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Effect of Fermentation Time on the Functional Properties, Proximate and Anti-Nutrients Composition of Groundnut Hull

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ABSTRACT **Original Research Article**

This study determined the functional, proximate and anti-nutrient content of fermented groundnut hull flour. 1kg of groundnut hull was sorted, washed, and then soaked in a bucket of 1 litre of water for one hour. A measured weight of 20g of tempeh yeast (Rhizopus oligosporus) was added, thoroughly mixed manually and divided into 7 equal portions (as per experimental design). They were put in plastic buckets, closed at a temperature of 29±2°C and allowed to ferment for 0, 8, 16, 24, 32, 40 and 48 h, respectively. The fermented hulls were dried for 12 h at 60°C, cooled, milled and stored in a high-density polyethylene. The results showed that proximate composition (%) of the samples ranged: moisture content 1.50 - 3.33, ash 2.00 - 4.50, fibre 6.80 - 12.00, fat 0.40 - 3.20, protein 0.50 - 1.24 and carbohydrate 78.53 - 86.00. The moisture content of the sample was significantly ($p < 0.05$) influenced by fermentation time and the fermented groundnut sample contained higher ash in comparison to the control sample (unfermented). Fermented samples had significantly ($p<0.05$) higher fiber (8.80 - 12.00%) and protein (0.79 - 1.24%). Functional properties of the samples showed that the water absorption capacity ranged from 1.00 - 1.50 mL/g, oil absorption capacity 0.47 - 2.33 mL/g and bulk density 0.56 - 0.63 g/mL, with high significance differences ($p < 0.05$) being observed when compared to the unfermented sample. All the samples were high in phytochemicals, tannin 579.94 - 586.87 mg/g and saponin 168.00 - 264.00 mg/g with high significance differences ($p < 0.05$). The high dietary flavonoid obtained in this study can help in reducing the risk of chronic conditions like cardiovascular disease, cancer, and neurodegenerative diseases. These improvements are essential for various food applications, making it a suitable ingredient for functional foods.

Keywords: Groundnut Hull, Arachis Hypogaea, Fermentation, Antinutrient.

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Introduction

Groundnut (Arachis hypogaea) is a specie in the legume or bean family (Fabaceae) and the most important legume in Africa (Olanipekun et al., 2019). It is an important crop of Brazilian origin, though now cultivated in tropical and temperate climates. Groundnut plant is a low growing, annual legume with central upright stem, they are grown all over Nigeria with major production in northern part of the country (Chikwendu et al., 2017). According to Shahidi (2018), groundnut provides an inexpensive source of high-quality dietary protein and oil. The vast food preparations incorporating groundnut to improve the protein level have helped in no small way in reducing malnutrition in developing Countries. Groundnut provides considerable amounts of mineral elements to supplement the dietary requirements of humans and farm animals. Gernah et al. (2019), reported that groundnuts are one of the most widely cultivated and consumed legumes globally, particularly in Africa and Asia.

Groundnut hull, also known as peanut shell, is the outer covering of the groundnut seed (Agarwal *et al*., 2018). It is a by-product of groundnut processing and makes up about 20–30% of the total pod weight. It is mainly composed of cellulose, lignin, and small amounts of protein and fat (Chikwendu *et al*., 2018). Groundnut hulls, a by-product of groundnut processing, account for approximately 20-25% of the total weight of the groundnut. Traditionally, groundnut hulls have been used as animal feed, compost, or discarded as waste. However, recent studies have shown that groundnut hulls are rich in nutrients, including dietary fiber, protein, and essential minerals like potassium, magnesium, and phosphorus (Halm, *et al*., 2019). The hulls also contain various phytochemicals, such as phenolic acids, flavonoids, and saponins, which have been linked to several health benefits, including antioxidant, anti-inflammatory, and antimicrobial activities (Ofori and Kyeibaffour, 2021). Polyphenols may serve as a natural preservative in foods, cell wall components, on the other hand, may ensure the controlled release of polyphenols in the human digestive system by protecting them (Bera et al., 2018). Recently, there has been a growing interest in lignin due to its functional and bioactive properties which could make it a potential food ingredient and food packaging agent (Udensi and Okoronkwo, 2023).

Despite the potential nutritional benefits of groundnut hulls, their utilization as a food ingredient is limited due to several factors. One major constraint is the high content of anti-nutrients, such as phytic acid, oxalic acid, and tannins, which can inhibit nutrient absorption and utilization. Additionally, groundnut hulls have a coarse texture and a bitter taste, making them unpalatable for human consumption. Groundnut hulls are often considered agricultural waste and contain various bioactive compounds, including antioxidants. While these antioxidants can be beneficial, there are situations where reducing their concentration may be desired for certain applications (e.g., animal feed formulation, food processing) (Nigam, 2015). Heating the hulls can reduce moisture and may also lead to the degradation of some antioxidants, roasting groundnut hulls at high temperatures can significantly decrease the levels of antioxidants, as exposure to heat can denature these compounds and the use of solvents such as ethanol, methanol, or aqueous solutions can selectively extract and reduce antioxidant compounds from groundnut hulls (Alaba et al., 2015). Also, fermenting groundnut hulls using specific microorganisms can result in the breakdown of some antinutrients and antioxidant compounds. The microbial activity can lead to the conversion of antioxidants into other metabolites (Katoch, 2021).

Fermented foods have historically been developed in every country in the world because of their properties that potentially promote health (Tortora et al., 2019). Food fermentation typically includes the conversion, sometimes under anaerobic conditions, of carbohydrates into carbon dioxide, organic acids or alcohol, utilizing bacteria or yeast. Several traditional fermented foods are produced in different countries of Africa (Molin, 2021). In order to improve the nutritional content and reduce the anti-nutritional contents of groundnut (Arachis hypogea) some common processing methods have been used such as soaking in water, heating in water, alkaline or acid solutions at elevated temperatures, germination, roasting, dehulling and fermentation (Esenwah and Ikenebomeh, 2018). Fermentation has proven over the years to be one of the most effective processing methods to practically limit anti-nutritional factors of legumes (Oluoba, 2020).

The method of reducing antinutrients and antioxidants in groundnut hull involves trade-offs regarding the preservation of other nutritional qualities and the feasibility of the method. The optimal processing method will depend on the desired end-use of the groundnut hulls and the specific antioxidants targeted for reduction. The knowledge of the optimum time combination to achieve the fermentation of the groundnut hull will give a clear profile about how to eliminate anti-nutrients and increase the utilization of this under-utilized crop.

Materials and Methods

Source of Materials

Groundnut (*Arachis hypogaea*) hulls were hygienically prepared and collected from a roasted groundnut vendor in Eke-Awka market, Anambra State, Nigeria. It was conveyed to the Food Science and Technology Department Laboratory, Nnamdi Azikiwe University, Awka, Anambra State for analyses.

Experimental Design

The Design method used for this research was a Completely Randomized Design (CRD).

Table 1: Experimental Design

S/N	Sample code	Fermentation time (h)
1	KAA	0
2	KAB	8
3	KAC	16
4	KAD	24
5	KAE	32
6	KAF	40
7	KAG	48

Production of Fermented Groundnut Hulls

For the production of fermented groundnut hull, the method described by Ezegbe *et al.* (2024) with little modification was adopted. 1kg of groundnut hull was sorted, washed, and then soaked in a bucket of 1 litre of water for one hour. A measured weight of 20g of tempeh yeast (*Rhizopus oligosporus*) was added, thoroughly mixed manually, then blended with 28.5 ml of vinegar to prevent the development of other undesirable bacteria and to adjust the pH to allow *Rhizopus oligosporus* to flourish easily. The content was divided into 7 equal portions, put in plastic buckets, and then closed to maintain a temperature of 29±2°C. The samples were then allowed to ferment for 0, 8, 16, 24, 32, 40 and 48 h, respectively. The fermented hulls were dried for twelve (12) hours at 60℃. They were then cooled, milled, sifted to trap any lumps, cooled and stored in a high-density polyethylene.

Determination of the Proximate Composition of the Fermented Groundnut Hull

The proximate composition (moisture, ash, crude fibre, crude protein, fat, and carbohydrate) of the groundnut hull samples was determined using standard procedures as described by Ezegbe *et al*. (2024). Moisture content was measured by oven drying, where samples were placed in a hot air oven (Gallenkamp 300 Plus Series, Widnes, Cheshire, U.K.) at 105 °C until a constant weight was achieved. Ash content was obtained by incinerating the samples in a muffle furnace (Hasthas, Servell Engineers, Chennai, India) at 600 °C for 5 h. Crude fibre was determined by sequential acid–alkali digestion: a defatted sample was first boiled in dilute sulfuric acid, filtered and washed, then digested in dilute sodium hydroxide solution. The residue was dried (105°C), weighed, incinerated (600°C) in a furnace, and reweighed; the loss in weight after ashing was recorded as crude fibre. Crude protein content was analyzed using the micro-Kjeldahl method with a nitrogen-to-protein conversion factor of 6.25. Fat was extracted using the Soxhlet method with petroleum ether (Loba Chemie, Mumbai, India) as the solvent. Carbohydrate content was then calculated by difference, expressed as 100 minus the combined values of moisture, ash, crude fibre, fat, and protein.

Determination of the Functional Properties of Fermented Groundnut Hull Flour

Water Absorption Capacity

Water absorption of flour was measured according to the centrifugation method described by Gernah *et al.* (2019). 1g of sample was weighed in a centrifuge tube and 10 ml of water was added and mixed thoroughly. The dispersion was allowed to stand for 30 min, followed by centrifugation for 15 mins at 3000 rpm. The sample was re-weighed; the amount of water retained in the sample was recorded as weight gained.

The water absorption capacity (WAC) was calculated as:

$$\text{WAC} = \frac{Volume\ of\ water\ absorbed}{Weight\ of\ sample\ used} \times 100 \qquad (1)$$

Oil Absorption Capacity

Oil absorption capacity was determined using the method described by Gernah *et al.* (2019). 1g of sample was mixed with 6ml of com oil in pre-weighed centrifuge tubes. The content was stirred for 1 minute with a thin brass wire to dispense the sample evenly in the oil. After a holding period of 30 minutes; the tubes were centrifuged for 25 minutes to drain the oil prior to reweighing. Oil absorption capacity (OAC) was weighed as weight of oil bound per weight of the sample on a dry basis.

The oil adsorption capacity (OAC) was calculated as:

$$OAC = \frac{Density\ of\ oil \times Volume\ absorbed}{Weight\ of\ sample} \times 100 \qquad (2)$$

Bulk Density

Bulk density (BD) was determined by the method described by Haug *et al.* (2020). 10 mL capacity graduated measuring cylinder was filled gently with each sample. The bottom of the cylinder was gently tapped on a laboratory bench several times until there was no further diminution of the sample level.

The Bulk density was calculated as:

$$\text{BD} = \frac{Weight\ of\ sample\ gml\ or\ g/ml}{Volume\ of\ sample\ after\ tapping\ (ml)} \qquad (3)$$

Determination of the Phytochemical Content of Groundnut Hull Flour

Determination of Tannins by Titration

The Tanin will be determined using the AOAC (2015) method. 10 grams of the sample was weighed in a conical flask and 100 mL of n- hexane was added and covered for 24 h. The sample was filtered and be allowed to stand for 15 minutes for the solvent to evaporate. It was re-extracted by soaking 100 mls of 1% acetic acid in ethanol for 4 hours. After which the sample was filtered and the filtrate was collected. 25 mL of ammonium hydroxide was added to the filtrate to precipitate. The remaining volume was measured and 5 mL was taken and 20 mL of ethanol was added to it. To be titrated with 0.1 M NaOH using 1mL of phenolphthalein as an indicator until a pink end point is reached.

The formula to calculate Tannin content is in percentage ($C_1V_1 = C_2V_2$) molarity (4)

Determination of Saponins

The Saponin was determined using the AOAC (2015) method. 10 grams of the ground sample was weighed into a thimble and transferred into the Soxhlet extractor chamber fitted with a condenser and flask. 250 ML of methanol was poured into the flask. Extraction continued for 1h, the saponin was exhaustively extracted by heating the flask on a heating mantle. The thimble and its content were removed and the methanol recovered leaving the saponin and little quantity of methanol in the flask. It was taken to an oven and kept at slanting position at a temperature of 70ºC to evaporate the residual methanol. The flask and content were weighed to know the difference between the flask plus saponin and flask alone.

The formula to calculate saponin is given as:

$$\% \boldsymbol{Saponin} = \frac{\boldsymbol{Total\ mass\ of\ saponin\ (mg)}}{\boldsymbol{Mass\ of\ sample\ (mg)}} \times \mathbf{100} \quad (5)$$

Determination of Flavonoids

The flavonoid was determined using the AOAC (2015) method. 10 grams of the plant sample was put in a beaker with 100 ML of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper. The filtrate was transferred into a crucible and evaporated to dryness over a water bath and was weighed to a constant weight.

Formula for calculating flavonoid:

$$\% \boldsymbol{Flavonoids} = \frac{\textbf{(Weight of crucible + residue)} - \textbf{(Weight of crucible)}}{\boldsymbol{Weight\ of\ sample\ analyzed}} \quad (6)$$

Statistical Analysis

The results were expressed as mean values and standard deviation of three (3) determinations. Data obtained from physicochemical, functional and phytochemical analyses were analyzed using a one-way Analysis of Variance (ANOVA) using Statistical Package for Social Science (SPSS) version 25.0 software to test the level of significance at 5% probability ($p<0.05$). Duncan's Multiple Range Test was used to separate the means.

Results and Discussion

Proximate Composition of Fermented Groundnut Hull Flour

Table 2 summarizes the result of the proximate composition of fermented groundnut hull flour. The result showed that moisture content ranged from 1.50 - 3.33, ash 2.00 - 4.50, fibre 6.80 - 12.00, fat 0.40 - 3.20, protein 0.50 - 1.24 and carbohydrate 78.53 - 86.00%. The moisture content of the sample was significantly ($p < 0.05$) influenced by fermentation time, ranging from 1.50 to 3.33%. Sample KAG (3.33%) fermented for 48 h had the highest moisture, while the unfermented sample KAA (1.00%) had the lowest. The moisture content obtained in this study was lower than 4.13 - 7.00% reported by Udensi and Okoronkwo (2023). The decrease could be attributed to low water absorption during the fermentation period and lack of substrate degradation by fermenting microbes, which may release moisture as one of their end products (Backer *et al.*, 2018). The low moisture content of unfermented sample (KAA) groundnut flour indicated that the flour could have a stable shelf life as Chikwendu *et al.* (2018) revealed that food samples with less moisture may have the added benefit of extending the products' shelf life.

The ash content of unfermented groundnut hull in this study ranged from 2.00% and 4.50%; the ash content was similar to the 2.03% reported by Molin (2021). The fermented groundnut sample contained higher ash in comparison to the control sample KAA (unfermented), although sample KAB and KAC fermented for 8 and 16 was not significantly different ($p < 0.05$) from the control sample KAA (unfermented) yet other fermented samples with higher fermentation time recorded higher ash content that was significantly different ($p < 0.05$) from the unfermented sample (KAA). The significant increase in ash content could be due to soaking of samples that resulted in retaining of minerals (Rao *et al.*, 2020). High ash content in fermented groundnut hull flour provides valuable minerals contributing to its potential role in managing metabolic diseases such as diabetes (Johnson *et al.*, 2019). Fermentation can increase overall mineral content and reduce anti-nutrients, enhancing the flour's nutritional profile and making the minerals more bioavailable.

The fibre content of the fermented groundnut hull flour ranged from 6.80 - 12.00%, with sample KAA (unfermented) having the lowest fibre (6.80%) while sample KAG fermented for 48 h had the highest fiber. Significant differences ($p < 0.05$) were observed in all the samples. Fermentation increased the fibre content of the groundnut hull from 8.80 - 12.00%.

Table 2: Proximate Composition (%) of Fermented Groundnut Hull Flour

S/No	Sample	Moisture	Ash	Fibre	Fat	Protein	Carbohydrate
1.	KAA	$1.50^{e}\pm0.00$	$2.00^{d}\pm0.00$	$6.80^{g}\pm0.10$	$3.20^{a}\pm0.00$	$0.50^{d}\pm0.10$	$86.00^{a}\pm0.14$
2.	KAB	$1.67^{e}\pm0.29$	$2.00^{d}\pm0.00$	$8.80^{f}\pm0.20$	$3.10^{a}\pm0.10$	$0.79^{c}\pm0.00$	$83.64^{b}\pm0.31$
3.	KAC	$2.17^{d}\pm0.29$	$2.17^{d}\pm0.29$	$9.60^{e}\pm0.00$	$2.10^{b}\pm0.10$	$0.82^{c}\pm0.02$	$83.15^{b}\pm0.56$
4.	KAD	$2.50^{cd}\pm0.00$	$3.17^{c}\pm0.29$	$10.00^{d}\pm0.20$	$1.60^{c}\pm0.10$	$0.88^{c}\pm0.00$	$81.85^{c}\pm0.31$
5.	KAE	$2.83^{bc}\pm0.29$	$4.00^{b}\pm0.09$	$10.40^{c}\pm0.20$	$1.20^{d}\pm0.00$	$0.91^{c}\pm0.02$	$80.66^{d}\pm0.35$
6.	KAF	$3.00^{ab}\pm0.00$	$4.33^{a}\pm0.29$	$11.60^{b}\pm0.10$	$1.00^{e}\pm0.00$	$1.05^{b}\pm0.08$	$79.02^{e}\pm0.36$
7.	KAG	$3.33^{a}\pm0.29$	$4.50^{a}\pm0.00$	$12.00^{a}\pm0.00$	$0.40^{f}\pm0.10$	$1.24^{a}\pm0.13$	$78.53^{e}\pm0.43$

Values are mean scores ± Standard deviation of three (3) replicates data in the same column bearing different superscript differ significantly ($p < 0.05$).

KEYS:
KAA (Control): (Not Fermented)
KAB: (Fermented for 8 h)
KAC: (Fermented for 16 h)
KAD: (Fermented for 24 h)
KAE: (Fermented for 32 h)
KAF: (Fermented for 40 h)
KAG: (Fermented for 48 h)

This result is in agreement with observations made by Costa *et al.* (2021). The increase in fibre contents in sample KAB and KAC fermented for 8 and 16 h respectively is an indication of softening of fibrous tissues and increased digestibility during fermentation due to activities of microorganisms which are known for the bioconversion of carbohydrates and lignocellulose into protein (Cui and Liu 2021). The most significant increase in crude fiber was achieved because the seed cover was not removed, because crude fiber is found mostly in the outer seed testa, its amount is dependent on the seed coat's thickness (Tina, 2021). The slight decrease in fiber content of sample KAB (8.80%) during fermentation could be attributed to the partial solubilization of cellulose and hemicellulosic material by microbial enzymes (Katoch, 2021). The high fiber content from the fermented hulls also offers benefits like improved digestion and reduced risk of cardiovascular diseases (Hall *et al.*, 2019).

The fat content decreased with increased fermentation period. The samples ranged from 0.40 - 3.20%. The unfermented sample (KAA) had the highest fat (3.20%) while sample KAG fermented for 48 h contained the lowest fat (1.00%). Significant ($p < 0.05$) differences were observed in all the samples except for sample KAB fermented for 8 h that was not significantly ($p < 0.05$) differences from the unfermented sample (KAA). The range was lower than 5.03 - 8.02% reported by Firdaus *et al.* (2021). The reduction in crude fat content of the samples with increased fermentation could be attributable to fat leaching into the processing water, oxidation, and hydrolysis during the fermentation period (Alaba *et al.*, 2015). The decrease in crude fat concentration in the fermented samples could be owing to an increase in lipolytic enzyme activities, which hydrolyze fat into free fatty acids and glycerol during fermentation, preventing the development of protein-lipid or carbohydrate-lipid connections that facilitate oil extraction (Agarwal *et al.*, 2018). The present finding was in agreement with other works reported by Oghbaei and Prakash (2020) in different legumes and pulses.

The protein content of the groundnut hull using the different fermentation times showed significant ($p < 0.05$) differences. The protein content of the fermented groundnut hull flour sample ranged from 0.50 - 1.24%, with sample KAG fermented for 48 h having the highest protein while the unfermented sample KAA (control) had the lowest protein. The low protein observed in the unfermented sample could be because protein is stored in the cotyledons, and its proportion increases when the cover, which contains a minimal quantity of protein, is removed but it increases during fermentation as reported by Madukwe *et al.* (2019). Proteolysis, which produces volatile ammonia as a byproduct of such a process in foods high in protein, may be responsible for the increase in protein concentration caused by fermentation (Udensi and Okoronkwo, 2023). Similar results were reported by Rao *et al.* (2020) for hull and their effect in fermentation.

Carbohydrates of the groundnut hull flour sample ranged from 78.53 - 86.00%, with sample KAG fermented for 48 h having the highest value while unfermented sample KAA had the lowest value of carbohydrate. There was no significant variance ($p > 0.05$) between some fermented samples (KAB and KAC) and (KAF and KAG). The rise in carbohydrate content may be due a significant reduction in other nutrients as reported by Gernah *et al.* (2019). Fermentation improves overall nutritional value and digestibility by reducing antinutrients and increasing the carbohydrate content of the flour which support digestive health and act as antioxidants, this also leads to better nutrient absorption and potential health benefits like improved blood sugar regulation, reduced cholesterol, and a lower risk of chronic diseases (Katoch, 2021).

Functional Properties of Fermented Groundnut Hull Flour

The result of the functional properties of fermented groundnut hull flour are shown in Table 3. Findings from the statistically analyzed data showed that the water absorption capacity ranged from 1.00 - 1.50 mL/g, oil absorption capacity 0.47 - 2.33 mL/g and bulk density 0.56 - 0.63 g/mL. Water absorption capacity represents the ability of the products to associate with water under conditions when water is limiting such as flour, doughs and pastes (Costa *et al.*, 2021).

The water absorption capacity of the fermented groundnut flour ranged 1.00 - 1.50 mL/g. It was highest in sample (1.50 mL/g) that was fermented for 48 h and lowest in the control sample (1.00 mL/g) that was not fermented. There was no significant ($p > 0.05$) difference in water absorption capacity between the control sample (1.00 mL/g) and the fermented samples (1.00 mL/g), (1.00 mL/g) and (1.00 mL/g) that were fermented for 8, 16 and 32 h respectively.

The ranged obtained in this study was lower than 2.24 - 3.02 mg/g reported by Katoch (2021) for fermented soybean hull flour due to the difference in the raw material used and fermentation time. According to Adebowale *et al.* (2022) difference in hydrophilic constituents may be responsible for the difference in water absorption capacity. The polar amino-acids are the preferred sites of the interactions between water and proteins. Firdaus *et al.* (2021) attributed high water absorption capacity to lose structure of starch polymers while low value as obtained in this study indicates compactness of the structure. Increase in fermentation time significantly increased the water absorption capacity of the groundnut hull flour samples because the microbial action during fermentation modifies the macromolecules, such as proteins and starches, exposing more hydrophilic (water-loving) regions (Cui and Liu 2021). The values obtained for water activity in all of the groundnut flour were low, water absorption capacity in the flour affects microbial and fungal contamination, and the higher the water absorption capacity in flour, the more contamination and spoilage (Agarwal *et al.*, 2018). Since the values for the water absorption capacity for all groundnut hull flour samples were low, this signifies that the flour could have a stable shelf life (Tortora *et al.,* 2019).

Table 3: Functional Properties of Fermented Groundnut Hull Flour

S/No	Sample	Water absorption capacity (g/ml)	Oil absorption capacity (g/ml)	Bulk density (g/ml)
1.	KAA	$1.00^{b}\pm0.00$	$0.47^{d}\pm0.01$	$0.56^{c}\pm0.00$
2.	KAB	$1.00^{b}\pm0.10$	$0.93^{c}\pm0.00$	$0.53^{d}\pm0.01$
3.	KAC	$1.00^{b}\pm0.20$	$0.93^{c}\pm0.00$	$0.59^{b}\pm0.01$
4.	KAD	$1.50^{a}\pm0.00$	$1.40^{b}\pm0.01$	$0.59^{b}\pm0.01$
5.	KAE	$1.00^{b}\pm0.00$	$1.40^{b}\pm0.01$	$0.56^{c}\pm0.02$
6.	KAF	$1.50^{a}\pm0.10$	$2.33^{a}\pm0.00$	$0.63^{a}\pm0.00$
7.	KAG	$1.50^{a}\pm0.20$	$2.33^{a}\pm0.00$	$0.63^{a}\pm0.02$

Values are mean scores ± Standard deviation of three (3) replicates data in the same column bearing different superscript differ significantly ($p < 0.05$).

KEYS:
KAA (Control): (Not Fermented)
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KAE: (Fermented for 32 h)
KAF: (Fermented for 40 h)
KAG: (Fermented for 48 h)

The Oil absorption capacity of the fermented groundnut hull flour increased as the fermentation time increased ranging from 0.47 - 2.33 mL/g, with the control sample KAA without fermentation time having the lowest oil absorption capacity while sample KAG (2.33 mL/g) that was fermented for 48 h had the highest oil absorption capacity. Significant ($p < 0.05$) difference was observed between the control sample KAA and the fermented samples with different time. There was no significant ($p < 0.05$) difference between (2.33 mL/g) and (2.33 mL/g) that was fermented for 40 and 48 hours respectively. This was because of the exposure of oil binding sites present on the side chain groups of proteins in a lipophilic environment as fermentation progressed (Temesgen 2022). The result was within the range 1.04 - 3.32 mL/g reported by Halm *et al.* (2019) in similar study with a higher fermentation time. Oil absorption capacity has been attributed to entrapment of oil and the binding of fat to the hydrophobic amino acid. High oil absorption is a prerequisite for the formulation of food (Backer *et al.*, 2018). The low oil absorption index obtained for the control sample in comparison to the fermented groundnut flour samples is expected as Agarwal *et al.* (2018) revealed that the low levels of hydrophobic proteins which exhibit higher lipid binding, could be the cause of the low oil absorption of groundnut hull flour.

The bulk density of the samples varied across different fermentation ranging from 0.56 - 0.63 g/ml, with the control sample KAA that was not fermented having the lowest bulk density while sample KAG that was fermented for 48 h having the bulk density. The bulk density of 0.48 g/mL and 0.62 g/mL reported by Hall *et al.* (2019) and Johnson *et al.* (2019), respectively for groundnut hull flour were within the values obtained for groundnut flour samples while 0.85 g/ml reported by Mlynekova *et al.* (2022) for groundnut flour was higher than the values obtained for the fermented groundnut flour samples. However, the range of values (0.64 –0.80 g/mL) reported by Adebowale *et al.* (2022) for Bambara groundnut flour was higher than the values obtained for fermented groundnut flour samples. The lowest bulk density was observed in groundnut flour that was not fermented (KAA). Groundnut hull flour could be useful in numerous food formulations due to low bulk density as Sadana and Chabra (2022) revealed that the flour with low bulk density is appropriate for high nutrient density food formulations.

Phytochemical Properties of Fermented Groundnut Hull Flour

The result of phytochemical properties of fermented groundnut hull flour is presented in Table 4. Tanin ranged 579.94 - 586.87 mg/g, Saponin 168.00 - 264.00 mg/g and flavonoid 1.95 - 2.15 mgQE/g. Tannin contents of the samples ranged from 579.94 - 586.87 mg/g, with sample KAG that was fermented for 48 h containing the lowest tannin while the control sample KAA (unfermented) had the highest tannin. Significant ($p < 0.05$) differences were observed in all the samples. Tannins can be reduced by leaching into water because they are water-soluble phenolic compounds and mostly located in seed covering (Halm *et al.*, 2019). Various enzymatic activities could be responsible for the reduction of tannin during fermentation, the enzyme tannase, for example, is well known for breaking down tannins during fermentation (Omafuvbe *et al.*, 2021). Fermented groundnut hull flour (KAA) had lower tannin levels because fermentation involves microorganisms breaking down anti-nutrients like tannins into simpler, less harmful compounds. The specific organisms used and fermentation time influence the extent of this reduction; generally, longer fermentation times result in greater tannin removal, leading to lower levels in the final fermented product compared to the unfermented sample (Agarwal *et al.*, 2018).

Table 4: Phytochemical content of Fermented Groundnut Hull Flour

Sample	Tannin (mg/g)	Saponin (mg/g)	Flavonoid (mgQE/g)
KAA	586.87^{a}±0.18	264.00^{a}±0.00	2.15^{a}±0.00
KAB	584.35^{b}±0.18	252.00^{b}±1.00	2.13^{b}±0.00
KAC	583.73^{c}±0.18	234.00^{c}±0.00	2.11^{c}±0.01
KAD	583.20^{d}±0.00	216.00^{d}±1.00	2.10^{d}±0.00
KAE	582.67^{e}±0.18	198.00^{e}±0.00	2.09^{e}±0.00
KAF	580.68^{f}±0.00	182.00^{f}±0.00	2.02^{f}±0.00
KAG	579.94^{g}±0.18	168.00^{g}±1.00	1.95^{g}±0.00

Values are mean scores ± Standard deviation of three (3) replicates data in the same column bearing different superscript differ significantly ($p < 0.05$).

KEYS:
KAA (Control): (Not Fermented)
KAB: (Fermented for 8 h)
KAC: (Fermented for 16 h)
KAD: (Fermented for 24 h)
KAE: (Fermented for 32 h)
KAF: (Fermented for 40 h)
KAG: (Fermented for 48 h)

Saponin content of the samples ranged from 168.00 - 264 mg/g, with sample KAG that was fermented for 48 h having lowest saponin while the unfermented sample (KAA) had the highest saponin. Significant ($p < 0.05$) differences were observed in all the samples. The range in this study was higher than 121.00 - 161 mg/g reported by Halm *et al.* (2019). Increase in the fermentation time gradually decreased the saponin content of the groundnut hull flour because microorganisms produce enzymes that hydrolyze saponins, breaking them down into simpler, less complex compounds. This enzymatic activity is a natural process occurring within the complex metabolic activities of fermenting microbes, leading to the degradation of these anti-nutritional compounds over increased fermentation time (Tina, 2021). According to Madukwe *et al.* (2019) high-saponin diets act as antioxidants and anti-inflammatory agents, and potentially reducing cancer risk by protecting cells and enhancing the immune response.

The flavonoid content of the samples ranged from 1.95 - 2.15 mgQE/g. Sample KAG that was fermented for 48 h had the lowest flavonoid content, while the unfermented sample had the highest flavonoid content. Fermentation reduced the flavonoid content of the samples. Adebowale *et al.* (2022) reported lower flavonoid (0.92 mgQE/g) for the fermented sample compared to the value obtained in this study. High dietary flavonoid intake offers strong antioxidant, anti-inflammatory, anti-cancer, and neuroprotective effects, potentially reducing the risk of chronic conditions like cardiovascular disease, cancer, and neurodegenerative diseases. (Shuaibu, 2021). Gernah *et al.* (2019) in similar research, observed low flavonoid with increased fermentation period and attributed to microbial metabolism, enzymatic reactions, and oxidation can break down or modify flavonoids into less active compounds or polymers over time.

Conclusion

This study of fermented groundnut hull flour reveals significant findings regarding its proximate composition, functional properties and anti-nutrient content. The proximate analysis indicates that fermented groundnut hull flour is rich in dietary fiber (8.80 - 12.00%) making it a valuable component for improving dietary quality. The study also shows that fermentation improves the ash and protein content of groundnut hulls. The fermentation process helps in breaking down complex carbohydrates, which improves the metabolism. The fermentation process enhances the functional properties (oil absorption capacity and water absorption capacity) of groundnut hull flour. These improvements are essential for various food applications, making it a suitable ingredient for functional foods and nutraceuticals. Fermentation is shown to significantly reduce the levels of anti-nutrients such as tannins. This reduction enhances the wholesomeness of the groundnut flour, making the flour more suitable for consumption and other food product applications.

Ethical Approval

Not applicable.

Competing Interests

Authors have declared that no competing interests exist.

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