GBF were not altered by micronization when observed with Fourier transform infrared spectroscopy. Scanning electron microscopy showed changes in the surface morphology of starch granules after micronization and these were dependent on temperature.

CONCLUSION: Overall, micronization at 120 °C showed the best improvement in functional properties of GBF and this makes it suitable for potential application for the manufacture of instant breakfast products, baked goods and pasta. In addition, the micronized GBF cultivars retained high RS, suggesting potential health benefits for people with diabetes and obesity.
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Keywords: micronization; green banana flour; functional properties; resistant starch

INTRODUCTION

The increase in socioeconomic, demographic changes and rapid urbanization of the population has resulted in poor eating habits and low physical activity, which contribute to the increased risk of obesity and type 2 diabetes.[1] Previous studies reported that frequent intake of food with a low glycemic index (GI), as well as those high in antioxidants, is efficient for the prevention of non-communicable diseases such as obesity and type 2 diabetes.[2,3] Thus, functional food products that are convenient and impart health benefits are becoming increasingly popular among consumers. As non-communicable diseases such as obesity and type 2 diabetes become more prevalent, consumers are becoming more interested in healthier foods.[4,5]

Green banana flour (GBF) has attracted interest from the food industry because of its high resistant starch content and low GI, which are both believed to promote colon health.[6,7] GBF is gluten-free and has other health-promoting properties such as its high antioxidant activity.[8] GI signifies how rapidly the blood glucose level increases after the consumption of a food product.[9] In addition to the type of starch, the amylose-to-amylopectin ratio, and lipids and proteins in the food matrix are some of the many factors that affect GI.[10,11]

Native starch has not been widely adopted in the food industry because of its poor thermal, shear and acid stability, and retrogradation during storage.[12] A variety of modifications can be applied to enhance these properties, including chemical, physical or enzymatic ones. Among the modifications commonly employed, physical modification represents an interesting approach for improving native starch properties as chemical reagent residues are not present. A variety of physical modifications have been

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used to modify the properties of native starch, including microwave heating, annealing, gamma rays and micronization. The micronization process uses electromagnetic radiation in the 1.8–3.35 μm infrared region. It is often used to pre-gelatinize starch, as well as to improve the functional properties and nutrition properties of starch, thus rendering it suitable for numerous applications within the food industry.

Even though numerous studies have examined the impact of thermal modification on the functional characteristics and digestibility of different starches, including wheat starch,[13] no comprehensive investigation of the thermal effects of micronization on the properties of banana flour has been published. Additionally, a number of publications on various techniques for thermally altering different starch sources have produced contradictory results regarding the effects on particular properties, making it challenging to select an acceptable modification technique for GBF. This study's objectives included producing immediate GBF using micronization, which will address consumer demand for quick-to-prepare, nutritious foods, as well as assessing how micronization affects the physicochemical properties of GBF.

MATERIALS AND METHODS

Materials

Five GBF cultivars (Grande Naine, Pisang Awak, Finger Rose, FHIA-01 and Du Roi) were kindly provided by the Tropical and Subtropical Crops branch of the Agricultural Research Council (ARC), Nelspruit, Mbombela, South Africa. Unless otherwise stated, all reagents were analytical grade, purchased from Sigma-Aldrich Pty, Johannesburg, South Africa.

Banana starch extraction

GBF starch was prepared by the water–alkaline extraction method, as described in a previous study.[14] Briefly, a 100-mesh screen was used to sieve 100 g GBF after macerating at a low speed in 1 L distilled water for 20 min. Soluble fiber was removed by adding NaOH (0.2%, w/v), followed by centrifuging at 4000 × g for 10 min. Water was added to the starch sediment and stirred for 5 min, then allowed to rest for 2 h.

Micronization of GBF

GBF samples were preconditioned to 30% moisture content over a period of 6 h. The water required to reach the desired moisture content was calculated as follows:[15]

$$\text{Weight of } H_2O = \frac{\text{Weight}\,\{\text{sample}\times[H_2O\,(\text{target})\%H_2O\,(\text{original})]\}}{100\%H_2O}$$

Approximately 100 g GBF was placed in a plastic zipper bag, with the appropriate amount of deionized water and steeped at 4 °C. A bench-top micronizer with a tubular quartz infrared lamp (115 V, 500 W) and a tungsten filament enclosed in a ceramic casing (Technilamp Pty, Johannesburg, South Africa) was used to treat 100 g batches of GBF. Prior to micronization, the micronizer was preheated for 20 min. Micronization was carried out to a final surface temperature of 90, 120 and 150 °C for 30 min. All the micronized samples were spread on the bench-top (on foil paper) and allowed to cool to room temperature for 1 h before placing in zipper bags. The samples were then stored at 4 °C until further analysis.

Effect of micronization on water absorption capacity (WAC) of GBF

With slight adjustments, the approach outlined in a previous report[16] was used to determine the WAC of GBF. Briefly, 0.5 g GBF was placed in a centrifuge tube (50 mL), followed by addition of 5 mL distilled water. The mixture was vortexed and rested for 1 h at room temperature (21 ± 2 °C). Thereafter, centrifugation of the samples was done at 3000 rpm for 30 min (25 °C), and WAC was determined as the amount of water absorbed per gram of GBF.

Effect of micronization on the moisture content of GBF

The moisture content was assayed using a vacuum oven dryer at 60 °C for 16 h using 2–3 g of sample, according to a previous report.[17]

Effect of micronization on GBF water solubility and swelling power

The method previously reported[16] was used to determine the water solubility index and swelling power. GBF (0.2 g) was mixed with distilled water (5 mL) and stirred for 30 s. The mixture was then heated for 20 min at 50, 70 and 90 °C, cooled and centrifuged at 3000 rpm for 10 min. The supernatant was evaporated in an oven for 16 h at 105 °C. The difference between the dried supernatant mass and that of flour mass was used to determine the solubility index, which was expressed as grams per 100 g dry weight (DW). The filtrate was weighed to determine the swelling power after centrifugation.

Structural properties

Scanning electron microscopy (SEM) of starch isolated from native and micronized GBF

The microstructure of the GBF starch granules was examined using SEM with energy-dispersive X-ray spectroscopy (SEM model JSM 7500F, JEOL, Tokyo, Japan). The GBF starch was mounted on aluminum cylinders that had been double-sided taped and then coated with carbon.[18] The microstructure of the samples was observed using an electron beam with a resolution set at a particle size range of 20–200 μm.

X-ray diffraction (XRD) and relative crystallinity of micronized GBF

A Philips X'Pert XRD apparatus was used to determine the XRD analysis of the GBF (Malvern PANalytical, Almelo, The Netherlands). The power source's parameters were set to 40 kV, 40 mA and a 5°/min scanning rate. $2\theta = 5$–$90°$ was the scanning range.[18]

Fourier transform infrared spectroscopy (FTIR) of micronized GBF

A 4000 FTIR spectrophotometer was used to measure the attenuated total reflectance (ATR)–FTIR spectra of GBF samples (JASCO, Midrand, South Africa). The ATR instrument had a diamond crystal plate with a scan rate of 16 runs per scan and a resolution of 4 cm^{-1} in wavenumbers from 500 to 4000 cm^{-1} to identify the functional groups of the isolated compound.[19]

In vitro starch digestion of unmicronized and micronized banana starch

The rapidly digestible, slow digestible, resistant and total starch were determined using Megazyme Kits (Megazyme Ltd, Bray, Ireland) according to the manufacturer. Enzymes (pancreatic amylase and amyloglucosidase) were mixed together in maleate

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buffer (pH 6.0) and added to the GBF sample (80 mg) (K-RNTDF; AOAC Method 2017.16).[20]

Effects of micronization on GBF amylose content

The amylose and amylopectin contents were determined using a mylopectin/amylose kit from Megazyme. The basic premise of this method is the separation of amylopectin and amylose. The precipitation of amylopectin is then carried out on concanavalin-A and then removed by centrifugation.[21]

Statistical analysis

Data analysis was carried with Statistica statistical software (Version 13.0/September 2015). One-way analysis of variance (ANOVA) was performed. The mean and standard deviation were used to express results. Fisher's least significant difference (LSD) tests were used to determine differences between means ($P \leq 0.05$). All the experiments were performed in triplicate.

RESULTS AND DISCUSSION

Effect of micronization on WAC of GBF

WAC indicates the extent to which starch granules swell in excess of water, and it is an important factor in beverage applications.[22] In this study, FHIA-01 cultivar at 150 °C had the highest WAC (89%), while Finger Rose at 90 °C recorded the least WAC (53%) among the micronized GBF samples. WAC increased with an increase in micronization temperature except for FHIA-01 and Grand Naive cultivars at 120 and 150 °C (Table 1). In fact, the WAC of all five GBF cultivars increased by approx. 2–3 times at 150 °C micronization compared to control (unmicronized GBF). The observed increase in WAC can be attributed to the weakening of the starch molecule that occurs when the temperature rises. Increases in WAC following infrared heating were also reported[13] on cowpea starch. The low WAC of the unmicronized samples can be explained by the weak interaction between amylose and amylopectin chains with water at standard room temperature.[23] The relatively high WAC of the FHIA-01 following micronization suggests the cultivar could be suitable as an ingredient for a value-added product that provides volume, bulkiness and a desirable texture. Also, it must be noted that a lower WAC is preferred for food with a thinner consistency.[24] A previous study[13] reported an increase in WAC with an increase in moisture levels and micronization heating time.

Effect of micronization on GBF moisture content

The effect of micronization on the moisture content of GBF cultivars grown in South Africa is shown in Table 1. The moisture content of all the unmicronized GBF was not significantly different and ranged from 9.40 to10.55 g 100 g^{-1} DW. Similarly, micronization at 90 °C did not cause a significant reduction in moisture content when compared to unmicronized GBF. However, micronization at higher temperatures (120 and 150 °C) significantly decreased the moisture content in all the GBF samples. The highest decrease in moisture content after micronization occurred with Grand Naine – 150 °C cultivar. The moisture content of GBF cultivars reported in the current study is within the range generally reported in the literature for unripe/GBF. Similar to the present study's findings, Kumar *et al.*[25] recorded 8.59% moisture content in green Grand Naine banana flour. Utrilla-Coello *et al.*[26] reported 7.03% moisture content for the unripe Enano cultivar and 8.96% for the unripe Valery banana cultivar. The moisture content of flour products is critical as it can have an influence on both the physical and chemical properties of foods.

Table 1. Water absorption capacity and moisture content of native and micronized banana flour cultivars

Flour type	Water absorption capacity (%)	Moisture content (g 100 g^{-1} DW)
FHIA-01	58.01 ± 0.31b	9.40 ± 1.34a
FHIA-01 − 90 °C	81.00 ± 1.55f	9.42 ± 0.34a
FHIA-01 − 120 °C	85.00 ± 0.71f	7.51 ± 0.11b
FHIA-01 − 150 °C	89.00 ± 0.91f	7.50 ± 0.60b
Grande Naine	43.18 ± 0.10a	10.50 ± 0.7a
Grande Naine − 90 °C	56.80 ± 0.38b	10.50 ± 0.39a
Grande Naine − 120 °C	64.05 ± 0.01c	8.43 ± 0.19b
Grande Naine − 150 °C	66.81 ± 0.87c	8.40 ± 0.39b
Pisang Awak	67.11 ± 0.00c	9.50 ± 0.33a
Pisang Awak − 90 °C	73.31 ± 0.94e	9.50 ± 0.50a
Pisang Awak − 120 °C	76.06 ± 0.71e	7.60 ± 0.67b
Pisang Awak − 150 °C	84.00 ± 0.67f	7.62 ± 0.10b
Finger Rose	40.00 ± 0.58a	10.55 ± 0.84a
Finger Rose − 90 °C	53.90 ± 0.33b	10.50 ± 0.14a
Finger Rose − 120 °C	59.65 ± 0.71b	8.59 ± 0.22b
Finger Rose − 150 °C	64.71 ± 0.89c	8.57 ± 0.78b
Du Roi	50.12 ± 69b	9.50 ± 0.51a
Du Roi − 90 °C	70,43 ± 0.06d	9.48 ± 0.91a
Du Roi − 120 °C	74.79 ± 0.80d	7.60 ± 0.10b
Du Roi − 150 °C	85.51 ± 0.63f	7.58 ± 0.48b

Note: Averages and standard deviations of three triplicates ($N = 3$) are presented. ANOVA indicates significant differences ($P < 0.05$) between values in each column, denoted by different letters.

Effect of micronization on GBF water solubility and swelling power

The extent to which the amorphous (amylopectin) and crystalline (amylose) regions of the starch molecule interact is defined by the solubility index and swelling power.[27] The solubility index of GBF ranged from 0.95% (Du Roi – 150 °C) to 15.01% (FHIA-01) (Table 2). The solubility of micronized GBF decreased with an increase in micronization temperature. This trend was observed with all the studied GBF cultivars. The observed reduction in solubility could be attributed to the initial gelatinization of the starch during micronization. In another study, a decrease in solubility accompanied by amylose leaching was positively correlated with swelling power.[28] It is worth noting that the solubility of GBF increased with an increase in temperature in this study. The swelling of starch granules is attributed to the disruption of hydrogen bonds between hydroxyl groups in the double helices of starch molecules during gelatinization.[29] Solubilization increases because of starch molecules leaching out of the granules' inner parts.

Swelling power is generally used to determine the extent to which a substance can be hydrated.[30] As expected, the swelling power of all cultivars was low when determined at a low temperature (50 °C) compared to that determined at high temperatures (70 and 90 °C) (Table 3). This was thought to be due to weakening of the intragranular binding forces of GBF, thus enabling more swelling and enhanced leaching of granular particles, which led to increased swelling power. Micronization significantly

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Table 2. Effects of micronization on water solubility and swelling index of green banana flour samples

Banana flour sample	Solubility (%)			Swelling power (g g^{-1})		
	50 °C	70 °C	90 °C	50 °C	70 °C	90 °C
FHIA-01	6.49 ± 0.73e	9.5 ± 0.71f	15.01 ± 0.71f	0.29 ± 0.71a	0.42 ± 0.71a	0.52 ± 0.95a
FHIA-01 – 90 °C	5.12 ± 0.82d	1.7 ± 0.43a	1.64 ± 0.61b	2.63 ± 0.42c	7.32 ± 0.31d	8.32 ± 0.58b
FHIA-01 – 120 °C	3.63 ± 0.80c	1.68 ± 0.71a	1.49 ± 0.70a	2.99 ± 0.22c	7.52 ± 0.87e	8.44 ± 0.11b
FHIA-01 – 150 °C	3.23 ± 0.12b	1.65 ± 0.78a	1.39 ± 0.50a	4.25 ± 0.53e	7.62 ± 0.09e	8.51 ± 0.61b
Grande Naine	7.40 ± 0.00f	9.61 ± 0.34f	10.21 ± 0.59d	0.50 ± 0.19b	0.67 ± 0.71b	0.75 ± 0.00a
Grande Naine – 90 °C	3.80 ± 0.58c	4.55 ± 0.90e	1.87 ± 0.02b	0.41 ± 0.73b	7.19 ± 0.55d	9.70 ± 0.91d
Grande Naine – 120 °C	3.38 ± 0.94b	3.91 ± 0.77d	1.39 ± 0.22a	0.33 ± 0.15a	6.99 ± 0.00d	8.59 ± 0.83c
Grande Naine – 150 °C	3.00 ± 0.23b	3.55 ± 0.46d	1.15 ± 0.65a	0.30 ± 0.52a	7.26 ± 0.62d	8.41 ± 0.95b
Pisang Awak	6.50 ± 0.32e	8.47 ± 0.58g	11.40 ± 0.58e	0.33 ± 0.21a	0.38 ± 0.44a	0.53 ± 0.01a
Pisang Awak – 90 °C	4.76 ± 0.42d	2.77 ± 0.65c	1.38 ± 0.86a	2.53 ± 0.57d	5.75 ± 0.71c	8.42 ± 0.55b
Pisang Awak – 120 °C	4.44 ± 0.71d	2.5 ± 0.77c	1.27 ± 0.32a	2.61 ± 0.62c	5.84 ± 0.32c	9.40 ± 0.23c
Pisang Awak – 150 °C	3.77 ± 0.99c	2.13 ± 0.85b	1.09 ± 0.28a	3.31 ± 0.78d	6.53 ± 0.41	10.41 ± 0.66e
Finger Rose	7.0 ± 0.08f	9.01 ± 0.34h	10.21 ± 0.59d	0.41 ± 0.79a	0.67 ± 0.71b	0.79 ± 0.04a
Finger Rose – 90 °C	3.38 ± 0.54b	4.12 ± 0.41e	1.23 ± 0.32a	0.31 ± 0.93a	7.19 ± 0.55d	9.50 ± 0.71d
Finger Rose – 120 °C	3.53 ± 1.31c	3.61 ± 0.97d	1.39 ± 0.32a	0.33 ± 0.75a	7.38 ± 0.29d	8.60 ± 0.85c
Finger Rose – 150 °C	3.13 ± 0.71b	3.88 ± 0.76d	1.09 ± 0.65a	0.41 ± 0.92a	7.36 ± 0.62d	8.53 ± 0.08c
Du Roi	5.50 ± 0.71e	7.59 ± 0.06f	8.03 ± 0.53c	0.38 ± 0.24a	0.63 ± 0.27b	0.83 ± 0.54a
Du Roi – 90 °C	4.16 ± 0.23c	3.61 ± 0.31d	1.14 ± 0.31a	3.03 ± 0.38d	6.77 ± 0.81d	10.42 ± 1.71e
Du Roi – 120 °C	3.38 ± 0.81b	2.78 ± 0.74c	1.04 ± 0.37a	3.33 ± 0.34d	7.50 ± 1.11e	11.01 ± 0.61f
Du Roi – 150 °C	2.48 ± 0.76a	2.1 ± 0.11b	0.95 ± 0.52a	4.21 ± 0.85e	7.60 ± 0.41e	12.21 ± 0.57g

Note: Significant differences ($P < 0.05$) are depicted by values with different letters in a column. The results are presented as means on a dry weight basis ($n = 3$).

($P < 0.05$) increased the swelling power of all GBF cultivars when compared to control, while with some cultivars an increase in micronization temperature resulted in a further increase in swelling power. The highest swelling power (12.21 g g^{-1}) was observed with Du Roi – 150 °C when determined at 90 °C, while the least (0.29 g g^{-1}) occurred with FHIA-01 at 50 °C. The observed increase in swelling power on the micronized flours was attributed to the loosening of intragranular bonds, which occurs when starch granules are heated in the presence of moisture. When starch granules reach temperatures above 70 °C, intermolecular hydrogen bonds break in amorphous areas, resulting in rapid swelling.[31] These results suggest that micronization improves the swelling power of GBF and such an increase is dependent on the temperature of micronization and cultivar. Flours with high swelling power, such as Duroi cultivar of this study, are preferred for making dough with high elasticity and can potentially be used in the manufacture of pasta and bread.

Structural properties

SEM of starch isolated from native and micronized GBF

SEM images were used to retrieve critical information about the size, shape, surface morphology, and structural integrity of starch granules. The micrographs of native and micronized GBF starch granules observed by SEM are shown in Fig. 1. Du Roi particle structure and starch morphology changed significantly after micronization. Unmicronized Du Roi starch granules appeared to be thin rods. However, as soon as the starch granules were micronized at 90 °C, the starch granules changed to an oval shape. This can be attributed to the WAC of Du Roi – 90 °C, which increased by approximately 20.31% in comparison to the unmicronized Du Roi sample, demonstrating that weakening of the starch granule occurred when the starch was subjected to micronization. It was also observed that with an increase in micronization temperature the size of some starch granules appeared to expand. This may be attributed to the increase in micronization temperature during gelatinization, which could lead to starch particles absorbing more water. Subsequently, the starch granules increase in size and decomposition of the internal structure occurs.[32] It is, however, worth noting that not all the micronized starch samples presented a similar profile with respect to size. With regard to Finger Rose, there were no significant changes in particle structure and starch morphology after micronization. It can be observed that the Finger Rose starch granules maintained their rod shape and size, with a slight expansion in size when micronized at 150 °C. This was expected as the Finger Rose had the lowest WAC, indicating that the starch granules only swelled to a limited extent in excess of water. The effects of micronization on Finger Rose are comparable to the results reported by Cahyana *et al.*[33] on the effects of annealing (a thermal treatment that alters the physical or chemical properties of starch), who reported that no pronounced change occurred in the granule morphology of unripe banana flour when annealing was applied. The surface structure of unmicronized and micronized GBF FHIA-01 and Pisang Awak starch granules was of irregular spheres or oval-like appearance and they exhibited dense surfaces with debris. This result was consistent with the finding reported by Padhi *et al.*[34] who observed spheroidal as well as oblong structures in unripe banana flour starch granules. Micronized Grand Naine–150 °C starch granules were agglomerated, and some of the starch granules had disintegrated. This could be an indication of complete gelatinization of starch. Other studies[13,35] also reported the negative effect of thermal treatment on the integrity of starch granules

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Table 3. Rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS) and amylose content of native and micronized GBF starches

Sample	RDS (%)	SDS (%)	RS (%)	Amylose (%)
Grande Naine	6.02 ± 0.11f	13.30 ± 0.00d	80.38 ± 1.41c	18.95 ± 0.98e
Grande Naine – 90 °C	6.00 ± 0.00f	13.53 ± 0.10e	80.31 ± 0.10c	18.25 ± 0.40e
Grande Naine – 120 °C	4.65 ± 0.03e	18.50 ± 0.55i	78.88 ± 0.70b	16.67 ± 0.84d
Grande Naine – 150 °C	3.75 ± 0.00d	20.91 ± 0.00j	76.00 ± 0.50a	15.98 ± 0.04c
Pisang Awak	5.50 ± 0.05f	11.73 ± 0.02c	84.35 ± 1.51e	23.00 ± 0.91h
Pisang Awak – 90 °C	5.92 ± 0.10f	10.87 ± 0.03b	84.35 ± 1.51e	22.70 ± 0.06h
Pisang Awak – 120 °C	2.54 ± 0.40c	15.87 ± 0.83g	82.59 ± 0.15d	21.56 ± 0.08h
Pisang Awak – 150 °C	2.50 ± 0.40c	16.85 ± 0.97h	79.59 ± 0.15b	19.65 ± 0.86f
Finger Rose	5.43 ± 0.31e	14.87 ± 0.01f	81.70 ± 1.21c	15.55 ± 0.90c
Finger Rose – 90 °C	5.09 ± 0.11e	14.50 ± 0.31f	81.59 ± 0.75c	15.55 ± 0.00c
Finger Rose – 120 °C	4.39 ± 0.91d	17.70 ± 0.51h	78.94 ± 0.14b	13.67 ± 0.84b
Finger Rose – 150 °C	2.69 ± 0.58c	20.72 ± 0.21j	76.82 ± 0.35a	10.43 ± 0.54a
FHIA-01	4.50 ± 0.22e	10.17 ± 0.61a	86.50 ± 0.21g	24.82 ± 0.00i
FHIA-01 – 90 °C	4.48 ± 0.90d	10.99 ± 0.00b	86.34 ± 0.22g	24.60 ± 0.03i
FHIA-01 – 120 °C	3.88 ± 0.42d	11.69 ± 0.85b	84.00 ± 0.97e	20.72 ± 0.13f
FHIA-01 – 150 °C	1.90 ± 0.23a	18.70 ± 0.32i	80.54 ± 0.65c	20.22. ± 0.03f
Du Roi	4.46 ± 0.82d	10.42 ± 0.51a	85.50 ± 0.40f	21.32 ± 0.16g
Du Roi – 90 °C	4.43 ± 0.99d	10.44 ± 0.87a	85.36 ± 0.94f	21.10 ± 0.40g
Du Roi – 120 °C	2.43 ± 0.74b	18.39 ± 0.77h	78.66 ± 0.26b	20.48 ± 0.53f
Du Roi – 150 °C	2.07 ± 0.09a	20.69 ± 0.66j	76.87 ± 0.33a	18.83 ± 0.61e

Note: Significant differences ($P < 0.05$) are depicted by values with different letters ($n = 3$).

as well as agglomeration of starch after the treatment. In general, using SEM did not show any changes in the GBF granule after micronization. Overall, our finding on the lack of change in granule morphology was in agreement with that found in other studies that focused on the effect of different thermal modifications on the physicochemical properties of GBF.[36,37]

XRD and relative crystallinity of micronized GBF

Figure 2 illustrates the results of an XRD analysis of the impact of micronization on the crystalline structure of banana starch granules. The XRD patterns for all GBF samples showed B-type crystals with three distinct peaks that were each present at a different angle: a narrow peak at 25.00°, a robust peak at 17.80° and a minor peak at 15.51°. B-type crystals in GBF are valued for their health benefits because they are highly resistant to digestion.[38] This observation was in line with that reported by Cahyana *et al.*[33] who found that native GBF displayed an XRD pattern with the characteristics of a B-type pattern: a narrow peak at 24.98° 2θ, strong peaks at 15.01° and 16.95° with shoulders around 17.80° and a small peak at 5.51°. The relative crystalline properties varied with GBF cultivar, while there were no observable alterations in the relative crystallinity of the starch granules with an increase in micronization temperature, which may suggest that there was no discernible impact on the chemical makeup of the examined GBF. The degree of relative crystallinity was inversely proportional to the amylose content. Finger Rose, which had the lowest amylose content (15.55%), had the highest relative crystallinity, whereas FHIA-01, with 24.82% amylose content, had the lowest relative crystallinity. The relative crystallinity of banana flours ranged from 44.91% (FHAI-01) to 49.41% (Finger Rose). It should be noted that the crystallinity of the starch granules can also be influenced by the flour's composition (ash, lipids and protein content).[39] The inability of micronization to alter the XRD patterns in the current investigation shows that infrared radiation in the 90 – 150 °C range is somewhat insufficient to cause a movement or loss of water on the pair of double helices.

FTIR of micronized GBF

In order to detect the several distinctive functional groups present in the GBF cultivars and to keep track of the alterations brought on during micronization, FTIR spectroscopy was used. Heat treatment typically does not create or destroy functional groups, but it can modify how distinctive peaks absorb energy.[19] In general, micronization did not alter the functional groups of all the GBF cultivars, as shown in (Fig. 3). This was somewhat supported by what was observed in the XRD patterns shown in Fig. 2. The current findings indicate that micronization did not affect the chemical bonds of GBF. Chizoba *et al.*[40] revealed similar findings when investigating the effect of microwave heating on flour. It was also observed that Grande Naine's cultivar characteristics matched those in earlier studies.[39-41] The GBF fingerprint zone is defined as the absorption bands between 800 and 1600 cm^{-1} in the Finger Rose cultivar.[42] Finger Rose cultivar had distinctive bands at 1002.80 cm^{-1} and bands between 990 and 1160 cm^{-1} that were attributable to the stretching of carbonyl group (═C═O) bonds.[43] The value of 1047/1022 cm^{-1} represents the ratio of the ordered crystalline region to the amorphous region in starch, which is called short-range order and recorded as degree of order of banana starches.[44] The presence of CH_2 groups is shown by the Du Roi absorption bands at 2368.16 cm^{-1}.[45] Our findings suggest that the unmicronized (control) Durio has stronger spectral bands than the micronized samples, which could be attributed to the relatively high concentration of amylose in the control sample. Pisang Awak displayed absorption bands from 500 to 4000 cm^{-1}; however, the peaks at about 1006.66 cm^{-1}, which is indicative of the alkene groups for ═C—H bending,[46] and

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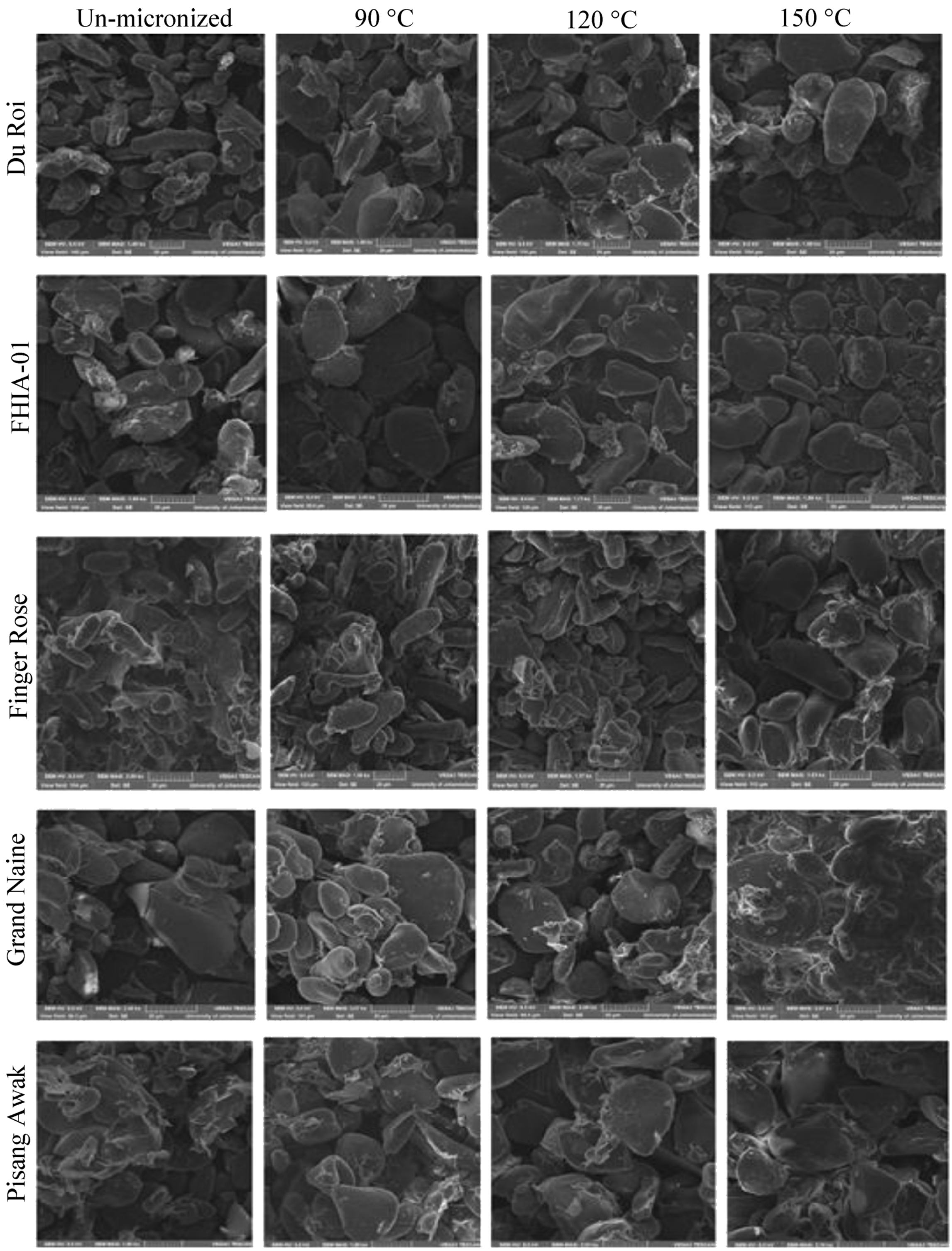

Figure 1. SEM micrographs of starch from unmicronized and micronized GBF at different temperatures (90, 120 and 150 °C).

2352.73 cm^{-1}, which is indicative of the CH_2 group, were stronger.[32] FHIA-01 revealed similarities in crystallinity by being correlated with the quantity of amorphous structure and, consequently, the amount of water interacting with intramolecular hydrogen bonds.[47] The FHIA-01 bands at 2985.27 cm^{-1} were within the region (2800–3000 cm^{-1}) where CH bond stretching normally occurs, as previously reported by Alimi *et al.*[48]

In vitro starch digestion and amylose content of unmicronized and micronized banana starch

The rapidly digestible starch (RDS) of banana starches isolated from unmicronized and micronized GBF varied substantially ($P \leq 0.05$), with the Grande Naine cultivar having the highest RDS (6.02%) and FHIA-01 – 150 °C cultivar having the lowest (1.90%) (Table 3). Micronization decreased the RDS content of all

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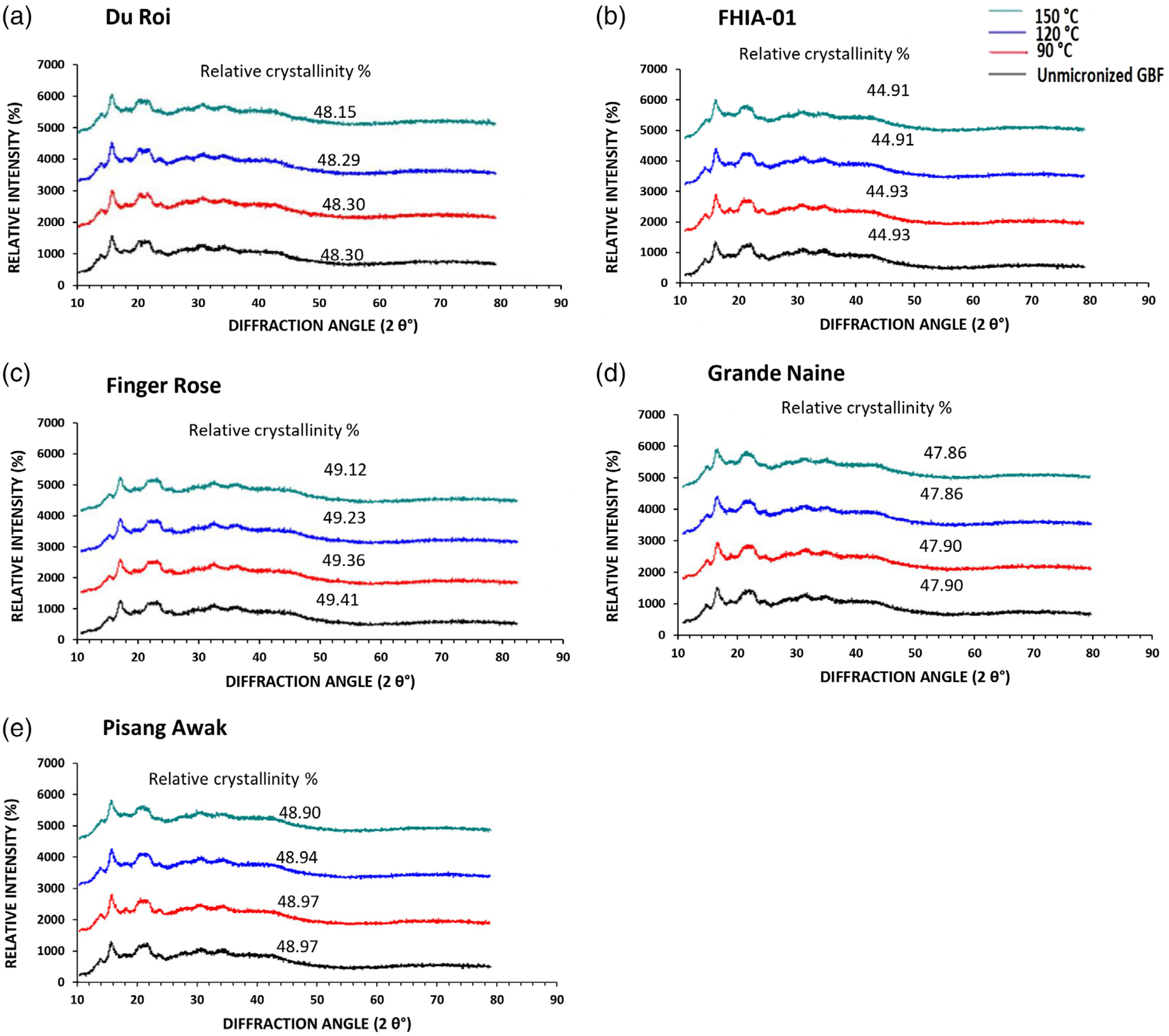

Figure 2. X-ray diffraction pattern of native and micronized GBF at different temperatures (90, 120 and 150 °C). (A) Du Roi; (B) FHIA-01; (C) Finger Rose; (D) Grand Naine; (E) Pisang Awak.

the studied GBF cultivars and this was probably due to the starch interactions with some proteins during micronization, which is believed to affect starch digestibility, thus causing a decrease in the availability of RDS.[49] The decrease in RDS implies a health benefit, as a high RDS content is associated with a rapid release of glucose in the blood.[50] Slowly digestible starch (SDS) ranged between 10.17% (FHIA-01) and 20.91% (Grande Naine – 150 °C). In general, an increase in micronization temperature resulted in an increase in the SDS for all five GBF cultivars, particularly with temperature increases from 120 to 150 °C. Other authors have reported similar findings and have attributed the increase in SDS to amylose–lipid complex formation, which may cause conversion of some of the RDS into SDS and/or resistant starch (RS).[33,45,51] High SDS content implies a health benefit as it is associated with sustained slow release of blood glucose as well as other benefits associated with low glycemic index and insulin response.[52]

GBF is well known for its high RS content.[22] In this study, FHIA-01 cultivar had the highest RS (86.50%), whereas Grande Naine – 150 °C contained the lowest RS (76.00%) (Table 3). Low-temperature (90 °C) micronization was found not to significantly ($P > 0.05$) affect the RS content in all cultivars, while micronization at high temperature slightly reduced the RS by up to 8.63% (Du Roi) when compared to unmicronized flour. The RS of the cultivars of this study was similar to that reported previously in plantain starch before cooking (87.50%), native GBF starch (88.70%),[53] and uncooked native plantain starch (85.00%), and.[32] A high RS content is associated with health benefits such as improved gut and digestive health.[54] Other benefits of RS include helping with weight loss and improved insulin sensitivity.[55,56] Even though micronization slightly decreased the RS content of the GBF here, it is important to mention that the RS of micronized GBF remained relatively high.

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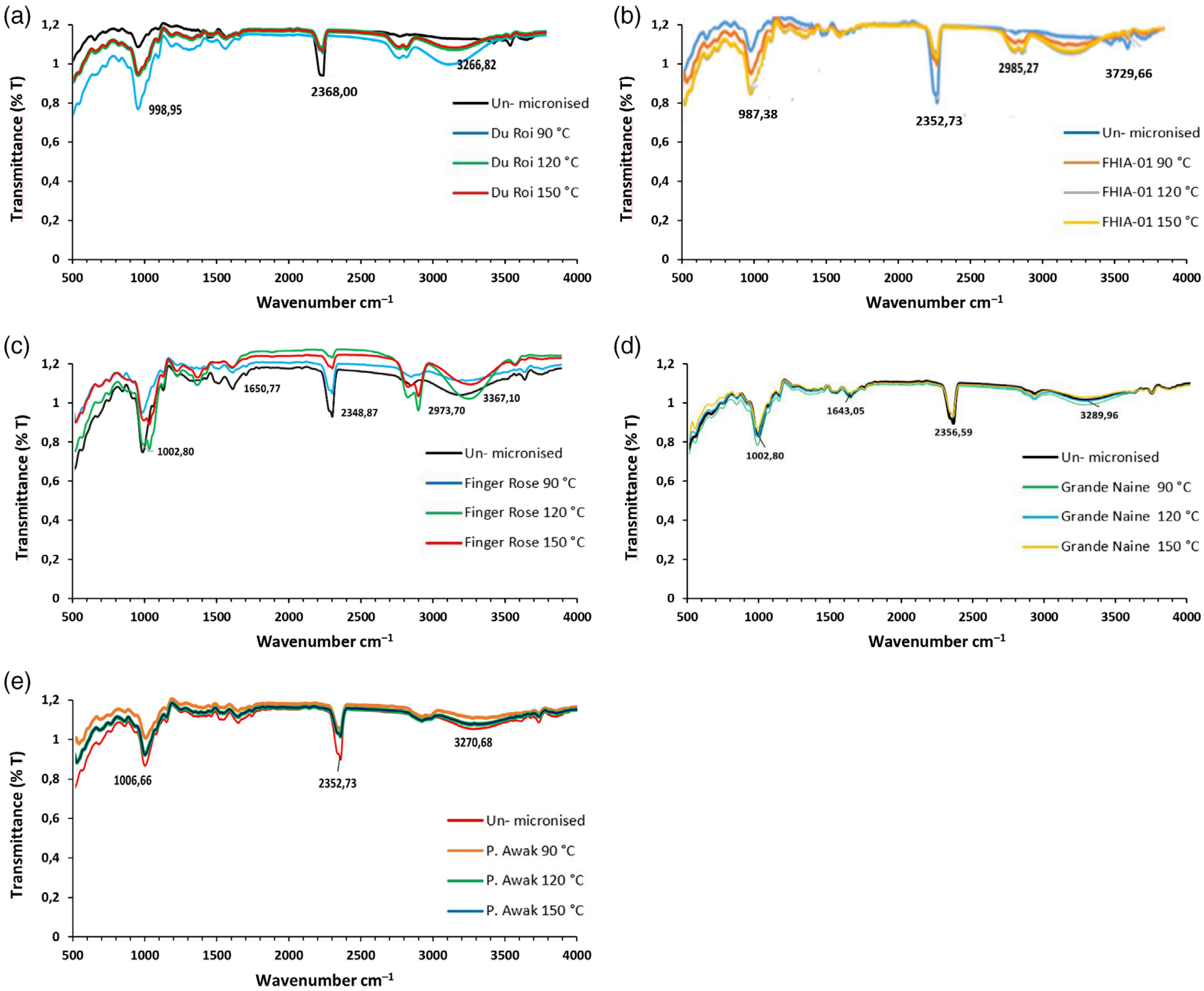

Figure 3. FTIR spectra of native and micronized GBF at different temperatures (90, 120 and 150 °C). (A) Du Roi; (B) FHIA-01; (C) Finger Rose; (D) Grand Naine; (E) Pisang Awak.

In terms of amylose, FHIA-01 had the highest (24.82%) and Finger Rose had the lowest amylose content (10.42%). With increase in temperature, micronization reduced the amylose concentration, and this was true for all cultivars. This implies that there was rupture of the starch granules and leaching of amylose brought about by the rise in micronization temperature. Amylose is known to inhibit the swelling of starch.[57] It is worth noting that FHIA-01, which had the highest amylose content, had the lowest swelling power (0.29 g g^{-1}), suggesting that amylose indeed inhibits the swelling of starch granules during gelatinization. According to previous reports, a positive correlation exists between the concentration of amylose and that of resistant starch.[51,55] Therefore, the above results have confirmed that the amylose and amylopectin concentration in GBF can influence its physical characteristics, including swelling, gelatinization, retrograde behaviour and swallowing.[58] In general, a high amylose content is associated with a low glycemic index.[44] Even though studies have shown that a decrease in amylose content results in high glycemic index, it is worth noting that the amylose content of all five GBF cultivars investigated in this study were relatively high when micronized at 90 °C.

Investigating the overall effect of micronization on all GBF cultivars: combining the effects of GBF type and GBF micronization temperatures

Principal component analysis was performed to further clarify the relationship among *in vitro* digestibility, amylose content, WAC, solubility, swelling power, FTIR and moisture content of micronized and unmicronized GBF. Figure 4 shows the combination of the score and loading plot. The eigenvalues of the first two principal components explains all the variances. The first principal component (PC1) was mainly contributed by the SDS content of micronized Finger Rose – 120 °C, Finger Rose – 150 °C, Grande Naine – 120 °C, Grande Naine – 150 °C and the RDS, solubility and moisture content of both native and micronized Grande Naine, Grande Naine – 90 °C, Finger Rose and Finger Rose – 90 °C. The second principal component (PC2) contained the RS, amylose content of FHIA-01, FHIA-01 – 90 °C, Pisang Awak, Pisang

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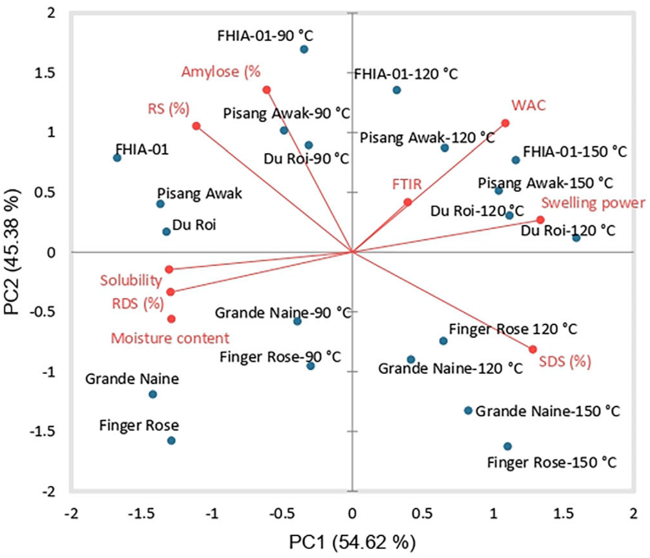

Figure 4. Principal component analysis showing effect of micronization on molecular, functionality, microstructure and *in vitro* starch digestibility properties of GBF cultivars.

Awak – 90 °C and Du Roi – 90 °C, as well as the WAC, swelling power and FTIR of FHIA-01 – 120 °C, FHIA-01 – 150 °C, Pisang Awak – 120 °C, Pisang Awak – 150 °C, Du Roi – 120 °C and Du Roi – 150 °C. The micronization process increased digestibility, due to the conversion of most RS into RDS and SDS. Notably, micronized Grande Naine – 150 °C still had a relatively higher SDS content. It is concluded that the *in vitro* digestibility of GBF was affected by micronization temperature, functionality, granule morphology and amylose content. The data provide important insights into the development of healthful, functional foods with micronized GBF.

CONCLUSIONS

This study has demonstrated that GBF can be modified physically using micronization. Micronized FHIA-01 cultivar appears to be a better cultivar when one considers functional properties such as a high WAC and swelling power, making it a potential ingredient for use in the baking industry. Moreover, FHIA-01 demonstrated a low RDS, and the highest RS and amylose content. The microstructure of the GBF, as observed with SEM and XRD, was not affected by micronization. Higher temperatures of micronization significantly induced lower content of RS and amylose, which is considered to have a negative effect on its application as a low-GI starch. However, micronization at 120 °C proved to be a promising method for improving the functional properties of GBF. Therefore, it is recommended for its desired positive effects on WAC, solubility, swelling, RDS and SDS. The findings of this study suggest that micronized GBF has potential for application in the food industry, particularly in the manufacture of instant breakfast beverage products, baked goods and pasta.

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AUTHOR CONTRIBUTIONS

Conceptualization: MK, EK and BCD; methodology: MK; software: MK; validation: EK, BCD and MK; formal analysis: MK; investigation: MK; resources: BCD; data curation: MK; writing original draft preparation, M.K.; writing – review and editing: EK and BCD; visualization: EK; supervision: BCD and EK; project administration: BCD; funding acquisition: EK and BCD. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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