



Physicochemical and pasting properties of unripe plantain (*Musa paradisiaca*) flour supplemented with bean pod (*Brachystegia eurycoma*)

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Received May 18, 2025, Revised October 1, 2025: Accepted November 7, 2025

This study investigated the physicochemical and pasting properties of composite flours formulated from unripe plantain (*Musa paradisiaca*) and bean pod (*Brachystegia eurycoma*) in order to enhance nutritional quality of the composite flour which could be nutritionally advantageous low-income earners, especially in the African Continent. Composite blends were prepared in varying ratios: 100% unripe plantain flour (URPF), 100% bean pod flour (BPF), and three formulations—URPF:BPF at 96:4, 92:8, and 88:12 respectively. Functional parameters such as bulk density, water absorption capacity, swelling power, solubility, amylose and amylopectin contents, free sugar, and starch were analyzed alongside pasting properties. Results showed that bulk density increased with the least BPF substitution, with UB recording the highest value (1.99 g/mL). Water absorption capacity was highest in 100% BPF (99.90%) and lowest in the UB blend (83.18%). Swelling power decreased with increasing BPF content, while amylose content reduced and amylopectin content increased. UB sample had the highest sugar and starch content, while pasting properties revealed that 100% BPF exhibited superior peak, trough, and final viscosities. However, the highest setback viscosity (257.86 RVU) was recorded in the 88% URPF:12% BPF blend, indicating better gel stability and reduced retrogradation. These findings suggest that supplementing unripe plantain flour with bean pod flour enhances its functional and pasting characteristics, offering potential for the development of improved, nutrient-rich composite flours for both industrial and household food applications.

Keywords: Unripe plantain flour (*Musa paradisiaca*), bean pod (*Brachystegia eurycoma*), physicochemical properties, pasting properties, composite flour.

INTRODUCTION

Plantain (*Musa paradisiaca*) is an important starchy staple and commercial crop in West and Central Africa, where approximately 50% of the world's plantain is produced. It is primarily cultivated and consumed in tropical regions of Africa, South America, Central America, and Asia (Adamu *et al.*, 2017). The plant is a tree-like, perennial herbaceous species, growing between 2 and 9 meters tall, with an underground rhizome or corm. It features long overlapping leaf stalks and a stem height ranging from 1.22 to 6.10 m

(Oladiji *et al.*, 2010). Taxonomically, plantain belongs to the family *Musaceae*, order *Plantaginaceae*, and genus *Musa*. The principal species include *Musa paradisiaca* (French plantain), *Musa acuminata*, and *Musa corniculata* (Horn plantain) (Adamu *et al.*, 2017). It is widely cultivated in the Eastern and Southern regions of Nigeria.

A diet rich in unripe plantain is filling and can be beneficial for weight management. Unripe plantain flour is a valuable source of essential nutrients, including iron,

zinc, potassium, and sodium (Mepba *et al.*, 2007; Zakpaa *et al.*, 2010). Adeniji *et al.* (2006) reported that iron content in plantain varies from 14.275 to 36.500 µg/g depending on the cultivar. Unripe plantain flour has been incorporated into composite flours with wheat, maize, and rice or used in combination with proteins to produce bread (Juarez-Garcia *et al.*, 2006), pasta (Patiño-Rodríguez *et al.*, 2019), and snacks (Flores-Silva *et al.*, 2017). Also, Patiño-Rodríguez *et al.* (2019) found that unripe plantain flour has minimal impact on the sensory properties of composite flours, making it acceptable to consumers.

In Nigeria and other African countries, plantain serves as an affordable source of dietary calories (Adegunwa *et al.*, 2012). Approximately 70 million people, especially in West and Central Africa, obtain over 25% of their carbohydrate intake from plantain (Olumba, 2014). Unripe plantain fingers (UPF) are traditionally processed into flour, which is used in a variety of dishes including *akara*, *ukpo ogede*, soups, and baked products. The flour can also be reconstituted in boiling water to prepare *amala*, a thick paste consumed with soups across Nigeria (Awoyale *et al.*, 2020; Onuoha *et al.*, 2014). *Amala* is commonly made from dried yam, cassava, sweet potato, or unripe plantain flour, which is stirred into boiling water to form a smooth paste (Abiodun and Akinoso, 2015).

Brachystegia eurycoma, commonly known as bean pod, is a leguminous plant native to tropical Africa (Okwu and Okoro, 2006). It typically grows along river banks and swampy areas in Western and Eastern Nigeria. It is widely used as a flavoring and thickening agent in soups and is consumed as a food crop. Locally, it is known by various names: *okung* (Efik), *eku* (Esan), *achi* (Igbo), *ekalado* or *eku* (Yoruba), *okweri* (Edo), and *akpakpa* or *taura* (Hausa). Over the past three decades, the use of legume seed flours has increased due to growing awareness of their nutritional and functional benefits (Kisambira *et al.*, 2015). According to Okwu and Okoro (2006), the fruits of *Brachystegia* species ripen between September and January and are dispersed explosively.

The seed flour of *Brachystegia eurycoma* is traditionally used in southeastern Nigeria as a condiment, flavoring, and thickening agent in soups such as *egusi* (melon), *ofe onugbu* (bitter leaf), *oha*, and *nsala*. Beyond culinary use, it also serves as a stabilizing and emulsifying agent in soups and is increasingly being applied in bakery and meat-based products due to its water absorption capacity (Ikegwu *et al.*, 2010).

The physicochemical properties of food describe the complex interactions between molecular composition, structural configuration, and environmental factors, and they significantly influence the functional performance of food ingredients (Dzandu *et al.*, 2024). Both unripe plantain and bean pod have been recognized for their commercial potential in food formulation. However, there is limited literature on the physicochemical and pasting properties of composite flour made from these two materials.

Bean pod flour is known to contain a variety of bioactive compounds including flavonoids, phenolics, alkaloids, saponins, and tannins. In addition to its nutritional value, different parts of the plant have demonstrated various biological and pharmacological properties such as analgesic, anti-inflammatory, antimicrobial, wound-healing, antioxidant, anticancer, and hypoglycemic effects. It has also shown potential for modulating lipid profiles and liver enzyme activities. These attributes are important in determining food quality and functional performance.

Starch is primarily responsible for gelatinization, browning, gelation, and dextrinisation, while proteins contribute to emulsification, coagulation, foaming, and denaturation. Fats play key roles in emulsification, aeration, shortening, and texture development. Although some components may have overlapped or minor influences, each contributes uniquely to the overall functional properties of food (Godswill, 2019).

Functional properties refer to the physical and chemical characteristics of ingredients that affect their behavior during processing, storage, and cooking, ultimately influencing food quality, texture, appearance, and consumer acceptability. These include bulk density, water and oil absorption capacities, emulsification and swelling capacities, foam stability, solubility, gelatinization temperature, jelling, shortening, plasticity, moisture retention, aeration, and sensory attributes, among others (Godswill, 2019).

As consumers increasingly demand foods that offer both nutritional value and additional health benefits, the food industry is challenged to develop innovative products with functional attributes. In response, this study investigates the physicochemical and pasting properties of unripe plantain flour supplemented with bean pod flour. The findings aim to provide valuable information for industrial applications, particularly in the development of affordable, natural, and functional composite flours that may be blended with wheat flour to produce health-enhancing food products.

MATERIALS AND METHODS

Collection of samples

Bunches of unripe plantain were bought at a local market in Malete, Kwara State, Nigeria, while bean pod seed sample was bought at a local market in Ilorin, Kwara State, Nigeria.

Chemicals and reagents

Ethanol, iodine solution, sulphuric acid, phenol solution, acetic acid (57.75 ml, glacial acetic acid in 1000 ml), NaOH 1N (40 g in 1000 ml), distilled water and perchloric acid were obtained from the Department laboratory, Malete, Kwara State, Nigeria and the reagents were of analytical grade.

Preparation of sample composite

Unripe plantains were cut from the branch, rinsed and peeled; the pulp were cut into reasonable sizes and air dried for a week while the

bean pod were rinsed and left to dry for the same period (Okafor and Nwogu, 2020). The air-dried plantains and bean pod were ground into flour using a standard laboratory blender, after which they were packed in an airtight plastic container and stored at room temperature for further analysis.

Subsequently, percentage by mass of both the unripe plantain and bean pod were weighed out using a standard laboratory analytical balance as follows:

- 100 (g) of unripe plantain flour (URPF)
- 100 (g) of bean pod flour (BPF)
- 96 (g) of unripe plantain flour (URPF:96)
- 92 (g) of unripe plantain flour (URPF:92)
- 88 (g) of unripe plantain flour (URPF:88)
- 4 (g) of bean pod flour (BPF:4)
- 8 (g) of bean pod flour (BPF:8)
- 12 (g) of bean pod flour (BPF:12)

Unripe plantain flour (URPF:96 g) and bean pod flour (BPF:4 g), (URPF:92 g) and (BPF:8 g), (URPF:88 g) and (BPF:12 g) were blended to homogeneity using a standard blender respectively to produce a composite formulation of unripe plantain and bean pod flour mixture, while 100 (g) of unripe plantain flour (URPF) and 100 (g) of bean pod flour (BPF) were used as control samples

- URPF; BPF (96:4)
- URPF; BPF (92:8)
- URPF; BPF (88:12)
- URPF (100)
- BPF (100)

DETERMINATION OF FUNCTIONAL PROPERTIES

Determination of amylose content

Amylose content of samples was determined by the method described by Juliano *et al.* (1981). Briefly, 100 mg of the flour samples was mixed with 1 mL of 95% ethanol and 9 mL of 1 N NaOH, and the mixture was heated in a water bath at 100°C for 10 min to gelatinize the starch. After cooling to room temperature, 0.5 mL of diluted extract was mixed with 0.45 mL of distilled water, 0.1 mL of 1N acetic acid solution, 0.2 mL of iodine solution (0.2% I₂ in 2% KI) and 9.2 mL of distilled water was diluted to 10 mL. The reaction mixture was incubated at room temperature for 20 min, after which the absorbance was measured at 620 nm in a UV-Vis spectrophotometer. Subsequently, amylose content of the sample was calculated by the difference, using the formula reported by Torruco *et al.* (2006).

$$\text{Amylopectin (\%)} = 100 - \text{amylose (\%)}$$

Determination of bulk density

The method described by Onwuka (2005) was adopted to determine the bulk density (BD) of flour samples. A graduated cylinder 100 mL was weighed dry and gently filled with 10 g of samples. The bottom of cylinder was then tapped gently on a laboratory bench several times. This was continued until there is no further diminution of the samples in the cylinder, after filling to the mark, was observed. Weight of cylinder plus flour was measured and recorded (Nwakaudu *et al.*, 2017). The bulk density was calculated as shown below:

$$\text{Bulk density} = \text{Weight of sample (g)} / \text{Volume of sample (mL)}$$

Determination of water absorption capacity (WAC)

The procedure described by Onwuka (2005) was followed to determine the water absorption capacity of flour samples. One gram (1 g) of sample was mixed with 10 mL of distilled water in a graduated

centrifuge tube for 30 sec. The suspension was left for 30 min at room temperature, and then centrifuged at 5000 rpm for 5 min. The supernatant was decanted and the tube was tipped up to drain for 10 min. After which the centrifuge tube, with content was weighed to determine the difference in the amount of water held by the samples.

$$\text{Calculation: water binding capacity (WBC \%)} = \frac{\text{gram bound water} \times 100}{2.5}$$

Determination of swelling power and solubility

The method by Leach *et al.* (1959) was adopted for swelling power and solubility determination; briefly, one gram (1 g) of the sample was mixed with 15 mL of distilled water in a 100 mL conical flask. The suspension was shaken for 5 min at low speed. It was then transferred into a water bath and heated for 40 min at 80-85°C with constant stirring. Thereafter, 7.5 mL of distilled water was added and centrifuged at 10,000 rpm for 5 min. The supernatant was carefully decanted into a pre-weighed container and dried at 100°C to constant weight. The container was allowed to cool to room temperature and weighed. The sediment with the centrifuge tube was also weighed.

$$\text{Swelling power} = \frac{\text{Weight of sediments}}{\text{Weight of sample} - \text{Weight of soluble}}$$

$$\text{Solubility index} = \frac{\text{Weight of soluble} \times 100}{\text{Weight of sample}}$$

Determination of total free sugar and starch

Total free sugar and starch content of the samples were quantified following the phenol sulphuric acid method as reported by Onitilo *et al.* (2007). Total free sugar was extracted from 0.02 g of sample using 80% hot ethanol. The mixture was then centrifuged at 2000 rpm for 10 min, after which the supernatant was decanted and used for free sugar analysis, while the residue was used for starch analysis. For free sugar analysis, 0.2 mL of the diluted supernatant was mixed with 0.5 mL of phenol solution (5%) and 2.5 mL of concentrated sulphuric acid (H₂SO₄). The mixture was allowed to cool to room temperature, and the absorbance was read at 490 nm.

The residue was hydrolyzed with 7.5 mL of perchloric acid for 1 h, diluted to 25 mL with distilled water and filtered through Whatman filter paper (No. 2). Then 0.05 mL of the filtrate was mixed with 0.5 mL of phenol solution (5%) and 2.5 mL of conc. H₂SO₄.

The mixture was allowed to cool to room temperature and the absorbance was read in a spectrophotometer (Milton Roy Company, USA; model: spectronic 601) at 490 nm. Starch and total free sugar contents of sample were calculated from a glucose standard curve prepared along with the sample.

Determination of pasting properties

The pasting properties of flour was measured in triplicate in a Brabenders micro-visco amylograph (Brabender, Duisburg, Germany), using a slightly modified procedure of Marti *et al.* (2017). An aliquot of flour (12 g) was dispersed in 100 ml of distilled water, scaling both flour and water weight on a 14% flour moisture bases. The pasting properties were evaluated under constant conditions (speed 250 rpm, sensitivity 300 cmg) by using the following time temperature profile; heating from 30 to 95°C for 20 min; cooling from 95 to 30°C. The heating and cooling phases were carried out with a temperature gradient of 3°C/min. pasting parameters were calculated by using specific software.

Table 1. Functional physical properties of samples.

Sample	BD (g/mol)	WAC (%)	SWP	Solubility
100% URPF	1.50 ± 0.01 ^d	91.89 ± 0.16 ^c	8.32 ± 0.01 ^a	10.15 ± 0.07 ^a
100% BPF	1.54 ± 0.01 ^c	99.90 ± 0.14 ^a	3.67 ± 0.01 ^c	10.15 ± 0.21 ^a
96% URPF;4% BPF (UB)	1.99 ± 0.01 ^a	83.18 ± 0.01 ^d	9.06 ± 0.01 ^a	9.85 ± 0.01 ^b
92% URPF; 8% BPF (RB)	1.96 ± 0.01 ^b	86.46 ± 0.14 ^c	6.19 ± 0.01 ^b	9.69 ± 0.01 ^c
88% URPF; 12% BPF (PB)	1.92 ± 0.01 ^d	89.74 ± 0.01 ^b	3.33 ± 0.01 ^c	9.53 ± 0.01 ^b

Data represent the mean ± standard deviation of duplicate readings; values with the same lower case superscript letter along the same column are not significantly different ($p < 0.05$). **URPF**– Unripe plantain flour, **BPF**– Bean pod flour, **BD**–Bulk density; **SWP**– Swelling power; **WAC**– water absorption capacity. NB: Samples with 96% URPF;4% BPF = **UB**, Samples with 92% URPF; 8% BPF = **RB**, Samples with 88% URPF; 12% BPF = **PB**.

Data Analysis

All measurements were done in triplicates and results were presented as mean ± standard error of mean (SEM). Data were analyzed using one-way analysis of variance (ANOVA) and differences between means were determined using the least significant difference on Statistical Package for Service Solutions (SPSS) version 20. The significance level was set at $p < 0.05$.

RESULTS AND DISCUSSION

Functional (physicochemical) properties of the samples

The functional and physicochemical properties of the samples (100% URPF, 100% BPF, 96% URPF: 4% BPF, 92% URPF: 8% BPF and 88% URPF: 12% BPF) are presented in Tables 1 and 2. The results in Table 1 (functional physical properties) show that 100% unripe plantain flour (URPF) had the lowest bulk density (1.50 ± 0.01 g/mL), while the 96% URPF:4% bean pod flour (BPF) blend recorded the highest value (1.99 ± 0.01 g/mL). The 92% URPF:8% BPF and 88% URPF:12% BPF blends had bulk densities of 1.96 ± 0.01 g/mL and 1.92 ± 0.01 g/mL, respectively, while 100% BPF recorded 1.54 ± 0.01 g/mL. All samples showed significant variation in bulk density ($p < 0.05$). Interestingly, the increase in bulk density for 100% URPF contrasts with the findings of Adenugwa *et al.* (2017), who reported a decrease. Similarly, the bulk density of 100% BPF in this study was higher than that reported by Irondi *et al.* (2017). Variations may be attributed to differences in drying, handling, and sample preparation techniques (Olawuni *et al.*, 2013). Additionally, bulk density can be influenced by starch content—higher starch typically increases bulk density (Godswill, 2019)—as well as by physical factors such as geometry, particle size, surface properties, and solid density (Iwe *et al.*, 2016).

Previous studies using legumes in plantain flour formulations (Falade and Olugbuyi, 2010; Falade and Oyeyinka, 2015) reported minimal impact on bulk density, unlike the notable variations observed with BPF supplementation in this study. The increase in bulk density

with rising BPF content indicates improved functionality and better packing potential of the flour.

The water absorption capacity (WAC) of 100% URPF in this study falls within the range of $131.75 \pm 8.84\%$ to $78.75 \pm 8.13\%$ reported by Adenugwa *et al.* (2017). Among all samples, 100% BPF exhibited the highest WAC ($99.90 \pm 0.14\%$), although this is lower than the $223.61 \pm 3.68\%$ previously reported by Irondi *et al.* (2017). The lowest WAC ($83.18 \pm 0.01\%$) was recorded in the 96% URPF:4% BPF blend. The observed increase in WAC with rising BPF content indicates enhanced hydrophilic properties, likely due to the presence of fiber and protein which suggests improved water-binding potential for formulations requiring moisture retention.

Swelling power was highest in 100% URPF (8.32 ± 0.01 g/g), which is significantly lower than the 38.38 ± 0.27 g/g reported by Adenugwa *et al.* (2017). The lowest swelling power was observed in the 88% URPF:12% BPF blend (3.33 ± 0.01 g/g), followed closely by 100% BPF (3.67 ± 0.01 g/g), which was also lower than the 12.30 ± 0.42 g/g reported by Irondi *et al.* (2017). The progressive reduction in swelling power with increasing BPF content suggests a dilution of starch granule swelling ability, likely due to the high fiber and protein content of BPF. This decline in swelling capacity with higher BPF inclusion reflects a functional shift towards reduced water absorption and gelation potential, which may affect the flour's suitability for products requiring high thickening properties.

The results in Table 2 (functional chemical properties) shows that the amylopectin content increased with the level of BPF substitution, with 100% BPF recording the highest value (94.30%), and 96% URPF:4% BPF the lowest ($81.68 \pm 1.67\%$). Conversely, amylose content decreased with increasing BPF levels, ranging from $17.30 \pm 2.74\%$ in 100% URPF to 5.70% in 100% BPF. This inverse relationship aligns with previous findings by Ratnawati *et al.*, (2019), who observed similar trends in cassava flour substituted with *B. eurycoma* seed flour. Since amylose and amylopectin influence functional properties such as gelatinization, water holding, and retrogradation, their proportions are critical to both food texture and industrial application (Irondi *et al.*, 2017).

Table 2. Functional chemical properties of samples.

Sample	Free sugar (%)	Free starch (%)	Amylose content (%)	Amylopectin content (%)
100% URPF	47.11 ± 0.29 ^c	30.71 ± 0.07 ^a	17.30 ± 2.74 ^a	82.70 ± 2.74 ^b
100% BPF	54.20 ± 0.03 ^b	21.40 ± 0.50 ^b	5.70 ^d	94.30 ^a
96% URPF;4% BPF (UB)	168.61 ± 0.58 ^a	34.38 ± 0.07 ^b	18.32 ± 1.67 ^a	81.68 ± 1.67 ^b
92% URPF; 8% BPF (RB)	129.80 ± 0.14 ^b	26.47 ± 0.07 ^d	17.89 ± 3.28 ^a	82.11 ± 3.28 ^b
88% URPF; 12% BPF (PB)	90.98 ± 0.21 ^b	18.55 ± 0.07 ^b	17.47 ± 2.37 ^b	82.53 ± 3.17 ^b

NB: Samples with 96% URPF;4% BPF = **UB**, Samples with 92% URPF; 8% BPF = **RB**, Samples with 88% URPF; 12% BPF = **PB**

Table 3. Pasting properties results of Sample

Sample	Peak 1 viscosity (RVU)	Trough 1 viscosity (RVU)	Breakdown viscosity (RVU)	Final viscosity (RVU)	Setback viscosity (RVU)	Peak Time (Min.)	Pasting Temp. (°C)
100% URPF	193.25	159.17	34.08	243.83	84.67	5.60	83.25
100% BPF	1023.50	878.92	144.58	1064.08	185.17	7.00	84.10
96% URPF;4% BPF (UB)	118.98	112.98	6.00	191.00	78.02	5.71	85.01
92% URPF; 8% BPF (RB)	188.38	184.06	4.33	352.00	167.94	6.35	84.37
88% URPF; 12% BPF (PB)	257.78	255.14	2.67	513.00	257.86	6.99	83.73

Data represent the mean ± SD (standard deviation). One-way analysis of variance was used to test for significant group differences. **URPF**- Unripe plantain flour. **BPF** - Bean pod flour.

NB: Samples with 96% URPF;4% BPF = **UB**, Samples with 92% URPF; 8% BPF = **RB**, Samples with 88% URPF; 12% BPF = **PB**

The solubility of both 100% URPF (10.15 ± 0.21%) and 100% BPF (10.15 ± 0.07%) did not differ significantly ($p < 0.05$). However, solubility decreased as BPF content increased in the composite samples, indicating limited dispersion capability likely due to the fiber-rich composition of BPF.

Regarding sugar content, the 96% URPF:4% BPF blend had the highest value (168.61 ± 0.58%), followed by 92% URPF:8% BPF (129.80 ± 0.14%) and 88% URPF:12% BPF (90.98 ± 0.21%). The lowest sugar content was found in 100% URPF (47.11 ± 0.29%) and 100% BPF (54.20 ± 0.23%). These results suggest that moderate BPF inclusion enhances free sugar content, which may be beneficial in sweetened or baked products.

Free starch content followed a similar trend. The highest value (34.38 ± 0.07%) was recorded in the 96% URPF:4% BPF blend, while the lowest (18.55 ± 0.07%) was observed in the 88% URPF:12% BPF blend. The decline in starch content with increasing BPF content reflects the lower starch profile of bean pod flour compared to plantain.

Pasting Properties Results of the Samples

The pasting properties of the samples (100% URPF, 100% BPF), (96% URPF, 4% BPF), (92% URPF, 8% BPF) and (88% URPF, 12% BPF) are presented in Table 3. It shows that 100% BPF exhibited the highest values in all pasting parameters, except for setback viscosity and pasting

temperature. The highest setback viscosity (257.86 RVU) occurred in the 88% URPF:12% BPF blend, while the 96% URPF:4% BPF blend had the highest pasting temperature (85.0°C). Setback viscosity across all samples ranged from 78.02 to 257.86 RVU, exceeding the 17.21 ± 0.41 to 149.83 ± 1.77 RVU range reported by Adenugwa *et al.* (2017).

An increase in setback viscosity with higher BPF content suggests reduced retrogradation and improved stability of the gelatinized paste upon cooling, reducing the likelihood of staling. The high pasting characteristics observed in 100% BPF align with the findings of Irondi *et al.* (2021) and support its potential application in various food products. Among the composite blends, the 96% URPF:4% BPF sample had the lowest peak and trough viscosities, while the 92% URPF:8% BPF and 88% URPF:12% BPF blends showed significant variations, further highlighting the impact of BPF inclusion on pasting behavior which aligns with the findings of Ratnawati *et al.*, (2019).

Conclusion

The evaluation of unripe plantain flour supplemented with bean pod flour revealed significant improvements in both physicochemical and pasting properties of the composite flours. The increase in bulk density and amylopectin content, along with a reduction in amylose and swelling power, reflects structural modifications that favor improved

shelf life, water retention, and stability. The observed enhancement in pasting viscosity parameters, especially the increased setback viscosity in higher BPF blends, suggests reduced staling and improved paste quality. Notably, the 96% URPF:4% BPF blend demonstrated the best balance of starch, sugar, and viscosity for functional application, while the 100% BPF showed superior peak and final viscosity. These attributes highlight the industrial relevance of bean pod flour as a functional ingredient in flour-based products. This study provides valuable baseline data for future food formulation involving legume-enriched starchy flours and supports the inclusion of under-utilized crops like bean pod flour in composite flour development.

Conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical statement

The authors declare that no human or animal specimens were involved in this research.

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