

Optimizing extrusion for maximum resistant starch: Unlocking the potential of banana flour

Stellamaris Kembabazi^{a,b,c}, Martin Mutambuka^a, Norhasnida Zawawi^{c,d},
Ediriisa Mugampoza^a, Radhiah Shukri^{c,*}, Florence Isabirye Muranga^b

^a Department of Food Science and Technology, Faculty of Science, Kyambogo University, Kampala, Uganda

^b Banana Industrial Research and Development Centre, BIRDC, Bushenyi, Uganda

^c Functional Carbohydrate and Protein Laboratory, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

^d Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, Malaysia

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ABSTRACT

Banana starch, particularly rich in resistant starch type 2 (RS2), is known for its physiological benefits, but it lacks thermal stability compared to resistant starch type 3 (RS3). This study explored the use of twin-screw extrusion to modify native starch into the more thermally stable RS3 in banana flour derived from East African Highland cooking bananas. Using a central composite design, four variables; banana cultivar, feed moisture, barrel temperature, and screw speed were optimized. The highest RS content (38 %) was achieved under the following conditions: cultivar *Enyeru*, 18 % feed moisture, 90 °C barrel temperature, and 300 rpm screw speed. Post-cooking evaluation of the optimized extrudate showed significant RS retention and improved thermal stability. The increased RS positively impacted the functional properties: solubility, swelling power, water absorption capacity, and oil absorption capacity. Extrusion further improved the banana flour attributes with better paste stability against shear and heat, as well as reduced bulk density and tannin. These findings highlight the potential of extrusion technology to enhance the RS3 content in banana flour, offering new opportunities for functional food applications.

1. Introduction

The global rise in metabolic disorders like diabetes and obesity has raised concerns about the quality of carbohydrate consumption worldwide (WHO, 2022). Excessive intake of refined and low-quality carbohydrates, particularly from processed foods with high glycemic index, is associated with the prevalence of diet-related non-communicable diseases [1]. To address this, researchers are focusing on developing starch-based foods with low glycemic index to promote better health outcomes [2]. One approach involves incorporating resistant starch (RS) ingredients into food formulations, due to the observed health benefits and the availability of novel RS ingredients [3]. However, many RS ingredients lack thermal stability, hindering their effectiveness in increasing RS content in final products (Roman & Martinez, 2019). Innovative strategies are required to increase RS levels and convert resistant starch type 2 (RS2) from native starch granules into a thermally stable form, resistant starch type 3 (RS3) that is retrograded starch. RS3 is one of the types of RS used in the food industry for its thermal stability

and ease of fermentation by the intestinal microflora [4,5]. RS3 usually contains melted and retrograded amylose structures. These structures can bind bile salts, thus preventing reabsorption in the ileum, stimulating their liver production and increasing cholesterol utilization [4,6].

Extrusion has emerged as a prominent method for increasing RS levels in food products due to its efficiency, versatility, and high productivity [7]. This technique utilizes high-shear conditions to depolymerize starch molecules, leading to the production of retrogradable straight chains through gelatinization and retrogradation processes. Extrusion has successfully increased RS3 formation in high-amylose native starches, reaching levels up to 71 % [8,9]. Extrusion was applied to different types of starches, such as potato [10] and bean [11], demonstrating an increase in RS. Additionally, extruded starches and flours showed improved solubility and water holding capacity. The extrusion parameters such as temperature, screw speed, and feed moisture content play a crucial role in modifying the structural composition of the final food product. These modifications influence key characteristics, including nutrient availability, starch gelatinization,

* Corresponding author.

E-mail address: radhiah@upm.edu.my (R. Shukri).

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improved in-vitro digestibility, and reduced anti-nutritional factors [12, 13].

RS2 is found in native banana starch, which is limited for application in the food industry because of its thermal instability, where the RS breaks down during heat processing and thus loses its physiological potential [14,15]. It is thus required to modify native banana starch to improve the physicochemical properties, gelatinization, and thermal stability. RS3 is formed through retrogradation of gelatinized starch, which requires controlled heating and cooling [16] and is influenced by extrusion parameters, namely barrel temperature, feed moisture content, and screw speed. Optimized extrusion parameters ensure maximum RS3 production while maintaining structural integrity and functionality for food applications [17]. Although previous research by García-Alonso, [18]; Srichuwong et al., [19], and Zięba et al., [20], have reported success in increasing RS3 in banana flour through extrusion cooking, challenges such as low yield due to complete dextrinization remained. By varying parameters such as botanical source, moisture content, screw speed, and barrel temperature, the study sought to achieve optimal gelatinization conditions for amylose and amylopectin retrogradation thus improving RS3 yield. While some studies have shown promising results in increasing RS3 yield in banana flour using extrusion cooking, none has specifically optimized extrusion for East African Highland Cooking Bananas (EAHCB) cultivars that is higher in amylopectin (waxy starch). Thus, the objective of this study is to investigate the effects of different banana cultivars, screw speed, feed moisture content, and barrel temperature on RS3 formation in EAHCB. The findings from this study are expected to shed light on the mechanisms underlying extrusion cooking's role in enhancing RS3 yield in flour derived from EAHCB, providing valuable insights for future food processing endeavors.

2. Materials and methods

2.1. Materials

Flour from four EAHCB cultivars (*Enyeru*, *Bukumu*, *Mbwazirume*, and *Mpologoma*) was obtained from the Banana Industrial and Research Development Centre, Bushenyi, Uganda.

2.2. Proximate and chemical composition of banana flour

Moisture content, crude fat, crude protein, carbohydrates and ash were determined using AOAC standard methods. Kjeldahl's method was used to estimate protein content (conversion factor 6.25) using AOAC method number 984.13. Crude fat was determined using the Soxhlet method, AOAC method number 996.06. Moisture content was determined by AACC method 4-19 [21]. Ash content was determined by incinerating the sample in a muffle furnace at 500 °C overnight, [21]. Amylose content of starches was determined by the modified Concanavalin (Con) A method using the Megazyme Amylose/Amylopectin Kit (CAT NO: AMYL, Wick low, Ireland). Resistant starch was determined using Megazyme Kits K-RSTAR, CAT.NO. K-RAPRS according to AACC standard method No 32-40.01 (Megazyme International Ireland Limited, Wick low Ireland).

2.3. Extrusion

Extrusion was carried out using a co-rotating and intermeshing twin-screw extruder (Process 11, Coperion Stuttgart, Germany) connected with a rectangular cooling die (5 × 55 mm, $H \times W$) at the end of the extruder barrel. The barrel diameter was 54 mm with a length of 1200 mm. All trials were operated at a screw speed of 200–300 rpm, moisture level of 18–25 %, and barrel temperature of 90–130 °C. The entire extrusion cooking process used water (20 °C) as the cooling medium.

The feed material consisted of banana flour at varied moisture content (18–25 %). Response surface methodology (RSM) was used to

generate an optimization design using Design Expert Software (Stat-Ease, Minneapolis, MN, USA). Thirty-nine experimental runs were generated using a Central Composite Design (Table 2). Extrudates were collected, air-dried, ground, and sieved through a 200- μ m mesh sieve and stored in airtight plastic bags at 4 °C until use.

2.3.1. Cooking of the extruded flour

Cooking was done using a method described by Arcila & Rose [22] with minor modifications.

Banana flour (1 g) was suspended in 15 mL of distilled water in a 50 mL centrifuge tube. The tube was immersed in a boiling water bath (Julabo, SW22, Germany) for 20 min. During the first 5 min of cooking, the slurry was mixed by vortex, and the cap was vented several times to relieve steam pressure buildup. After cooking, the samples were cooled for 5 min at room temperature and then oven-dried at 60 °C for 24 h.

2.3.2. Relative crystallinity of the cooked extruded flour

The relative crystallinity of the flour was determined using an X-ray Diffractometer (Shimadzu XRD 6000, Tokyo, Japan) fitted with Cu-K α radiation ($\lambda K\alpha_1 = 1.5406\text{\AA}$) from $2\theta = 5^\circ - 80^\circ$ with increments of $\Delta 2\theta: (0.034^\circ)$, voltage of 40 kV, current of 1.6 KW and counting time of 6"/min. Crystallinity was calculated using the following formula:

$$RC\% = \left(\frac{AC}{(AC + Aa)} \right) \times 100$$

RC is the relative crystalline, Ac is the crystallized area on the X-ray diffractogram, and Aa is the amorphous area on the X-ray diffractogram.

2.3.3. Thermal properties of the cooked extruded flour

The thermal properties of the cooked optimal extruded flour were analysed using Differential Scanning Calorimetry (Mettler Toledo, DSC 822, Tokyo, Japan). Water (14 μ L) was added with a micro syringe to the flour sample (6 mg dwb) in the DSC pans, which were then sealed, reweighed, and kept at room temperature for 24 h to ensure equilibration of the starch sample and water. The samples were scanned from 35 °C to 150 °C at a heating rate of 10 °C/min, and an empty pan was used as a reference. The values of onset (T_o), peak (T_p), and conclusion (T_c) gelatinization temperatures and enthalpy (ΔH) were obtained from the thermographs of the samples using Universal Analysis 2000 3.9A software.

3. Characterization of the extruded flour

3.1. Granular properties of the extruded flour

A scanning electron microscope (JOEL, JSM-6400; Tokyo, Japan) was used to analyze the microstructure of samples. Flour samples were attached to the sample holders and sputter-coated with gold. Images were then taken under a high vacuum with an acceleration voltage of 15 kV and a magnification of 100 and 1000x

3.2. Functional properties of the extruded flour

3.2.1. Bulk density, particle size, and colour

The bulk density of the flour was determined using a method by Chandra et al. [23]. Flour (100 g) was weighed into a measuring cylinder (250 mL) after tapping the cylinder on a wooden plank until no visible decrease in volume was noticed, and based on the weight and volume, the apparent (bulk) density was calculated.

The granular size distribution of the flour was determined using a laser-light particle size analyzer (Mastersize, SCIROCCO 2000, Tokyo, Japan) equipped with a delivery system for powder samples (SCIROCCO 2000, Tokyo, Japan).

The colour of the extrudate was measured using a hand-held Chroma meter (Konica Minolta, Tokyo, Japan). Colour was reported in terms of

the sample's lightness (L^*), redness (a^*), and yellowness (b^*).

3.2.2. Swelling power, solubility, and water absorption capacity

The swelling power of the flour was determined using a method described by Tan et al., [24]. A suspension of the flour solution prepared with distilled water (1:50, w/v) was heated in a water bath at 40, 50, 60, 70, 80, and 90 °C for 30 min with continuous stirring. The gelatinized starch samples were cooled rapidly to 25 °C in an ice water bath and centrifuged at 7000 g for 20 min. The supernatant (soluble starch) was dried at 105 °C to constant weight.

$$\begin{aligned} \text{Swelling power} &= \text{weight of starch precipitate (g)}/\text{weight of dry starch sample} \\ \text{Solubility} &= \text{solid content in the supernatant}/\text{sample in dry weight} \times 100 \end{aligned}$$

$$\begin{aligned} \text{Water Absorption Capacity} &= \text{weight of wet sediment}/(\text{weight of dry sample} \\ &\quad - \text{weight of the dry solids}) \end{aligned}$$

3.2.3. Oil binding and emulsifying capacity

The flour's oil binding capacity was measured using a method adapted from Ramlan et al. [25]. The flour (4 g) was added to 20 mL corn oil in a 50 mL centrifuge tube. The content was then stirred for 30 s every 5 min, and after 30 min, the tubes were centrifuged at 1600 g for 25 min. The free oil was then decanted, and the absorbed oil was determined by difference. The oil binding capacity was expressed as absorbed oil per gram sample.

The emulsifying capacity of the extrudate was measured according to the method of Ramlan et al. [25]. Aqueous dispersion (25 mL) of 7 % of the extrudate was mixed with 20 mL of corn oil and blended in a Waring blender for 5 min. An aliquot was then centrifuged at 3000 g for 5 min. The percentage of the total mixture that remained emulsified after centrifugation was expressed as the stability index. The stability index of a suitable emulsion would be greater than 94 %, while that of a poor emulsion would only be 50 % [25].

Table 1

Resistant starch, amylose, amylopectin, and proximate compositions of East African Highland cooking banana flour.

Parameter/ Cultivar	Mpologoma	Enyeru	Mbwazirume	Bukumu
Resistant Starch (%)	68.60 ± 2.78 ^c	72.57 ± 3.32 ^a	69.71 ± 4.86 ^b	50.80 ± 5.69 ^d
Amylose (%)	8.17 ± 0.65 ^c	7.30 ± 0.32 ^d	9.29 ± 0.04 ^b	11.32 ± 0.78 ^a
Amylopectin (%)	91.83 ± 1.57 ^a	92.70 ± 1.78 ^a	90.71 ± 0.09 ^b	88.68 ± 1.49 ^c
Moisture (%)	14.87 ± 0.09 ^a	13.92 ± 0.01 ^{ab}	13.22 ± 0.08 ^b	10.85 ± 0.63 ^c
Ash (%)	2.60 ± 0.30 ^b	2.38 ± 0.14 ^c	2.20 ± 0.23 ^d	2.80 ± 0.57 ^a
Fat (%)	0.20 ± 0.08 ^b	0.26 ± 0.12 ^a	0.24 ± 0.03 ^a	0.19 ± 0.07 ^b
Protein (%)	0.17 ± 0.03 ^c	0.29 ± 0.16 ^a	0.23 ± 0.04 ^b	0.14 ± 0.09 ^d

Values are expressed by mean ± standard deviation ($n = 3$). Means with different letters in the same row differ significantly ($p < 0.05$). Means with no letters are not significantly different.

3.3. Pasting properties of the extruded flour

The pasting properties of the extrudate were determined using a Rapid Vasco Analyzer (Newport Scientific Pty. Ltd, syarikar, 295,514-U, Warriewood, Australia), using a general method STD1 profile. Data were collected directly from the pasting curve using ThermoLine for Windows v3.0 (TCW3) software for the RVA.

3.4. Sugar content and anti-nutrient composition of the extruded flour

Sucrose, d-fructose, and d-glucose were determined using a Sucrose, d-fructose, and d-glucose assay kit (CAT.NO.K-SURFRG, Ireland). The phytate content of the extrudate was determined following the protocol of Duguma et al. [26]. Flour (0.5 g) was extracted with 10 mL of 2.4 % HCl in a mechanical shaker for 1 hour at ambient temperature and

Table 2

Experimental design for extrusion of banana flours with the process variables and the response function as obtained from Response Surface Methodology.

Run	Process Variables				Response Function Resistant starch (%)
	Feed Moisture (%)	Barrel Temperature (°C)	Screw Speed (rpm)	Banana Cultivar	
1	25	117.2	250	Mbwazirume	17.3
2	18	90	300	Enyeru	39.9
3	18	90	200	Bukumu	21.6
4	21.2	113.8	300	Bukumu	24.3
5	18	102.8	250	Enyeru	33.8
6	18	130	250	Bukumu	21.3
7	18.9	105.4	200	Enyeru	31.3
8	21.9	125.2	250	Mpologoma	19.8
9	21.7	130	200	Mbwazirume	19.7
10	18	130	200	Mpologoma	21.1
11	21.3	97	250	Bukumu	21.3
12	18.9	130	250	Mbwazirume	23.0
13	25	90	300	Bukumu	21.0
14	18	90	250	Mpologoma	22.1
15	25	106	300	Mbwazirume	24.8
16	25	92	200	Mbwazirume	23.4
17	23.7	90	200	Enyeru	28.4
18	18	118.4	300	Mbwazirume	21.2
19	18.1	130	300	Enyeru	38.6
20	18.8	112.2	200	Bukumu	20.3
21	25	111	200	Mpologoma	18.6
22	18	104	200	Mbwazirume	23.4
23	23.7	110.4	300	Enyeru	32.6
24	18.1	107	300	Mpologoma	25.1
25	25	90	250	Mpologoma	18.0
26	18.6	90.8	250	Mbwazirume	20.1
27	22.1	92.2	300	Mbwazirume	24.8
28	25	130	200	Enyeru	31.4
29	24.2	100	300	Mpologoma	25.7
30	22.4	130	300	Mbwazirume	29.8
31	23.5	124.3	200	Bukumu	42.1
32	25	116.6	250	Bukumu	24.2
33	25	130	250	Enyeru	37.4
34	25	130	300	Mpologoma	25.7
35	20.4	90	200	Mpologoma	18.4
36	19.4	130	200	Enyeru	28.4
37	18	129.2	300	Bukumu	21.2
38	21.9	125.2	250	Mpologoma	20.9
39	22.8	95.6	250	Enyeru	22.4

Table 3

Model terms, their corresponding p-values, and the optimal extrusion conditions for optimal resistant starch (RS) yield.

Terms	p-value	Optimal extrusion conditions
Model	< 0.0001	Optimal RS yield: 38.8 %
X ₁ (Feed Moisture)	0.3366	18 %
X ₂ (Barrel Temperature)	0.8334	90 °C
X ₃ (Screw Speed)	0.0052*	300 rpm
X ₄ (Banana Cultivar)	<	<i>Enyeru</i>
X ₁ × X ₂ (Feed Moisture X Barrel Temperature)	0.0001**	
X ₁ × X ₄ (Feed Moisture X Banana Cultivar)	0.8353	
X ₂ × X ₄ (Barrel Temperature X Banana Cultivar)	0.1303*	
R ²	0.9101	
Desirability		0.95

**Significant at $p < 0.01$; *Significant at $p < 0.05$; ns - Non significant ($p > 0.05$).

centrifuged (Heraeus IDNr. 20,057,821–9) at 3000 g for 30 min. The clear supernatant (3 mL) was collected and mixed with 2 mL of Wade reagent (0.03 % FeCl₃·6H₂O and 0.3 % sulfosalicylic acid in water). The mixture was homogenized and centrifuged at 3000 g for 10 min. A series of phytic acid standard solutions were prepared. The mixtures were centrifuged at 3000 g for 10 min, and the absorbance of the solutions (both the sample and standard) was measured at 500 nm using a UV–Vis spectrophotometer (Biospec, Mini, A115251, Shimadzu Corp, Tokyo, Japan) using deionized water as a blank.

The condensed tannin content of the extrudate was determined using a method adopted from Duguma et al. [26]. Flour (2 g) was added to a screw cap test tube and extracted with 10 mL of 1 % HCl in methanol for 24 h at room temperature with mechanical shaking. The solutions were centrifuged at 1000 g for 5 min. The supernatant (1 mL) was mixed with 5 mL of vanillin-HCl reagent. d-catechin was used as a standard for condensed tannin determination. The absorbance of the sample and standard solutions was measured at 500 nm using a UV–Vis spectrophotometer.

The saponins content of the extrudate was determined following the method of [27]. The vanillin-sulphuric acid assay was used to determine the total saponin content using the aescin standard. Extrudate flour (0.25 g), aescin standards and reagent blank were incubated with 0.25

mL of 8 % (w/v) vanillin in ethanol and 2.50 mL of 72 % (v/v) sulphuric acid in water for 15 min at 60 °C in a shaking water bath. After cooling in water at the ambient temperature for 5 min, the absorbance of the standards and sample was measured at 560 nm using a UV–VIS spectrophotometer.

3.5. Statistical analysis

The experiments were conducted in independent triplicates, and the results were presented as mean values ± standard deviations (SD). Statistical differences were analysed using one-way analysis of variance (ANOVA) at a 5 % significance level using Duncan's test as a post-hoc test. All statistical analyses were performed using SPSS 28.0 (SPSS Inc., Chicago, USA). Data presented as models were analyzed using Response Surface Methodology (RSM) procedures. Design-expert statistical software (DX6.0; Stat-Ease, Inc., MN, USA; 2003) was used in all data analyses.

4. Results and discussion

4.1. Resistant starch in banana flour

Table 1 showed native EAHCB flours contained 88.70–92.7 % amylopectin and minimal amount of ash (2–3 %), fat and protein (<0.3 %). The RS content (50.8–72.6 %) was significantly different between cultivars and was lower than that reported by Chang et al. [28] for EAHCB varieties of Tanzania. RS content of the native banana flour is in the form that is naturally found in the granular structure and is thus, regarded as RS2 [29]. After extrusion, it is expected that RS2 in banana flour will undergo gelatinization, recrystallization, and transformation into RS3 [30].

Extruded flour's RS content indirectly measures retrograded starch, potentially categorized as RS3 [7]. Extrusion at different feed moisture, barrel temperature and screw speed reduced the RS, as depicted by the response function in Table 2. Extrusion typically reduces RS2 content due to gelatinization, similarly observed in corn, potato, and pea starches [31]. This decrease could be attributed to the gelatinization process and subsequent degradation of the granular structure. Upon gelatinization, starch undergoes hydrolysis into smaller fragments capable of retrogradation [32]. Gelatinization leads to starch hydrolysis and subsequent retrogradation, not only enhancing RS3 but also slowly

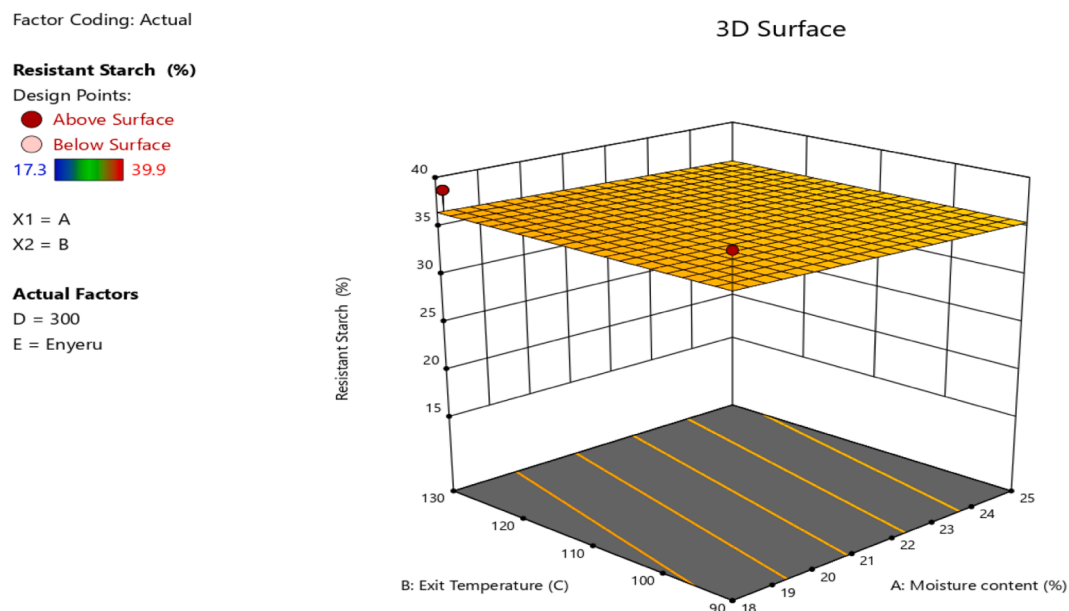


Fig. 1. Effect of barrel temperature and moisture content on resistant starch formation in *Enyeru* cultivar at screw speed of 300 rpm.

digestible starch (SDS) [33].

Results obtained from this study showed that screw speed and banana cultivar significantly affected the formation of RS3 (Table 3). The optimal RS yield was 39.9 % from *Enyeru* cultivar at 300 rpm, 18 % feed moisture content, and 90 °C barrel temperature at a desirability of 0.950. Feed moisture content and barrel temperature interaction positively influenced RS3 formation (Fig. 1); however, not as much as their individual interactions negatively affected it. The interaction between feed moisture content and barrel temperature influences starch transformation during extrusion by affecting gelatinization, dextrinization, and retrogradation [34]. Individually, high moisture content and elevated barrel temperatures promote excessive starch gelatinization and dextrinization, reducing the availability of amylose and linear amylopectin chains necessary for retrogradation into RS3 (Lund & Lorenz, 2009). However, when these parameters interact, a balance may be achieved where partial gelatinization occurs, allowing some amylose and amylopectin chains to reassociate upon cooling, leading to RS3 formation [35]. Higher barrel temperatures cause extensive starch swelling and breakdown of crystallinity. Excessive feed moisture can further accelerate these effects, leading to a reduction in retrogradable starch [35]. In addition, amylose and amylopectin reorganization, whereby a moderate interaction between temperature and moisture can facilitate partial gelatinization, which provides sufficient leached amylose for retrogradation while preventing excessive dextrinization [36,37]. Furthermore, competition between hydrothermal breakdown and recrystallization, where the degree of shearing and heat exposure dictates whether amylose and or amylopectin chains remain long enough to retrograde or are broken into shorter dextrans, which are less likely to form RS3 [38,39].

Several studies highlight that interactions between moisture and temperature can mitigate extreme negative effects, but they do not fully counteract them because thermal degradation thresholds exist once exceeded, starch molecular integrity is lost [40]. Excessive moisture reduces shear intensity, leading to less granular disruption and limiting starch rearrangement into an RS3-favorable structure [19]. Competing pathways, some starch molecules retrograde, while others degrade beyond recovery, leading to only a partial regain of RS3 yield [41]. This result aligns with previous studies where lower feed moisture content enhances starch molecular degradation, leading to increased starch digestibility [42,43]. Yet, high feed moisture content has been linked to low RS3 formation [44,17]. Our findings contrast with studies showing a positive correlation between feed moisture content and RS in certain flours [45,46].

Feed moisture facilitates amylose solubilization and starch granule swelling, promoting RS formation, especially in high amylose content flours [47]. However, the examined native EAHCB flour had lower amylose content (Table 1) compared to green banana flour used in previous studies, which may affect RS formation. The effect of feed moisture content on RS formation likely depends on the amylose content of the flour. In high-amylose starches, increased moisture facilitates greater amylose leaching and retrogradation, which are critical for RS formation [48]. In contrast, banana flour with lower amylose-to-amylopectin ratio, may not experience the same degree of amylose solubilization, limiting the potential for RS formation [49]. Instead, the starch modification in banana flour primarily relies on the retrogradation of amylopectin to form RS [14]. As such, while moisture content is still important for gelatinization, the effectiveness of this mechanism in RS formation is reduced due to the lower amylose content in banana flour [14].

Barrel temperature negatively influenced the formation of RS. This is consistent with the findings of Sahu et al. [17], Von Borries-Medrano et al. [50], and Ali et al. [51]. High temperatures frequently promote starch gelatinization and the destruction of starch crystallites during extrusion [30]. However, higher temperature results in greater starch gelatinization and, thus, higher starch digestibility. Complete gelatinization causes dextrans, which may increase rapidly digestible sugar

Table 4

Effect of cooking on resistant starch, amylose, and thermal properties of native and extruded and extruded banana flours from *Enyeru* cultivar.

	Banana flour		Extruded banana flour	
	Before cooking	After cooking	Before cooking	After cooking
Resistant Starch (%)	72.57 ± 3.32 ^a	4.49 ± 0.29 ^b	38.80 ± 0.41 ^b	12.78 ± 0.06 ^c
Amylose (%)	7.30 ± 0.32 ^c	5.68 ± 0.07 ^d	10.43 ± 0.29 ^a	7.95 ± 0.43 ^b
Thermal properties				
To (°C)	64.58 ± 0.69 ^c	60.00 ± 0.97 ^d	66.13 ± 0.53 ^b	70.58 ± 0.59 ^a
Tp (°C)	70.17 ± 0.87 ^c	67.35 ± 0.67 ^d	74.50 ± 0.76 ^b	77.43 ± 0.55 ^a
Tc (°C)	72.33 ± 0.80 ^c	71.17 ± 0.60 ^c	78.83 ± 0.70 ^b	80.17 ± 1.17 ^a
ΔH (Jg ⁻¹)	9.67 ± 0.32 ^c	8.38 ± 0.07 ^c	11.15 ± 0.10 ^b	14.82 ± 0.18 ^a

Values are expressed as mean ± standard deviation ($n = 3$). Means with different letters in the row differ significantly ($p < 0.05$).

(RDS) compared to RS [30].

Screw speed significantly increased the RS3 content (Table 3). A screw speed of 300 rpm, which is a moderate screw speed, leads to higher RS, SDS, and less RDS [52,30]. Screw speed directly influences the residence time within the extruder. High screw speed produces high shearing on starch molecules, causing retrogradation at a lower residence time [46]. The increase in screw speed causes molecular degradation of the amylose and amylopectin and the consequent splitting of chains that occurs at high screw speed [53]. This property of gelatinization and recrystallization at high screw speed has also been addressed in the work of [54,55] and [56].

Banana cultivar influenced RS3 formation in extruded banana flour as depicted in Table 3. Depending on the starch sources, differences in gelatinization temperatures cause differences in retrogradation properties and, thus, considerable variance in digestibility [57]. Ssonko et al. (2017) reported that *Enyeru* and *Mbwazirume* starch has higher gelatinization temperatures than *Mpologoma* and *Bukumu* starch. These authors further reported differences in amylose content among the starch varieties. The difference in the gelatinization temperatures and amylose content could influence the differences in the yield of RS3 in the different cultivars.

The next parts of the paper will compare the characterization of *Enyeru* native banana flour with extruded banana flour with the optimal RS yield (39.9 %) from *Enyeru* cultivar that was extruded at the screw speed of 300 rpm, 18 % feed moisture content, and 90 °C barrel temperature.

4.2. Effect of cooking on the properties of native and extruded banana flours (*Enyeru*)

Table 4 shows the effect of cooking on the RS, amylose, and thermal properties of native banana flour and extruded banana flour. Cooking significantly reduced the RS of native banana flour from 72.57 % to 4.49 % (93.8 % reduction), but extruded flour retained one-third-of its RS after cooking (38.8 % to 12.78 %). These findings were similar to studies by Liu et al. [58]. This could be explained by the almost complete gelatinization of starch molecules in the native banana flour, which was dispersed without retrogradation [58]. On the other hand, extruded banana flour undergo retrogradation, which is more heat stable and retained RS close to the recommended daily intake (25–30 g) after cooking at a similar temperature. This finding is similar to the findings of Liu et al. [58]. After cooking and cooling, amylose and amylopectin chains tend to retrograde. Retrograded starch has a more ordered structure than gelatinized starch, reducing its susceptibility to

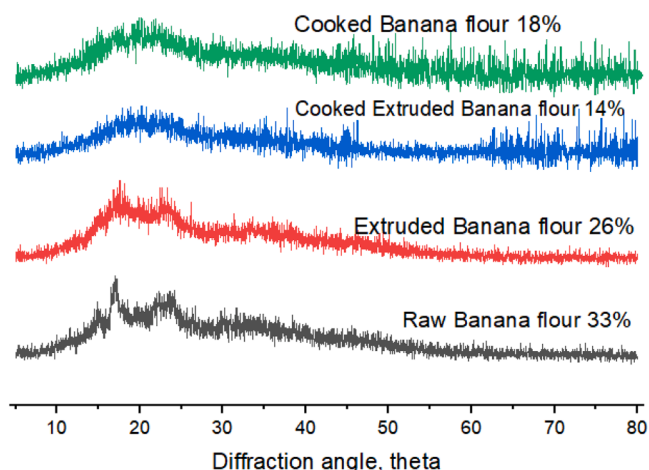


Fig. 2. The X-ray diffractometer graphs and the crystallinity (%) of the cooked and uncooked native and extruded banana flours from *Enyeru* cultivar.

enzymatic digestion [59]. Overall, the combination of the ordered structure, resistance to gelatinization, formation of retrograded starch, and limited accessibility to digestive enzymes contribute to the low digestibility [32].

The amylose of banana flour was altered to ~43 % more after extrusion (from 7.30 % to 10.43 %), mostly due to structural starch modifications. Some of the branches of amylopectin were likely to be cleaved by shear during extrusion, forming straight chains that portrays amylose properties. This finding is similar to that of Bresciani et al. [60] and Liu et al. [9]. Cooking significantly reduced the amylose of both native banana flour and extruded banana flour, attributed to amylose leaching during cooking. Although amylose has been implicated in increasing RS in gelatinized starches, it depends on the starch's botanical sources [61,62]. The current study shows direct correlation of amylose content with the RS in the banana flours – the lower the amylose content, the lower the RS. However, the RS in the extruded banana flour is a representative of retrograded starch and possibly amylose content.

Cooked extruded banana flour exhibited significantly higher gelatinization temperatures (T_o - onset, T_p - peak and T_c - conclusion temperatures) and enthalpy (ΔH) than the cooked native banana flour, indicating better stability against high-temperature processing. These observations aligned with previous reports [63,58]. This phenomenon could be attributed to the weakening of the crystalline structure and the unwinding of the double helix during extrusion [64]. These changes are followed by the degradation of amylose molecules during gelatinization in the cooking process, resulting in the formation of shorter amylose

Table 5

Physical and chemical properties of native and extruded banana flours from *Enyeru* cultivar.

	Native banana flour	Extruded banana flour
Physical Properties		
<i>Particle Size (μm)</i>		
0.1	16.60 \pm 0.18 ^b	72.58 \pm 4.32 ^a
0.5	41.22 \pm 0.69 ^b	234.60 \pm 10.87 ^a
0.9	151.30 \pm 4.78 ^b	486.30 \pm 28.08 ^a
<i>Bulk density (g/mL)</i>		
	0.91 \pm 0.00 ^a	0.51 \pm 0.00 ^b
<i>Color</i>		
Lightness (L)	69.28 \pm 1.48 ^a	63.59 \pm 0.98 ^b
Redness (a*)	2.42 \pm 0.03 ^b	5.17 \pm 0.01 ^a
Yellowness (b*)	12.63 \pm 0.19 ^b	24.67 \pm 0.37 ^a
Chemical Properties		
<i>Dietary Fiber</i>		
Total dietary fiber (%)	11.47 \pm 0.55 ^a	12.40 \pm 1.28 ^a
Soluble fiber (%)	3.37 \pm 0.35 ^b	8.20 \pm 0.47 ^a
Insoluble fiber (%)	8.10 \pm 0.20 ^a	4.20 \pm 0.8 ^b
<i>Reducing sugars</i>		
Fructose (mg/g)	0.01 \pm 0.00 ^b	4.83 \pm 0.06 ^a
Sucrose (mg/g)	2.21 \pm 0.53 ^b	6.53 \pm 0.03 ^a
Glucose (mg/g)	0.75 \pm 0.19 ^b	3.09 \pm 1.02 ^a
<i>Anti-nutrients</i>		
Phytates (mg/g)	1.77 \pm 0.08 ^a	1.66 \pm 0.03 ^a
Saponins (mg/g)	2.80 \pm 0.16 ^a	2.59 \pm 0.01 ^b
Condensed tannin (mg/g)	0.56 \pm 0.17 ^a	0.38 \pm 0.14 ^b

Results are expressed as means \pm standard deviation ($n = 3$). Values with the different superscript letters in the same row are significantly different ($P < 0.05$).

fragments, which can then interact with the outer branches of amylopectin during cooling and retrogradation [65] hence the elevated T_o , T_p , and T_c temperatures. The findings align with those of Jafari et al. (2017), who report lower gelatinization enthalpy at high feed moisture and die temperature, and more stable residual starch granules after heating (after gelatinization). The interaction between amylose and amylopectin branches stabilizes the crystalline region, suggesting a higher melting temperature after retrogradation (Jafari et al. 2017; [58]).

Short amylose fragments during cooking also plays a crucial role in the formation of double helices, which contribute to the increased ΔH values as they reassociate with amylopectin and promote more efficient packing [66,67]. However, the observed increase in ΔH could be due to other factors, such as changes in the crystalline structure or enhanced molecular ordering during retrogradation. In addition to the amylose-amylopectin interactions, the organization of amylopectin itself, with its long-branched structure, could also contribute to the enthalpy change. Therefore, while amylose degradation is an important factor, the increase in ΔH values is likely a result of both

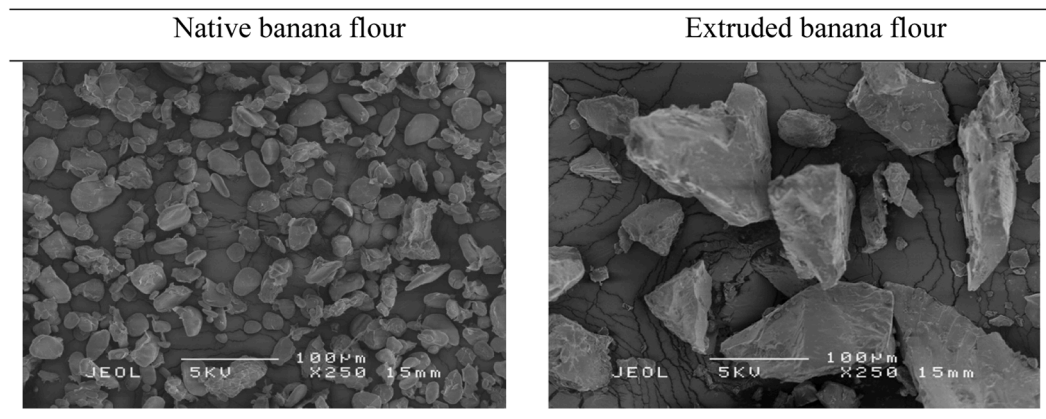


Fig. 3. SEM images (250 \times magnification) of native and extruded banana flours from *Enyeru* cultivar.

amylose-amylopectin interactions and amylopectin self-association, leading to the formation of more stable starch structures.

Extrusion significantly enlarged the amorphous regions of the starch in the extruded banana flour (Fig. 2). Both cooked native banana flour and cooked extruded banana flour had lost the distinctive peaks with reduced crystallinity. When starch granules are gelatinized, they exhibit an amorphous XRD pattern (with no peaks) as opposed to native or partially gelatinized starch that displays XRD profiles with peaks [68]. Similar findings of loss of crystallinity resulting in an amorphous material were reported in banana, corn, and potato starches upon extrusion [69,31]. The rupture of starch particles might be a possible explanation for the reduced crystallinity. However, the crystalline structure may be reformed upon retrogradation [64,70].

Studies have shown that high RS-starch sources are suitable for gluten-free bread [71], unlike low and moderate RS-starch sources, which are preferred for cakes and pudding due to ease of hydration [71, 72]. Extrudates thus serve as valuable functional ingredients suitable for diverse cooking methods, including frying or baking, expanding their potential utility in the food industry [73,74].

4.3. Comparative study on properties of native and extruded banana flour (Enyeru)

4.3.1. Morphological properties

Fig. 3 depicts the structural morphology of native banana flour and extruded banana flour at 250 × magnification. Native banana flour granules showed irregular, intact, and smooth surface granules morphology with varied particle sizes. The native starch has limited usage in the food industry due to poor functional properties [75]. In contrast, extruded banana flour exhibited larger particle size, disrupted structure, and surface irregularities with sharp edges, indicating transformation caused by high shear and high temperature during extrusion. The result is similar to the morphology of extruded breadfruit flour in the findings of Huang et al. [76]. Extrusion altered the structure of banana flour by inducing gelatinization, starch fragmentation, improving hydration and digestibility properties, as noted in prior research [77, 78]. These morphological changes directly affect the functional properties of banana flour, such as water absorption, solubility, and digestibility, making the extruded flour suitable for enhancing texture, expansion, and crispiness in gluten-free products, as well as serving as a thickener or binder in various formulations [79].

4.3.2. Physical and chemical properties

The particle size distribution, bulk density, color, dietary fiber profiles, reducing sugar profile and anti-nutrient profile of the native and extruded banana flours are shown in Table 5.

Extrusion significantly increased the particle size and reduced the bulk density of banana flour. The particle sizes at 0.1, 0.5 and 0.9 μm increased up 4 to 6 times higher, whereas the bulk density reduced by 44 % in extruded banana flour. The results were attributed to the expansion and disruption of starch and fiber matrices during extrusion. Extrusion caused granule swelling, rupture, and aggregation, leading to coarser particle structures and lower packing density [80,81]. Low bulk density benefits complementary food formulation, ensuring lightweight products for easy reconstitution, as seen in infant cereal production [82,83].

Extrusion significantly altered the color profile of banana flour, with decreased lightness and increased redness and yellowness. The color changes resulted from Maillard reaction that is enhanced by extrusion's high processing temperatures and banana flour's low moisture content [56].

The interaction between reducing sugars and amino acids produces melanoidins, a brown pigmented substance [84]. Additionally, high temperature during extrusion causes sugar caramelization in banana flour, particularly glucose and fructose [85]. High shear during extrusion could also break down cell walls of banana flours, releasing bound carotenoids that lead to elevated yellowness [86]. However, dark colors

Table 6

Functional and pasting properties of native and extruded banana flour from *Enyeru* cultivar.

Parameter	Native banana flour	Extruded banana flour
Functional Properties		
Oil Absorption Capacity (%)	7.42 ± 0.08 ^a	5.17 ± 0.17 ^b
Oil Emulsification Capacity (%)	4.19 ± 0.09 ^a	2.29 ± 0.44 ^b
Pasting Properties		
Pasting Temperature (°C)	75.92 ± 0.04 ^a	66.57 ± 0.62 ^b
Peak Viscosity (cP)	3810.00 ± 78.50 ^a	2044.00 ± 4.15 ^b
Through Viscosity (cP)	105.00 ± 13.54 ^a	843.00 ± 1.53 ^b
Breakdown Viscosity (cP)	1036.00 ± 58.98 ^a	1327.00 ± 2.84 ^b
Final Viscosity (cP)	3923.00 ± 11.84 ^a	1115.00 ± 7.35 ^b
Setback (cP)	831.00 ± 14.29 ^a	273.70 ± 1.85 ^b

Results are expressed as means ± standard deviation ($n = 3$). Values with the different letters in the same row are significantly different ($p < 0.05$).

in extrudates are undesirable, as they cannot be masked by food dyes, potentially reducing consumer acceptance [77].

The dietary fiber profile of the extruded banana flour is shown in Table 5. Although insignificant, extrusion increased the total dietary fiber of banana flour. On the other hand, extrusion significantly increased the soluble dietary fiber and decreased the insoluble dietary fiber which was similar to other findings [87,35]. High shear and high temperature during extrusion disrupt the structure of covalent and non-covalent bonds and insoluble fiber into smaller fragments of lower molecular weight water soluble fiber, thereby increasing the soluble dietary fiber [86]. The slight increase in total dietary fiber could be due to the formation retrograded starch, also known as RS3, that resists enzymatic digestion and classified as dietary fiber [88].

Extrusion significantly increased the fructose, sucrose and glucose concentrations in the extruded banana flour (Table 5), as agreed by previous findings [34,32]. Under conditions of high temperature, shear and pressure, starch - particularly amylopectin molecules - is hydrolysed predominantly around the branching points, resulting in increased glucose and other reducing sugars [30,89]. The severe condition during extrusion also caused cell walls disruption that releases bound sucrose [90]. The high temperature during extrusion also facilitates sucrose hydrolysis, resulting in increased glucose and fructose [85]. Saponins and condensed tannins decreased significantly after extrusion, while phytate levels showed a slight, non-significant decline (Table 5). The significant decrease in saponins and tannins are caused by thermal degradation under high temperature and high shear during extrusion, most likely due to their heat-labile nature [91]. This reduction is beneficial because tannin is known to interfere with nutrient digestibility, whereas saponin causes bitterness. On the other hand, phytate is more heat-stable [92], which explains the insignificant reduction observed. The reduction in anti-nutrient compound indicates improvement of nutrient bioavailability and quality of extruded banana flour.

4.3.3. Functional properties

Extrusion significantly decreased the oil absorption capacity (OAC) of the banana flour from 7.42 % to 5.17 % (Table 6). Formation of RS3 in extruded banana flour has a more ordered and compact crystalline structure caused by structural rearrangement during retrogradation, which in turn limits the availability of amorphous regions within the starch granules that bind oil [93], hence decreasing OAC. OAC is crucial for flavor retention, improved palatability, and extended shelf life, which are essential for baked products [94]. Low OAC in flour has the potential to reduce fat content, improve texture, extend shelf life, promote a healthier product profile, and enhance flavor retention in food products [95].

The oil-emulsifying capacity (OEC) profile of the extruded banana flour significantly reduced by ~45 % (from 4.19 to 2.29 %), as shown in Table 5. However, these findings were contrary to reports which suggested that the flexibility and surface hydrophobicity of the protein

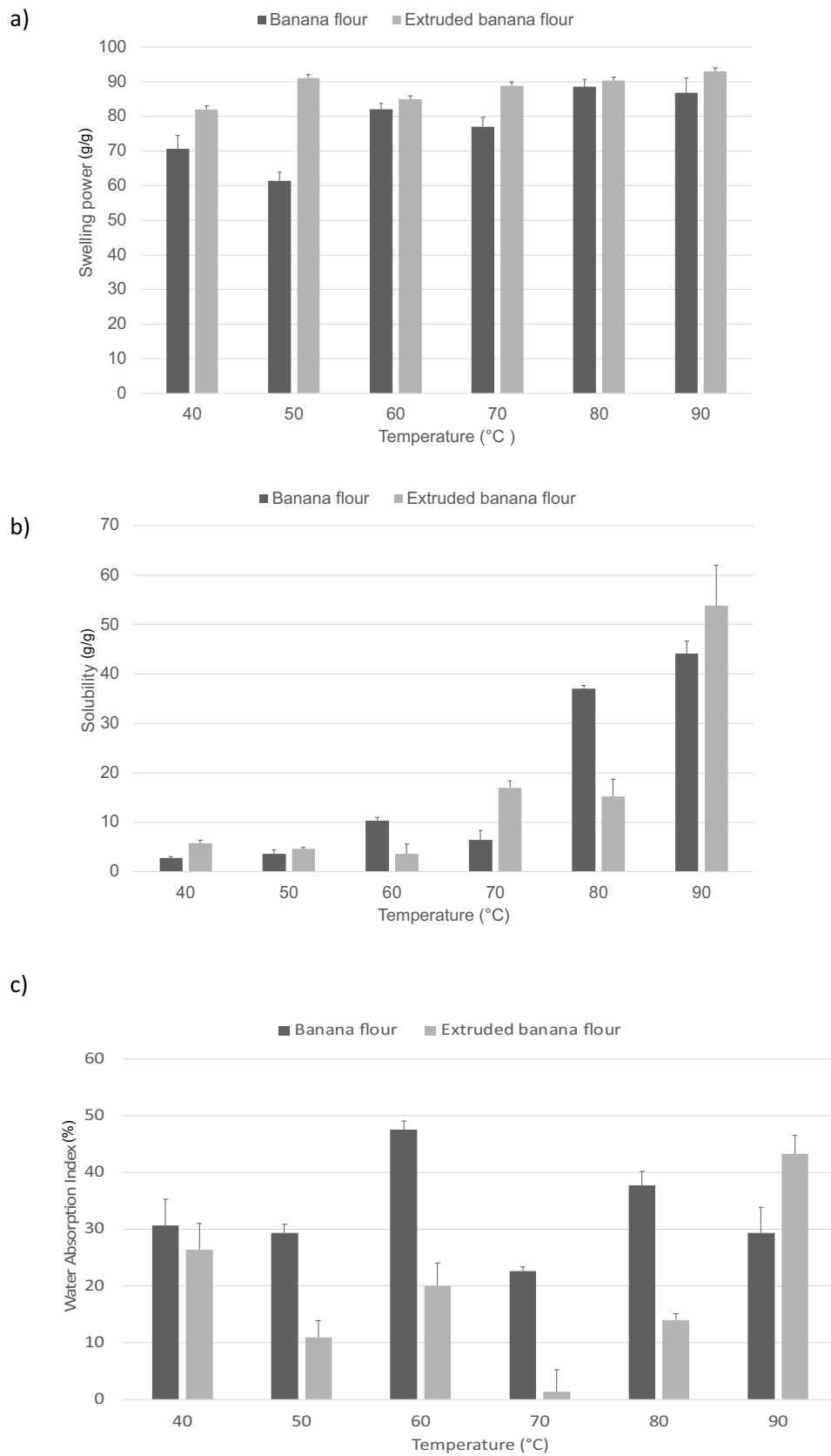


Fig. 4. The a) swelling power, b) solubility, and c) water absorption index of native and extruded banana flours from *Enyeru* cultivar.

increases with the denaturation of the air-water interface when the protein is extruded and produces a suitable emulsifying property [96,97], which in this case, applies to starches with a high protein content, unlike bananas, which are poor protein sources. While the low protein content in banana flour could be a contributing factor to the reduced OEC, structural modifications in starch, including increased gelatinization or changes in amylose fractions, could also play a significant role [65]. Gelatinization can lead to the loss of the starch granule's native structure, which may affect its ability to interact with oils and stabilize emulsions [98]. Additionally, extrusion formed larger and aggregated particles (Fig. 3) that are less effective in stabilizing oil. Protein denaturation during extrusion can increase surface hydrophobicity and improve emulsifying properties [99]. However, the absence of sufficient protein content in banana flour limits its emulsifying potential. Starch gelatinization and the subsequent disruption of native starch structures can reduce emulsifying properties in starch-based systems, even in the presence of low protein content. These studies highlight that starch modifications, rather than protein alone, can significantly influence OEC in food products with low protein content [100–102].

The swelling power of extruded flour reflects starch gelatinization during extrusion cooking [94]. Extrusion cooking increased the swelling power of the extruded banana flour at the temperatures of 40 to 90 °C, but was more prominent at 40 and 50 °C (Fig. 4-a). This result is similar to the findings obtained by Huang et al. [76]. The increase is mainly due to the disruption of the crystalline structure during extrusion, where the intermolecular hydrogen bonds of starch were broken and the granular arrangement was less compact [70,103].

Extrusion significantly increased the solubility of the extruded banana flour, particularly at 70 and 90 °C (Fig. 4-b). These findings were similar to the findings of Giraldo-Gómez et al. [69], who reported an increase in the solubility of banana flour after extrusion. Similar findings were further reported in extruded soya bean hull [104]. This increase is possibly due to sheer mechanical degradation, leading to an increasing amount of soluble molecules and resulting in an increase in water solubility index values [69,105]. When the temperature increases, amylose and amylopectin chains are separated and form an expansible matrix, resulting in a higher solubility [30]. Generally, extrusion cooking increases the usability of the flour in instant foods such as instant porridge and soup (Tabibloghmany et al., 2017).

Extrusion cooking significantly decreased the water absorption capacity of the extrudate at 40–80 °C but increased significantly at 90 °C (Fig. 4-c). This result is similar to the findings of Tabibloghmany et al. [104]. This increase at higher temperature could be due to the unfolding and loosening of biopolymer chains, resulting in greater availability and more effortless penetration of structures by water molecules. A high water absorption value enables incorporation of more water into the dough, improving its handling characteristics and helping to retain moisture during storage. Increased water absorption is desirable in bakery products and instant foods, such as noodles, soups, and porridge, as it improves texture, mouthfeel, rehydration capacity and overall product quality.

4.3.4. Pasting properties of the extruded flour

The pasting profile of extrudate is shown in Table 6. Extrusion decreased the pasting temperature, peak viscosity, final viscosity, and setback viscosity, whereas through viscosity and breakdown viscosity were significantly increased. High shear, screw speed and temperature during extrusion cause pre-gelatinization of the starch granules, and as such, less energy is required for further gelatinization [106], hence the reduced pasting temperature. During extrusion, optimal feed moisture content and barrel temperature cause ideal gelatinization, with sufficient molecular integrity retained, leading to moderate viscosity reduction (Lund & Lorenz, 2009). High shear and temperature during extrusion also caused starch structural damage and molecular degradation, causing lower swelling capability as reflected through the reduced peak viscosity [30]. This result also supports the amylose and

RS stability against heat in extruded banana flour (Table 4). The through viscosity of the extruded banana flour was higher by four times than the native banana flour, most likely caused by improved paste stability against constant high temperature and shear. However, the lower molecular weight of depolymerized starch in extruded banana flour could be the reason of higher breakdown viscosity. In addition, extrusion cooking causes weak gel formation due to reduced starch molecules reassociation, thus reducing the final viscosity [106]. Extrusion cooking also disrupts amylose realignment due to limited retrogradation due to structural modifications causing a reduction in the setback viscosity [107]. Similar findings of reduction in peak viscosity, final viscosity, through viscosity, and breakdown with extrusion were reported in banana and corn starches [108,70]. The extruded banana flour shows improved paste stability, lower viscosity and lower starch degradation, allowing applications in infant food and starch-stable food products [109].

5. Conclusion

This study demonstrates that extrusion is an effective technique for enhancing both the structural and functional properties of banana flour. The process disrupted the native crystalline starch structure, lowered gelatinization temperature, and promoted starch retrogradation. As a result, resistant starch type 3 (RS3) formed post-extrusion exhibited improved thermal stability—even after cooking—making the flour suitable for high-temperature food applications.

Among the tested cultivars, *Enyeru* had the best ability to form RS3 under optimized extrusion conditions. Higher screw speeds showed elevated RS, while increased feed moisture and barrel temperature hindered RS3 formation. These findings underscore the importance of fine-tuning extrusion parameters to improve the nutritional quality of banana flour.

Functionally, the extruded flour showed reduced anti-nutritional property and oil absorption capacity, with improved solubility, swelling and water absorption. It has potential use in a variety of food applications including snacks, cereals, bakery items, noodles, and infant foods. Future research should explore the extruded banana flours incorporation into real food systems, focusing on sensory quality, stability, and consumer acceptance. Overall, this study highlights extrusion as a valuable strategy for developing nutrient-rich, functional ingredients from underutilized banana cultivars.

CRedit authorship contribution statement

Stellamaris Kembabazi: Writing – review & editing, Visualization, Software, Writing – original draft, Validation, Project administration, Formal analysis, Investigation, Conceptualization. **Martin Mutambuka:** Project administration, Supervision, Conceptualization. **Norhasnida Zawawi:** Project administration, Validation. **Ediriisa Mugampoza:** Methodology, Project administration. **Radhiyah Shukri:** Writing – original draft, Validation, Methodology, Conceptualization, Writing – review & editing, Visualization, Supervision, Data curation. **Florence Isabirye Muranga:** Project administration, Conceptualization, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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