



ORIGINAL RESEARCH ARTICLE

THE EFFECT OF FERMENTATION ON THE PROXIMATE COMPOSITION, PHYSICAL AND FUNCTIONAL PROPERTIES OF GUNA (*Citrullus vulgaris*) SEEDS
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ARTICLE INFORMATION

Received: 16th August 2025
Revised: 29th August 2025
Accepted: 29th August 2025

Keywords:

Guna seeds
Fermentation
Protein
Physical properties
Functional properties

ABSTRACT

Guna (Citrullus vulgaris) is a type of melon seed traditionally extracted from its gourds after fermentation. This study investigated the effect of fermentation on the physical, proximate, and functional properties of guna seeds. Fresh gourds were divided into three treatments namely; control (0 day), 7-day fermentation, and 14-day fermentation and shade-dried. Standard analytical methods were employed for the analyses. The results showed that fermentation had no significant effect ($P > 0.05$) on seed length (6.05–6.07 mm), width (3.43–3.46 mm), thickness (1.46–1.51 mm), sphericity (0.51–0.52), arithmetic diameter (3.65–3.68 mm), geometric diameter (3.12–3.17 mm), aspect ratio (56.69–57.10), or surface area (30.54–31.48 mm²). However, 1000-seed mass (31.78–34.60 g) and bulk density (1.64–1.82 g/cm³) were significantly affected ($P < 0.05$), decreasing with longer fermentation time. Proximate analysis revealed moisture (4.63–4.78%), ash (3.11–3.37%), protein (23.70–29.95%), fat (25.71–28.63%), fibre (18.54–19.21%), and carbohydrate (17.67–20.72%). Fermentation significantly ($P < 0.05$) increased moisture, protein, and ash contents, while reducing fat, fibre, and carbohydrate values. Functional properties improved with fermentation time, including higher water absorption capacity (73.97–82.00%), oil absorption capacity (54.12–66.67%), foam capacity (17.22–21.11%), and foam stability (12.78–13.89%), alongside reduced flour bulk density (1.15–1.18 g/cm³). In conclusion, 14-days fermentation time enhanced protein and ash content, improved hydration and oil-binding properties, and lowered bulk density, indicating potential for guna seed protein supplementation in food applications.

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1.0 Introduction

The increasing global demand for sustainable and nutrient-rich foods has intensified interest in plant seeds as valuable sources of dietary proteins and essential amino acids (Etzbach et al., 2024). Oilseeds, in particular, are prized for their dual role in human nutrition and industrial applications, owing to their high protein, lipid, phytochemical and bioactive compounds contents (Kotecka-Majchrzak et al., 2020; Enujiugha et al., 2022; Nissar et al., 2025). *Guna (Citrullus vulgaris)*, a melon species within the *Cucurbitaceae* family, is traditionally consumed in Northeastern Nigeria, where its seeds are valued for their abundant proteins, essential amino acids, unsaturated fatty acids, and health-promoting phytochemicals (Penuel et al., 2013; Ndahi et al., 2023; Ndahi et al., 2024).

Beyond nutritional composition, the physical and functional properties of plant seeds such as size, shape, bulk density, water and oil absorption capacities, and foaming behavior, are critical for their suitability in food formulation, processing, and equipment design (Sahin and Sumnu, 2006; Awuchi et al., 2019). These parameters influence post-harvest handling and guide the engineering of machinery for harvesting, drying, milling, and storage (Pradhan et al., 2010).

Processing methods profoundly affect the nutritional and functional profiles of oilseeds. Among them, fermentation is particularly notable for its ability to modify macromolecular structures through microbial activity, thereby enhancing nutrient bioavailability, improving functional performance, and reducing anti-nutritional factors (Sharma *et al.*, 2020). Studies have shown that fermentation can significantly alter proximate composition and functional characteristics of plant seeds (Ogundele and Taylor, 2016; Mashau *et al.*, 2025).

Previous reports of fermentation effect on the proximate and functional properties of *Cucurbit* seeds have focused on methods such as: extraction and shelling of *Cucumeropsis manaii* seeds prior to fermentation (Omowaye – Taiwo *et al.*, 2015), boiling extracted seeds of *L. siceraria* and water melon seeds prior to natural fermentation (Ileola *et al.*, 2019; Akele *et al.*, 2022) and fermenting uncoated *C. vulgaris* (water melon) seeds with selected microbial strain (Chawla *et al.*, 2020).

In traditional practice, *guna* seeds are extracted from the fermented mass of *guna* gourds prior to drying, followed by other processes including cooking and milling (NEAZDP, 1992). However, despite their nutritional richness and cultural importance, there is limited scientific evidence on how prior fermentation of the gourds influences the physical, proximate, and functional properties of *guna* seeds. This study aims to evaluate the effects of fermentation durations on the physical properties, proximate composition, and functional properties of *guna* seeds, thereby providing insights for optimizing their food and industrial application.

2. Materials and Methods

2.1 Materials

Guna gourds were purchased from a farm at Hawul LGA, Borno state, Nigeria. The chemicals used for proximate composition analysis were from NAFDAC Maiduguri Office. Additionally, all equipment and tools used for physical and functional properties measurements were obtained from the Food Analysis Laboratory of the Department of Food Science and Technology, Faculty of Engineering, University of Maiduguri at Maiduguri, Borno state, Nigeria.

2.2 Sample Fermentation Treatment

The obtained *guna* gourds were divided into three (3) portions. A portion of the fresh gourds obtained was cut, seeds extracted and washed as control samples (CT). The second and third portions of fresh gourds were cut into halves, poured into a doubled-layered polyethylene and kept inside a rubber basin for support for 7 and 14 days as ST and FT samples respectively. Thereafter, the seeds were extracted and washed for each sample as locally practiced as shown in Plate 1.

2.3 Sample Drying Process

The extracted seeds from each treatment were washed thoroughly to remove adhering pulp and strained. The strained seeds of each treatment were thinly spread on perforated trays and shade-dried for 7 days at room temperature, through which they were redistributed every 24 hours for uniform drying. Thereafter, the physical properties were measured and each sample was milled, packaged in a polythene bag and kept in a cool dry place for further analyses.



A. Three portions of *guna* fruits

B. Two portions for fermentation treatment



C. Extracted *guna* seeds

Plate 1: (A) Three portions of *guna* fruits (B) Two portions for fermentation treatment (C) Extracted *guna* seeds

2.4 Determination of Physical Properties of *Guna* Seeds

2.4.1 Determination of geometrical properties

2.4.1.1 Size

For each sample, fifty (50) random *guna* seeds were selected and their length (L), width (W) and thickness (T) were measured using a digital vernier 150mm (made in China) with an accuracy of 0.01 mm as the size of the sample seeds.

2.4.1.2 Shape

The shape was expressed as **Sphericity** and **Aspect ratio**. For the sphericity (ϕ), the dimensions obtained for the 50 selected seeds were used to calculate the sphericity using equation 1 as adopted from Mehra *et al.* (2023):

$$\text{Sphericity } (\phi) = \frac{(L \times W \times T)^{1/3}}{L} \quad 1$$

where L = length (L) in mm; W = width (mm); and T = thickness.

The **aspect ratio (Ra)** was calculated using equation 2 (Khan and Saini, 2016):

$$\text{Ra } (\%) = \frac{W \times 100}{L} \quad 2$$

2.4.1.3 Arithmetic and Geometric Mean Diameter

The arithmetic mean diameter, D_a and geometric mean diameter, D_g of the seeds were calculated by using equations 3 and 4 respectively (Mehra *et al.*, 2023):

$$D_a = \frac{(L+W+T)}{3} \quad 3$$

$$D_g = (L \times W \times T)^{1/3} \quad 4$$

2.4.1.4 Surface Area

The surface area of seed was calculated using equation 5 as calculated by Bande *et. al.* (2012):

$$S \text{ (mm}^2\text{)} = \pi D_g^2 \quad 5$$

where, D_g = geometrical mean diameter (mm).

2.4.2 Determination of gravimetric properties of the seeds

2.4.2.1 Thousand Seed Mass

For each sample, hundred (100) random *guna* seeds were selected and weighed with an electronic balance (precision of 0.01g). The mass recorded was multiplied by 10 (Khan and Saini 2016). The thousand seed mass was expressed in grams (g).

2.4.2.2 Bulk Density

An empty cylindrical container was weighed (w_0), filled with seeds and tapped to a specific volume (v). The filled container was weighed (w_1) again and the bulk density(ρ_b) was calculated based on equation 6 (Khan and Saini, 2016);

$$\rho_b \text{ (g/cm}^3\text{)} = \frac{w_1 - w_0}{v} \quad 6$$

where, w_0 = Initial mass of the container (g); w_1 = mass of seeds and container; v = volume of the filled sample (cm^3).

2.5 Proximate Composition Analyses

Proximate composition of the seed samples was determined according to the procedure of AOAC (2000). All the seed flour samples were analyzed for moisture, protein, fats, ash and fibre contents while the carbohydrate content was obtained by taking the difference when all other components of proximate were added and was subtracted from hundred (equation 7).

$$\% \text{ Carbohydrate} = 100 - (\% \text{ Moisture} + \% \text{ Fat} + \% \text{ Protein} + \% \text{ Ash} + \% \text{ Fibre}) \quad 7$$

2.6 Functional Properties Determination

2.6.1 Determination of foaming properties

The foaming properties was determined following the method described by Khan and Saini (2016). One (1g) gram of the *guna* seed flour was mixed with 5 ml (v_1) of distilled water, shaken and poured into a 50ml graduated cylinder. The volume of the foam formed was noted (v_2) and calculated as the foam capacity (FC) (%) while the foaming stability (FS) (%) was noted (v_3) after 60mins. The FC and FS were calculated using equation 8 and 9 respectively:

$$\% \text{ FC} = \frac{v_2 - v_1 \times 100}{v_1} \quad 8$$

$$\% \text{ FS (after 60mins)} = \frac{v_3 - v_1 \times 100}{v_1} \quad 9$$

2.6.2 Determination of water absorption capacity (WAC)

The WAC of the flour samples was determined by modifying the procedure used by Ogundele and Taylor (2016). Using a weighed test tube (w_1), 1g of each sample was poured into separate test tube and weighed (w_2) then 10ml of water added and the suspension stirred for 5 minutes. The content was centrifuged at 3,500 rpm for 30 minutes. The supernatant obtained was measured in a 10 ml measuring cylinder while the residue in the test tube was weighed (w_3). The water absorbed by the seed flour was calculated using equation 10:

$$\% \text{ WAC} = \frac{w_3 - w_2 \times 100}{w_2 - w_1}$$

10

2.6.3 Determination of Oil Absorption Capacity (OAC)

The OAC of the flour samples was determined by modifying the procedure used by Ogundele and Taylor (2016). Vegetable oil (Refined groundnut oil) obtained from Baga road market, Maiduguri, was used. Using a weighed test tube (w_1), 1g of each sample was poured into separate test tube and weighed (w_2) then 10ml of oil added and the suspension stirred for 5 minutes. The content was centrifuged at 3,500 rpm for 30 minutes. The supernatant obtained was measured in a 10 ml measuring cylinder while the residue in the test tube was weighed (w_3). The oil absorbed by the seed flour was calculated using equation 11:

$$\% \text{ OAC} = \frac{w_3 - w_2 \times 100}{w_2 - w_1}$$

11

2.6.4 Determination of Bulk Density (BD)

The BD was determined using the method of AOAC (2010). Twenty grammes (20g) of each *guna* seed flour samples was poured into an initially weighed 25cm³ container (w_1) and was gently tapped. The new weight (w_2) was noted and the observed volume (v) was recorded. The bulk density of the samples was calculated using equation 12:

$$\text{BD}(\text{g/cm}^3) = \frac{(w_2 - w_1)}{\text{volume of sample}}$$

12

2.7 Statistical Analysis

The data generated were analyzed using Analysis of Variance and means separated using Duncan Multiple Range Test using Statistical Package for Social Science 23 version (SPSS) at 0.05 level of significance and chart trend was analyzed using MS Excel 2019.

3. Results and Discussion

3.1 The Effect of Fermentation on the Physical Properties of *Guna* Seeds

Table I presents the effect of fermentation on the physical properties of *guna* seeds. Fermentation had no significant ($P > 0.05$) effect on seed length, width, thickness, sphericity, arithmetic diameter, geometric diameter, aspect ratio or surface area. However, 1000-seed mass and bulk density (BD) differed significantly ($P < 0.05$) across treatments.

The measured *guna* seeds' length (6.05–6.07 mm), width (3.43–3.46 mm) and thickness (1.46–1.51 mm) were slightly higher in fermented seeds compared to the control. The slight increase in *guna* seeds' dimensions might be due to the modification of structural molecules that led to interaction with moisture and air causing elongation and swelling. These values align with ranges reported for melon seeds by Mansouri *et al.* (2017) and for flaxseeds by Khan and Saini (2016), but are lower than those observed in *Citrullus lanatus* and *Cucumis melo* species (Mehra *et al.*, 2023).

The observed variation in melon seeds sizes could be due to variety and geographical difference. Similarly, arithmetic (3.65–3.68 mm) and geometric (3.12–3.17 mm) diameters were highest in fermented seeds, consistent with the dimensional increase in length, width and thickness following fermentation (Table I). Such size parameters are crucial in the design of harvesting, cleaning, and milling equipment (Pradhan *et al.*, 2010).

Sphericity (0.51–0.52), aspect ratio (56.69–57.10%), and surface area (30.54–31.48 mm²) were marginally higher in fermented *guna* seeds (Table I). These values are comparable to those reported for the sphericity of *C. lanatus* (0.52) (Mehra *et al.*, 2023) and fall within the surface area range of melon seeds (18.46 – 39.80mm²) (Mansouri *et al.*, 2017). According to Garnayak *et al.* (2008), sphericity below 0.70 indicates a non-spherical shape and a tendency for *guna* seeds (0.51 – 0.52) to slide during handling, consistent with the flow behavior expected in both fermented and unfermented *guna* seeds.

The 1000-seed mass ranged from 31.78 to 34.60 g, with the control showing the highest value and 14-day fermented *guna* seeds the lowest. The reduction in seed mass could be attributed to the degradation of carbohydrate by fermenting microbes (Ogodo *et al.*, 2017). These results are within the range 27.69 - 40.28g for shelled and unshelled *guna* seeds reported by Abubakar (2004) but lower than *guna* seeds (41g) of Aviara *et al.* (1999), likely due to location difference. Bulk density ranged from 1.64 to 1.82 g/cm³, decreasing significantly with fermentation time. The reduction in bulk density of *guna* seeds likely reflects lower seed mass coupled with slight dimensional expansion. Bulk density and 1000-seed mass are important design parameters for storage, aeration and transport systems, as they influence load calculations and hopper design (Sahin and Sumnu, 2006).

Table I: The Effect of Fermentation on the Physical Properties of *Guna* Seeds¹

Physical Properties	Samples Code ²		
	CT	ST	FT
Length (mm)	6.05 ^a ± 0.14	6.06 ^a ± 0.17	6.07 ^a ± 0.17
Width (mm)	3.43 ^a ± 0.05	3.46 ^a ± 0.06	3.46 ^a ± 0.07
Thickness (mm)	1.46 ^a ± 0.06	1.51 ^a ± 0.04	1.51 ^a ± 0.05
Arithmetic Diameter (mm)	3.65 ^a ± 0.04	3.67 ^a ± 0.07	3.68 ^a ± 0.07
Geometric Diameter (mm)	3.12 ^a ± 0.04	3.16 ^a ± 0.05	3.17 ^a ± 0.06
Sphericity	0.51 ^a ± 0.01	0.52 ^a ± 0.01	0.52 ^a ± 0.01
Aspect Ratio (%)	56.69 ^a ± 1.47	57.10 ^a ± 1.45	57.07 ^a ± 2.20
Surface Area (mm ²)	30.54 ^a ± 0.46	31.36 ^a ± 1.07	31.48 ^a ± 1.17
1000 seeds mass (g)	34.60 ^a ± 0.90	33.20 ^a ± 1.10	31.78 ^b ± 1.09
Bulk Density (g/cm ³)	1.82 ^a ± 0.01	1.77 ^b ± 0.01	1.64 ^c ± 0.01

¹Values are means of triplicate analysis and their standard deviation. In any row, means bearing similar superscript are not significantly different ($P > 0.05$).

²CT = Dried Control Sample; ST = Dried 7-days Fermentation Period Sample; FT = Dried 14-days Fermentation Period Sample

In this study, fermentation induced slight physical changes in *guna* seeds; particularly reductions in bulk density and seed mass, that could influence downstream handling, processing efficiency and equipment calibration in food and feed industries.

3.2 The Effect of Fermentation on the Proximate Composition of *Guna* Seeds

Fermentation significantly ($P < 0.05$) influenced the proximate composition of *guna* seeds, affecting moisture, ash, protein, fat, fibre, and carbohydrate contents (Fig. 1).

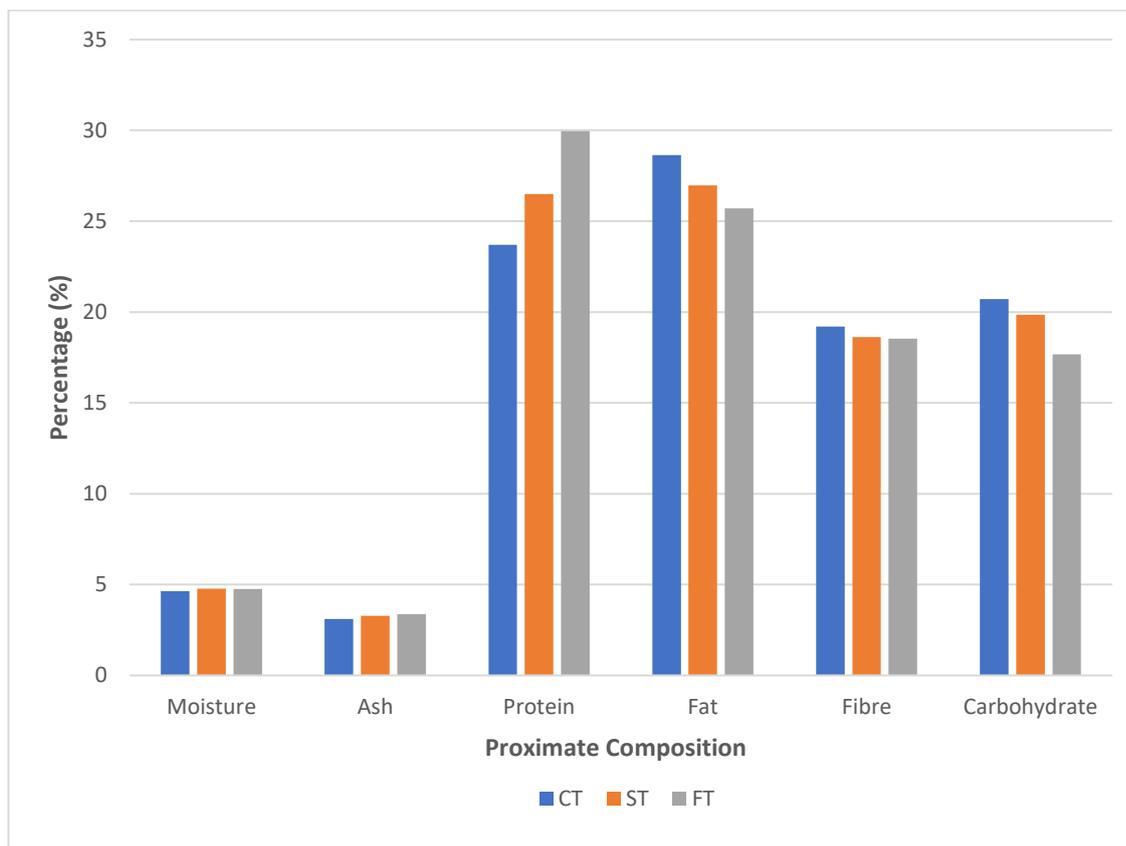


Figure 1: The Effect of Fermentation on the Proximate Composition of *Guna* Seeds

CT = Dried Control Sample; ST = Dried 7-days Fermentation Period Sample; FT = Dried 14-days Fermentation Period Sample

Moisture content ranged from 4.63–4.78%, with the highest value observed in 7-days fermented *guna* seeds (ST) and the lowest in the control (CT). Ash content increased progressively with fermentation, from 3.11% (CT) to 3.37% in 14-days fermented seeds (FT) (Fig. 1). These ash values align with the range reported for watermelon seeds (1.70–4.93%) (Mathew *et al.*, 2025) but are higher than those for some melon seeds (2.10–2.20%) (Saeed *et al.*, 2023). Similar trends have been reported in fermented watermelon seeds, where prolonged fermentation elevated both moisture and ash contents (Ejinkeonye *et al.*, 2018). The slight increase in moisture content could be due to the partial hydration of exposed hydrophilic site caused by hydrolysis of macromolecules (Mashau *et al.*, 2025) whereas the observed increase in ash content may be attributed to the relative concentration as microbes utilized and degrade organic matter of *guna* seeds during fermentation (Ogodo *et al.*, 2017).

Protein content of *guna* seeds increased significantly with fermentation duration from 23.70% in CT to 29.95% in FT (Fig. 1). These values are comparable to melon seeds (26.36–39.96%) (Enujiugha *et al.*, 2022) and corroborate earlier findings that fermentation enhances melon seed protein content (Akele *et al.*, 2022; Ileola *et al.*, 2019). The increase is likely due to microbial synthesis of proteins or concentration effects from the degradation of other macronutrients during fermentation (Sharma *et al.*, 2020).

The fat content of *guna* seeds decreased significantly with fermentation time from 28.63% in CT to 25.71% in FT and was within the typical range of fat content for oilseeds (24.00–56.56%) (Kotecka-Majchrzak *et al.*, 2020). This decline of fat observed in this study was consistent with reports for fermented *C. lanatus* seeds (Jackson *et al.*, 2013) and has been linked to microbial lipase activity breaking down triglycerides to free fatty acids and glycerol during fermentation (Mutshinyani *et al.*, 2020).

Fibre and carbohydrate contents also decreased significantly, from 19.21% to 18.54% and from 20.72% to 17.67%, respectively, over the fermentation period. The carbohydrate values are consistent with the 8–20% range reported for watermelon seeds (Nissar *et al.*, 2025). Similar reductions have been documented in fermented *C. lanatus* (Akele *et al.*, 2022) and are likely due to microbial utilization of complex carbohydrates and fibre as energy sources, leading to mass loss and altered *guna* seeds composition (Fig. 1) as observed by Ogodo *et al.*, (2017).

Remarkably, fermentation of *guna* seeds promoted protein and ash enrichment while reducing fat, fibre, and carbohydrate contents. These compositional changes reflect both microbial metabolism and biochemical transformations, enhancing the nutritional quality of *guna* seeds for potential food applications.

3.3 The Effect of Fermentation on the Functional Properties of *Guna* Seed Flour

Fermentation significantly ($P < 0.05$) influenced the bulk density (BD), water absorption capacity (WAC), oil absorption capacity (OAC) and foaming capacity (FC) of *guna* seed flour while foaming stability (FS) remained unaffected (Table 2).

Table 2 The Effect of Fermentation on the Functional Properties of *Guna* Seeds¹

Physical Properties	Samples Code ²		
	CT	ST	FT
Bulk Density (g/cm³)	1.18 ^a ± 0.01	1.17 ^a ± 0.01	1.15 ^b ± 0.01
Water Absorption Capacity (%)	73.97 ^b ± 1.18	81.00 ^a ± 1.00	82.00 ^a ± 2.00
Oil Absorption Capacity (%)	54.12 ^c ± 0.34	58.30 ^b ± 0.61	66.67 ^a ± 0.45
Foaming Capacity (%)	17.22 ^b ± 0.96	19.44 ^a ± 1.93	21.11 ^a ± 1.92
Foaming Stability after 60 min (%)	12.78 ^a ± 0.96	13.89 ^a ± 0.50	13.33 ^a ± 0.96

¹Values are means of triplicate analysis and their standard deviation. In any row, means bearing similar superscript are not significantly different ($P > 0.05$).

²CT = Dried Control Sample; ST = Dried 7-days Fermentation Period Sample; FT = Dried 14-days Fermentation Period Sample

The BD of *guna* seeds declined slightly from 1.18 g/cm³ in the control to 1.15 g/cm³ after 14-days fermentation. This reduction is likely due to hydrolysis and structural modification of macromolecules during fermentation, leading to a lower seed mass (Table 1). Although the BD values in this study were higher than those previously reported for *C. vulgaris* (0.39–0.72 g/cm³), the decreasing trend aligns with earlier work (Chawla *et al.*, 2020; Mashau *et al.*, 2025). Lower BD is advantageous in complementary food formulations, as it increases nutrient density per unit volume and reduces bulkiness during consumption (Suresh and Samsher, 2013).

From Table 2, the WAC of *guna* seeds increased markedly with fermentation time, reaching 82.00% in FT and 81.00% in ST compared with 73.97% in CT. This aligns with earlier findings that fermentation enhances the WAC of melon seed flours (Omowaye-Taiwo *et al.*, 2015; Ogundele and Taylor, 2016). The increase in WAC of fermented *guna* seeds flour likely results from protein modification and carbohydrate degradation, which expose additional hydrophilic sites (Mashau *et al.*, 2025). High WAC is desirable in products such as bakery goods, dough, and sausages, where water binding contributes to desirable texture and consistency (Awuchi *et al.*, 2019).

Similarly, OAC of *guna* seed flour increased from 54.12% (control) to 66.67% (14-days fermentation). The increase with fermentation time is supported by reports on *C. vulgaris* (Ogundele and Taylor, 2016; Chawla *et al.*, 2020), although contrasting trends have been observed in *L. siceraria* and Bambara groundnut flours, where OAC decreased (Ogundele and Taylor, 2016; Mashau *et al.*, 2025). The rise in OAC for fermented *guna* seeds might be due to increased protein content and unfolding of protein structures and its hydrolysis, which exposes hydrophobic sites (Sareen *et al.*, 2023). Enhanced OAC benefits applications such as pastries and sausages by improving flavor retention and mouthfeel (Zhang *et al.*, 2023).

Fermentation also increased foaming capacity (FC) of *guna* seeds with a maximum of 21.11% compared to 17.22% in the control, whereas foaming stability (FS) values of the seeds were relatively close (13.89%, 13.33% and 12.78% for ST, FT and CT, respectively) (Table 2). These results agreed with the observations for *L. siceraria*, where fermentation improved FC (Ogundele and Taylor, 2016), but contrast with studies on melon

seeds and legumes reporting reduced foaming ability (Adebowale and Maliki, 2011; Omowaye-Taiwo et al., 2015; Chawla et al., 2020). Protein hydrolysis was noted to reduce molecular weight of proteins thereby increasing surface activity whereas lower molecular weight might weaken the interactions hence reduce stability (Eckert et al., 2019). Thus, the fermentation effect on *gona* seeds likely reflects improved protein surface activity, enabling better air incorporation while the FS showed minimal change, possibly due to partial protein denaturation reducing the stability of entrapped air bubbles.

4. Conclusion

Fermentation of *gona* seeds for up to 14 days resulted in seed expansion, reduced mass, and significant compositional and functional changes. Prolonged fermentation increased ash and protein contents, water and oil absorption capacities and foaming capacity while reducing fat, fibre, carbohydrate contents, bulk density, and foaming stability. These improvements in protein quality and techno-functional properties highlight the potential of 14-day fermented *gona* seed flour as a value-added ingredient in diverse food formulations. To explore *gona* seeds nutrients applicability in food formulation, further research should be carried out on the effects of various bioprocessing methods on amino acids profile, micronutrients and functional properties of *gona* seeds flour.

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