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## Article

# Effect of De-Husking on Nutritional and Antioxidant Quality of Nigerian Millet Varieties

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**Abstract:** Millet is a staple food in Northern Nigeria, widely used for the preparation of various millet-based dishes. However, traditional processing methods, such as de-husking, can significantly affect the grain's nutritional and antioxidant properties. This study evaluates the antioxidant composition and nutritional value of four millet varieties cultivated in Northern Nigeria, with a particular focus on the impact of de-husking on these parameters. Our analysis encompasses the proximate composition, phytochemical content, antioxidant vitamins, mineral elements, amino acid profile, and antioxidant activities of the millet samples. The findings reveal that all millet varieties contain significant levels of antioxidant compounds, including phenolics, flavonoids, and vitamins, although their concentrations varied due to genetic differences and environmental factors. Notably, in the mineral composition, higher ( $p < 0.05$ ) levels of selenium (Se) and magnesium (Mg) were observed, while trace levels of iron (Fe), copper (Cu), and zinc (Zn) were present. The study demonstrated that de-husking significantly ( $p < 0.05$ ) reduced the levels of most nutritional and antioxidant parameters, underscoring the superior nutritional value of whole grains compared to their de-husked counterparts. This highlights the importance of consuming millets in their unprocessed form to maximise health benefits. Moreover, the presence of essential phytochemicals, amino acids, and dietary fiber in these grains, suggests their potential role in reducing the risk of chronic diseases such as cancer, cardiovascular diseases, obesity, and diabetes. These findings underscore the nutritional and functional potential of millet grains as valuable sources of essential nutrients, which can be utilised in nutraceutical formulations or as functional foods for promoting human health. This study emphasises the need to preserve the integrity of millet grains during processing to fully capitalise on their health benefits.

**Keywords:** millet; de-husking; antioxidants; phytochemicals; minerals; vitamins

## 1. Introduction

Millet is one of the most drought-resistant grains and is ranked as the sixth most economically important agricultural crop globally (Amadou *et al.*, 2013). It is notably resilient to pests and diseases, capable of thriving in low-fertility soils, and exhibits a rapid growth rate, making it a critical crop, particularly in regions with harsh climatic conditions (Pasha *et al.*, 2018). Millets can also produce high yields under heat and drought, outperforming many other major cereals in such environments (Pasha *et al.*, 2018). Millets belong to the *Poaceae* family and encompass various species, including Pearl millet (*Pennisetum glaucum*), which accounts for 40% of global millet production, along with foxtail millet (*Setaria italica*), proso or common millet (*Panicum miliaceum*), and finger millet (*Eleusine coracana*) (Amadou *et al.*, 2013).

Global millet production was estimated at 30.6 million tons in 2022, with cultivation spanning over 93 countries (Royal Tropical Institute, 2023). West Africa is the largest producer, with Nigeria

recently ranked as the third-largest millet-producing country globally, after India and China, and the leading producer in Africa, following Niger and Mali (FAO, 2023). However, other reports suggest that Nigeria is the second-largest producer in Africa, with an annual production of 2 million metric tons, following Niger's 3.4 million metric tons (Royal Tropical Institute, 2023). In Nigeria, millet is a staple food, especially in the Northern regions, where it is used to prepare traditional dishes such as “Kunu” (millet juice), “Fura” (fermented milk paste), “Masa” (fried millet cake), and “Tuwo” (thick binding paste) (Izge *et al.*, 2013). Despite its nutritional benefits, many communities in Northern Nigeria practice de-husking millet, which may impact its antioxidant content.

Beyond its nutritional value, millet has been associated with several health benefits, including wound healing, cardiovascular health, and reductions in blood glucose and cholesterol levels (Rajasekaran *et al.*, 2004; Hegde *et al.*, 2005; Shobana *et al.*, 2009; Lee *et al.*, 2010). These benefits are largely attributed to millet's rich antioxidant profile, which plays a critical role in mitigating oxidative stress—a condition linked to metabolic diseases such as cardiovascular disease, cancer, neurodegenerative disorders, arthritis, and diabetes (Ou *et al.*, 2002; Valko *et al.*, 2007; Halliwell, 2012). Antioxidants in millet, including carotenoids, phenolics, and tocopherols, contribute to the prevention of oxidative damage and the preservation of nutritional quality in food products (Zieliński and Kozłowska, 2000; Asharani *et al.*, 2010). Additionally, millet is well-balanced in protein content, particularly rich in essential amino acids, and contains metabolically active compounds such as vitamins A, B, and E (Shah *et al.*, 2021; Maharajan *et al.*, 2021).

Despite the wealth of research highlighting the health benefits of millet, particularly its high dietary fiber, polyphenol content, and other bioactive compounds, there remains limited data on the antioxidant activity of different millet varieties cultivated in Nigeria—one of the world's leading millet producers. This gap in knowledge is significant, especially in light of the rising incidence of metabolic diseases in Nigeria, attributed to oxidative stress from environmental factors such as pollution, poor diets, and chemical exposure (Caligiuri and Pierce, 2017; Mills, 2020). These diseases pose substantial threats to human health, economic productivity, and national development, underscoring the need for affordable and accessible nutraceuticals rich in antioxidants.

Given the potential impact of genetic, environmental, and processing factors on the bioactive compound composition of millets (Chethan & Malleshi, 2007; Zhang *et al.*, 2015; Liu *et al.*, 2018), this study aims to evaluate the antioxidant activity of various millet varieties cultivated in Northern Nigeria, with a specific focus on the effects of de-husking. By comparing the antioxidant capacity of whole grain and de-husked millet, this research seeks to identify a functional and cost-effective nutraceutical source that could enhance dietary antioxidant intake and promote human health.

## 2. Materials and Methods

### *Sample Collection and Preparation*

Millet samples for this study were collected from various states across Northern Nigeria, although the research was conducted in Sokoto State, Nigeria. Four (4) millet varieties were selected: Pearl millet, Finger millet, Foxtail millet, and Proso (common) millet. Proso millet, the main variety cultivated in Sokoto State, was sourced locally, while Finger millet was obtained from Kebbi State, and Pearl and Foxtail millets were collected from Yobe and Borno States, respectively. All samples were submitted to the Herbarium at the Department of Plant Science, Usmanu Danfodiyo University, Sokoto, where they were identified as *Pennisetum glaucum* (Pearl millet), *Eleusine coracana* (Finger millet), *Panicum miliaceum* (Proso millet), and *Setaria italica* (Foxtail millet), with corresponding voucher identification numbers UDUH/ANS/0957, UDUH/ANS/0958, UDUH/ANS/0959, and UDUH/ANS/0960, respectively.

Only freshly cultivated, non-preserved millet samples were selected for the study. Samples were carefully inspected to ensure they were free from physical damage or disease. To minimize bias, samples were randomly chosen from larger batches, ensuring each had an equal and independent chance of selection.

Each millet variety was then divided into two (2) groups: whole grains and de-husked grains. De-husking, also known as hulling, was performed using a hand-pounding mortar and pestle, with a small amount of clean water added to facilitate the removal of the fibrous bran. The de-husked grains were washed and air-dried at room temperature for three (3) days, with special care taken to prevent loss or damage. Both whole and de-husked grains were subsequently ground into fine powder using the same mortar and pestle, and the powdered forms were used for subsequent analyses.

#### *Sample Digestion and Mineral Elements Determination*

The digestion of samples for mineral element analysis was performed following the method described by Bhatti *et al.* (2006). Initially, 2 g of each sample was measured into a 50 mL conical flask, followed by the addition of 5 mL of 100 ppm Nitric acid (HNO<sub>3</sub>). The mixture was stirred and then heated on a hot plate, producing a strong yellow fume that dissipated after gentle heating for 2 minutes. The mixture was then allowed to cool for 30 minutes. Subsequently, 2.5 mL of 1.0 M perchloric acid (HClO<sub>4</sub>) was added, and the solution was reheated until it became colorless. After cooling, 20 mL of distilled water was added to the mixture, which was then filtered into plastic bottles. The digested samples were analyzed for mineral elements, including Fe, Zn, Se, Ca, Cu, Mg, Mn, K, and P, using Microwave Plasma Atomic Emission Spectroscopy (MP-AES) (Model: G8007A, Agilent Technologies, Australia).

#### *Proximate Analysis*

The proximate composition of the samples was determined using standard methods outlined by the Association of Official Analytical Chemists (AOAC, 2000). The moisture, ash, fibre, protein, and lipid contents were determined by oven drying, muffle furnace, acid/base digestion, micro-Kjeldahl, and Soxhlet extraction methods respectively while the carbohydrate content was determined by difference.

#### *Quantitative Determination of Phytochemicals*

The phytochemical content, including total flavonoids, alkaloid, saponins, tannin, and glycosides was quantitatively determined in both whole grains and de-husked samples of the four millet varieties. Total flavonoids of the samples were quantified using the method described by Boham and Kocipai-Abyazan (1974). Alkaloid content was determined using the gravimetric method outlined by Harbone (1998). The saponin, tannin, and glycoside contents were measured following the methods of AOAC (1990), Pearson (1976), and Onwuka (2005), respectively.

#### *Determination of Antioxidant Vitamins*

The antioxidant vitamins A, C, and E were quantified in the samples using established methods.

**Vitamin A** was determined using the method of Bassey *et al.* (1946). Briefly, 0.5 g of the sample was dissolved in 10 mL of distilled water, allowed to stand for 1 hour, and then filtered. The supernatant was centrifuged at 2000 rpm for 10 minutes, and 1 mL of it was read spectrophotometrically at 450 nm.

**Vitamin C** content was estimated using the method of Baker and Frank (1968). A 0.5 g sample was dissolved in 10 mL of distilled water, incubated at room temperature for 30 minutes, filtered, and centrifuged at 2000 rpm for 10 minutes. The absorbance was then measured at 700 nm.

**Vitamin E** was also determined using the method of Baker and Frank (1968). A 0.5 g sample was dissolved in 10 mL of distilled water, incubated for 30 minutes, filtered, and mixed with 0.5 mL of ethanol and 3 mL of xylene. After vigorous shaking and centrifugation at 2000 rpm for 10 minutes, the absorbance was measured at 539 nm.

### Determination of Amino Acids Profile

The amino acid profiles of the samples were determined using an amino acid analyser (120A PTH, Applied Biosystems Inc., USA) following the AOAC (2006) method. This analyser was selected for its ability to automatically analyse phenylthiohydantoin (PTH) amino acids, which are generated from the Edman degradation of proteins and peptides.

Prior to the analysis, the samples were dried to a constant weight and defatted using a chloroform/methanol mixture (2:1 ratio). Approximately 4g of each sample was then subjected to Soxhlet extraction for 15 hours, as per AOAC (2006) guidelines. After defatting, the samples were hydrolysed and concentrated using a rotary evaporator before being loaded into the amino acid analyser for profiling.

### Antioxidant Activity

The antioxidant activity of the samples was assessed using three parameters: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP), and total phenolic content (TPC), following the method described by Alyaqoubi *et al.* (2014).

#### 1. DPPH Radical Scavenging Activity:

A stock solution of DPPH was prepared by dissolving 40 mg of DPPH in 100 mL of methanol, which was then stored at -20°C. To prepare the working solution, 350 µL of the stock solution was mixed with 350 µL of methanol, and the absorbance was adjusted to  $1.0 \pm 0.01$  at 517 nm using a spectrophotometer (Epoch, Biotek, USA). For the assay, 100 µL of the sample extract was mixed with 1 mL of the methanolic DPPH solution and allowed to react in the dark for 2 hours. The DPPH scavenging activity was calculated using the formula:

$$\text{DPPH scavenging activity (\%)} = \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \times 100$$

where *A* represents absorbance.

#### 2. Ferric Reducing Antioxidant Power (FRAP):

The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ [2,4,6-tris(2-pyridyl)-s-triazine] in 40 mM HCl, and 20 mM FeCl<sub>3</sub>•6H<sub>2</sub>O in a 10:1:1 ratio. For the assay, 100 µL of sample extract was added to 1 mL of FRAP reagent, and the absorbance was measured at 595 nm after 30 minutes. A calibration curve using Trolox was established to express the results as mg of Trolox equivalents per 100 g of fresh sample (mg TE/100 g FW).

#### 3. Total Phenolic Content (TPC):

For TPC determination, 100 µL of the sample extract was mixed with 0.4 mL of distilled water and 0.5 mL of diluted Folin-Ciocalteu reagent. After incubating the mixture at room temperature for 5 minutes, 1 mL of 7.5% sodium carbonate (w/v) was added. The absorbance was measured at 765 nm after 2 hours. A calibration curve with gallic acid was used to express the results as mg of gallic acid equivalents per 100 g of sample (mg GA/100 g FW).

### Thiobarbituric Acid (TBA) Assay

The Thiobarbituric Acid (TBA) Assay was performed according to the method of Du and Bramlage (1992) to evaluate thiobarbituric acid-reactive substances (TBARS) as an indicator of lipid oxidation in millet samples. Millet samples were first homogenized and prepared for the assay. Thiobarbituric acid reacts with malondialdehyde (MDA), a product of lipid peroxidation, to form a red fluorescent 1:2 MDA/TBA adduct, which was measured spectrophotometrically at 532 nm.

To determine the antioxidant capacity of the millet samples, MDA levels were calculated using the following formula:

$$\text{MDA concentration (mM/g)} = \frac{\text{Absorbance of test} \times \text{Assay volume (1.5 ml)} \times 10^3}{\text{Molar extinction coefficient (1.56} \times 10^5) \times \text{Weight of sample (g)} \times \text{Sample volume (0.5 ml)}}$$



The assay was performed in triplicate to ensure accuracy, with appropriate blank controls included. Data were statistically analyzed to compare the antioxidant capacities across different millet varieties.

### Statistical Analysis

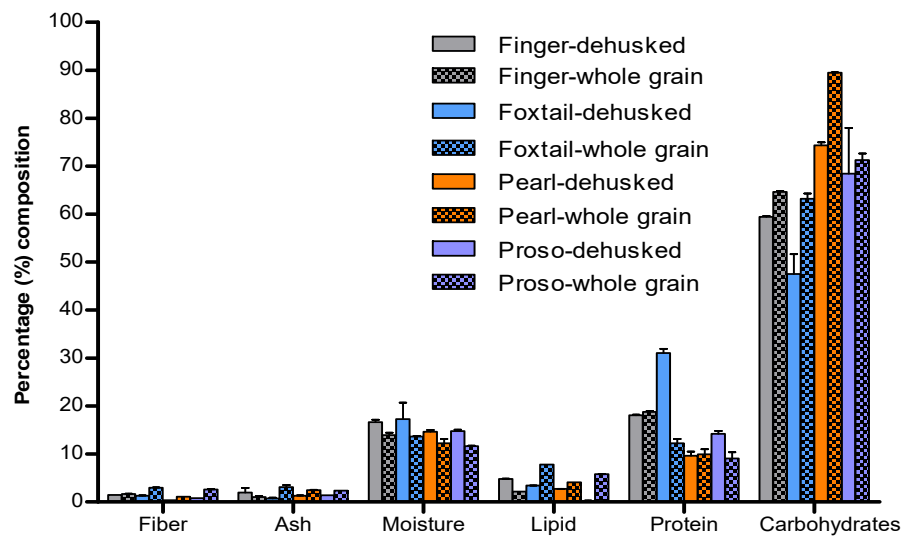
Data were expressed as mean  $\pm$  standard deviation (SD). Statistical analysis was performed using GraphPad Prism Software, version 6.01 (San Diego, USA). One-way analysis of variance (ANOVA) was conducted to assess statistical significance among the groups, followed by Tukey's multiple comparison post hoc test. A p-value of less than 0.05 ( $p < 0.05$ ) was considered statistically significant.

## 3. Results

### Proximate Composition of Millet Varieties

The percentage proximate composition of four millet varieties cultivated in the Sokoto region of Nigeria is presented in Figure 1. Carbohydrate content was significantly higher ( $p < 0.05$ ) than other proximate parameters across all samples. Protein and moisture contents were consistently higher than lipid levels in all the studied varieties, while fiber and ash contents were the lowest among the proximate components.

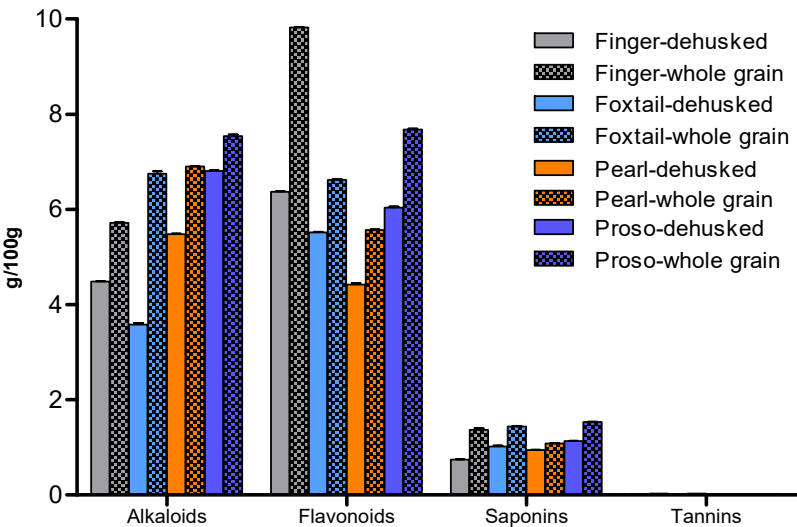
Among the varieties, whole grain Pearl Millet exhibited the highest ( $p < 0.05$ ) carbohydrate content. Dehusked Foxtail Millet had significantly higher ( $p < 0.05$ ) protein levels compared to other samples, while its whole grain variant showed elevated levels of lipid, ash, and fiber. Dehusking generally led to a reduction in carbohydrate, lipid, fiber, and ash contents across all millet varieties, except for Finger Millet, where lipid content significantly increased ( $p < 0.05$ ). Moisture content increased slightly upon dehusking in the Foxtail variety, but the change was not statistically significant ( $p > 0.05$ ). Conversely, dehusking significantly increased ( $p < 0.05$ ) protein levels in the Foxtail and Proso varieties.



**Figure 1.** Proximate composition of four varieties of millet grown in Nigeria. Bars represent the Mean triplicate values and error bars represent standard deviations.

Phytochemical Composition of Millet Varieties

Figure 2 illustrates the phytochemical composition—flavonoids, tannins, saponins, and alkaloids—of four millet varieties cultivated in the Sokoto region of Nigeria. All varieties exhibited high levels of both flavonoids and alkaloids. Saponins were present at moderate levels across the varieties, while tannins were detected only at trace levels. Dehusking the millet grains resulted in a significant reduction ( $p < 0.05$ ) in the levels of all analysed phytochemicals.



**Figure 2.** Phytochemical composition of four varieties of millet grown in Nigeria. The bars represent the Mean of triplicate measurements and error bars represent the standard deviation.

Amino Acid Composition of Millet Varieties

Table 2 presents the amino acid compositions of four millet varieties cultivated in the Sokoto region of Nigeria. Glutamic acid was the most abundant amino acid across all varieties, with concentrations ranging from 7.78 to 19.46 g/100g protein. Leucine was the second most prevalent amino acid, with levels between 7.41 and 11.21 g/100g protein.

Other amino acids present in high concentrations included alanine (4.44–7.82 g/100g protein), proline (2.13–6.50 g/100g protein), arginine (4.04–5.42 g/100g protein), valine (3.02–5.61 g/100g protein), phenylalanine (2.84–5.23 g/100g protein), serine (3.05–4.62 g/100g protein), and isoleucine (3.01–4.39 g/100g protein). Tryptophan was the least abundant amino acid, with concentrations ranging from 1.18 to 1.94 g/100g protein.

Overall, the dehusked millet samples exhibited lower amino acid levels compared to the whole grain samples across all varieties.

**Table 2.** Amino acid composition (g/100g protein) of four varieties of millet grown in Nigeria.

Amino Acid (g/100gprotein)	Finger-dehusked	Finger-whole grain	Foxtail-dehusked	Foxtail-whole grain	Pearl-dehusked	Pearl-whole grain	Proso-dehusked	Proso-whole grain
Alanine	7.51	7.82	4.44	7.40	7.17	7.50	5.84	6.71
Arginine	5.16	5.42	4.04	5.08	4.30	5.33	4.30	4.82
Aspartic acid	7.56	7.91	5.30	6.98	7.38	7.82	7.17	7.51
Cysteine	2.12	2.48	2.06	2.36	2.30	2.48	1.64	1.82
Glutamic acid	7.78	19.46	10.37	19.00	18.32	19.23	18.03	18.63

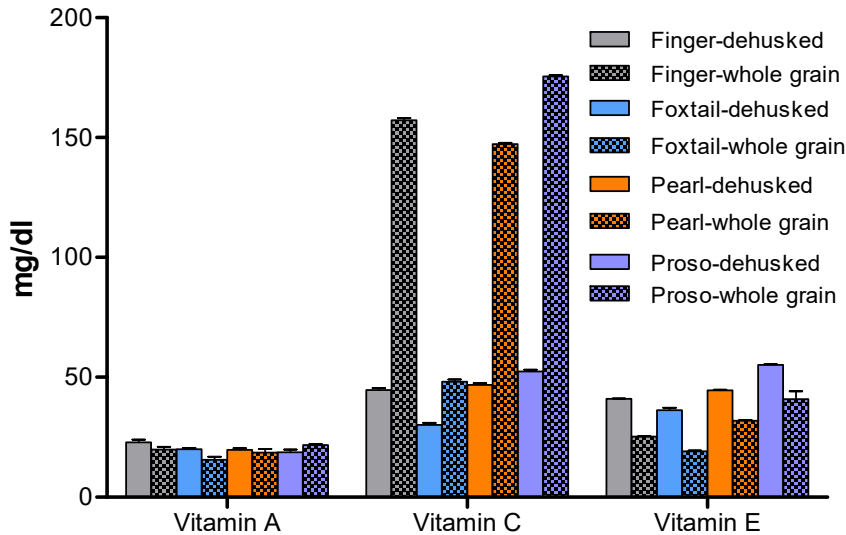
Glycine	3.66	3.80	2.87	3.71	3.61	3.82	3.30	3.52
Histidine	2.17	3.53	1.92	2.72	2.36	2.46	2.21	2.40
Isoleucine	4.06	4.39	3.01	4.16	3.63	4.26	3.64	3.90
Leucine	8.93	9.81	7.41	11.21	9.22	10.30	9.81	10.09
Lysine	3.08	3.63	2.25	3.69	3.13	3.58	3.34	3.61
Methionine	2.08	2.49	1.23	1.98	2.16	2.40	2.17	2.24
Phenylalanine	4.79	5.15	2.84	5.23	4.52	4.88	4.44	5.06
Proline	5.99	6.50	3.55	6.19	2.13	5.89	5.49	5.79
Serine	3.97	4.62	3.05	4.16	4.19	4.46	3.54	3.92
Threonine	3.16	3.75	2.19	3.50	2.97	3.80	3.39	3.61
Tryptophan	1.65	1.94	1.18	1.87	1.73	1.81	1.66	1.73
Tyrosine	3.27	3.61	2.58	3.44	3.10	3.61	3.61	3.96
Valine	5.14	5.50	3.02	5.61	5.44	5.55	5.00	5.20

Vitamin Composition of Millet Varieties

Figure 4 illustrates the vitamin C, A, and E levels in the millet varieties studied. Vitamin C levels were significantly higher than those of vitamins A and E across all varieties. The highest concentration of vitamin C was found in the whole grain of Proso millet, followed by Finger millet, with Pearl millet exhibiting slightly lower levels. Foxtail millet had the lowest vitamin C content among the varieties. Dehusking led to a significant reduction ( $p<0.05$ ) in vitamin C levels across all millet varieties.

In contrast, vitamin E levels were comparatively higher in the dehusked grains, particularly in Proso millet, which showed the highest increase. All dehusked varieties exhibited statistically higher vitamin E levels compared to their corresponding whole grains.

Vitamin A levels were significantly affected by dehusking in the Foxtail, Proso, and Finger varieties. Specifically, dehusking resulted in an increase in vitamin A levels in the Foxtail and Finger varieties, while a decrease was observed in the Proso variety. Notably, the vitamin A content in Pearl millet remained unchanged after dehusking.



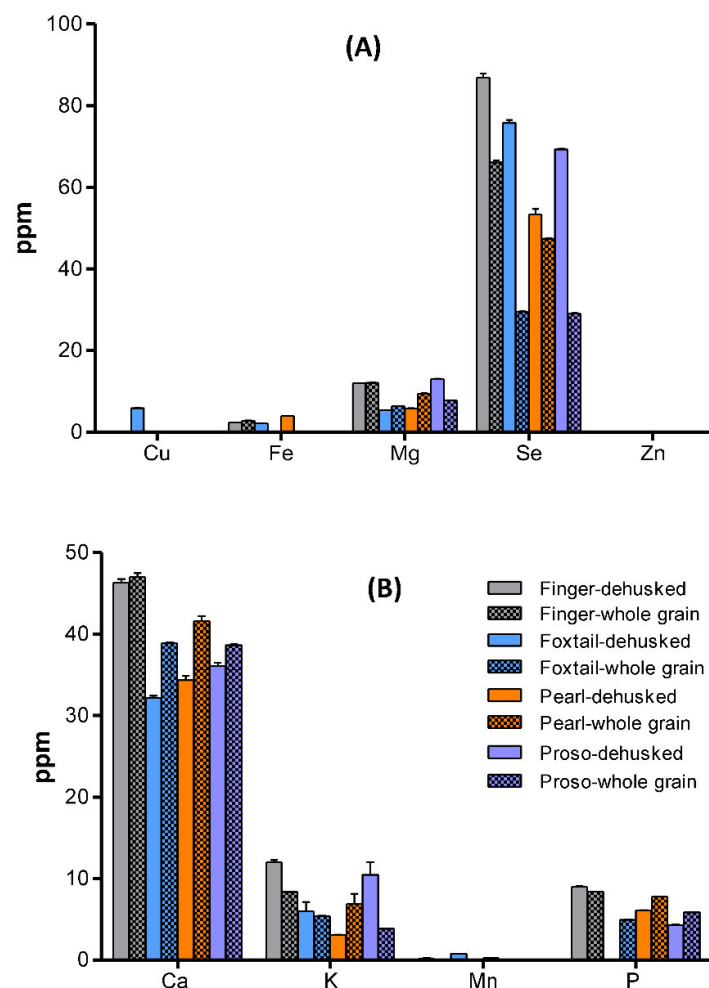
**Figure 3.** Antioxidant vitamin composition of four varieties of millet grown in Nigeria. The bars represent the Mean of triplicate measurements and error bars represent the standard deviation.



### Mineral Composition of Millet Varieties

Figure 4A and 4B present the levels of microelements (Cu, Fe, Mg, Se, and Zn) and macroelements (Ca, Mn, P, and K) in four millet varieties cultivated in Northern Nigeria. Among the microelements, selenium (Se) was significantly higher ( $p < 0.05$ ) than other trace elements, followed by magnesium (Mg), across all samples. Trace levels of iron (Fe), copper (Cu), and zinc (Zn) were detected in all varieties. The highest Se levels were found in the dehusked grain of the Finger millet variety, followed by dehusked Foxtail, dehusked Proso, whole grain Finger, dehusked Pearl, whole grain Pearl, whole grain Foxtail, and whole grain Proso millet, respectively. Overall, dehusking significantly increased ( $p < 0.05$ ) Se levels in all tested varieties. Notably, Mg levels in the Finger millet variety were unaffected by dehusking, while dehusking decreased Mg levels in Foxtail and Pearl varieties and increased them in Proso millet.

Regarding macroelements, calcium (Ca) was the most abundant, surpassing manganese (Mn), phosphorus (P), and potassium (K). Moderate levels of P and K were detected, whereas Mn was present only in trace amounts. Ca levels were notably higher in the Finger millet variety compared to the others. Dehusking led to a significant reduction ( $p < 0.05$ ) in Ca and P levels in all varieties except Finger millet. In contrast, K levels significantly increased ( $p < 0.05$ ) in dehusked Proso and Finger millet varieties, while a significant decrease ( $p < 0.05$ ) was observed in dehusked Pearl millet. No significant change in K levels was noted in the Foxtail variety after dehusking.



**Figure 4.** Micro (A) and macro (B) mineral elements composition of four varieties of millet grown in Nigeria. Data represent Mean  $\pm$  Standard deviation of triplicate values.

Antioxidant Activity and TBA Levels of Millet Varieties

Table 1 presents the thiobarbituric acid (TBA) levels, total phenolic content (TPC), ferric reducing antioxidant power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of the four millet varieties examined in this study. Dehusking significantly decreased ( $p < 0.05$ ) the TBA levels in Foxtail and Proso millet varieties, while it significantly increased ( $p < 0.05$ ) the TBA levels in Pearl millet. The TBA increase in the Finger millet variety after dehusking was not statistically significant ( $p > 0.05$ ).

In terms of TPC, a significant decrease ( $p < 0.05$ ) was observed after dehusking the whole grains of Foxtail and Finger millet. Conversely, Proso millet showed a significant increase ( $p < 0.05$ ) in TPC following dehusking, while Pearl millet exhibited a non-significant decrease ( $p > 0.05$ ).

Overall, FRAP activities decreased across all millet varieties due to dehusking, although the reduction was statistically significant ( $p < 0.05$ ) only in the Finger millet variety which also showed a 5-fold increase in FRAP compared to other varieties. Similarly, dehusking led to a significant decrease ( $p < 0.05$ ) in DPPH radical scavenging activity in all studied millet varieties.

Table 1. Antioxidant capacity of four varieties of millet grown in Nigeria.

Antioxidant Capacity	Finger-dehusked	Finger-whole grain	Foxtail-dehusked	Foxtail-whole grain	Pearl-dehusked	Pearl-whole grain	Proso-dehusked	Proso-whole grain
TBA (µg)	173.28±0.37 <sup>a</sup>	164.74±0.64 <sup>a</sup>	266.02±3.85 <sup>c</sup>	311.09±6.42 <sup>b</sup>	196.15±3.2 <sup>d</sup>	172.00±0.98 <sup>a</sup>	180.76±2.31 <sup>a,d</sup>	262.80±16.36 <sup>c,e</sup>
TPC (mg/GA100 of FW)	2.39±0.04 <sup>b</sup>	3.13±0.01 <sup>a</sup>	0.52±0.06 <sup>d</sup>	2.59±0.26 <sup>b,c</sup>	2.46±0.20 <sup>b,c</sup>	2.71±0.02 <sup>b,c,e</sup>	2.15±0.02 <sup>b,f</sup>	0.70±0.10 <sup>d,g</sup>
FRAP (mgTE/100g of FW)	23.99±0.62 <sup>b</sup>	25.74±0.44 <sup>a</sup>	3.41±0.10 <sup>c,d</sup>	4.18±0.18 <sup>c</sup>	5.49±0.26 <sup>c,f</sup>	5.66±0.18 <sup>c</sup>	4.59±0.31 <sup>c,f</sup>	5.42±0.09 <sup>c,f</sup>
DPPH (%)	55.57±0.40 <sup>b</sup>	63.84±0.54 <sup>a</sup>	54.04±0.67 <sup>b,d</sup>	57.99±0.42 <sup>c</sup>	49.85±0.90 <sup>f</sup>	90.13±0.10 <sup>e</sup>	60.18±0.99 <sup>g</sup>	64.61±0.59 <sup>a</sup>

Data represents Mean ± Standard deviation of triplicate values. Values with different superscript letters in a row represent statistically significant differences at  $p < 0.05$ .

4. Discussion

Despite the well-documented nutraceutical benefits of millets, these grains have historically received limited attention, leading the FAO (2017) to classify them as a “lost crop.” However, recent global challenges—such as climate change, sustainable food production, water scarcity, and overpopulation—have revitalized interest in millet. These resilient crops are now recognised as essential components in strategies aimed at addressing nutritional and agricultural challenges worldwide. The shift towards natural antioxidants in place of synthetic ones, due to concerns about potential health risks and toxicity, further underscores the importance of evaluating millet's nutritional and functional properties. This study aims to elucidate the nutritional and functional characteristics of different millet varieties cultivated in Nigeria.

Proximate Composition

The proximate composition of the four millet varieties cultivated in Nigeria revealed significant insights, particularly in fiber content. The fiber content was notably higher in whole grains compared to their de-husked counterparts. This result is consistent with the understanding that the bulk of dietary fiber is concentrated in the bran layer of grains, which is often removed during de-husking.

The fiber values observed in this study, while lower than those reported by Bot *et al.* (2021), are within the range reported by Sanusi (2019). Fibre is the non-digestible component of food and plays a crucial role in digestion, improving glucose tolerance, and increasing the bulk of faeces, making it an essential component of a healthy diet.

The ash content represents the inorganic mineral constituents food obtained after removal of water or organic substances through heating with oxidising agent (Mode *et al.*, 2023). This was relatively low across all the millet varieties. This low ash content could be attributed to factors such as the method of ashing, the mineral resources in the soil, and environmental conditions in the production areas. The values obtained align with previous studies (Twinomuhwezi *et al.*, 2020), which reported that fresh foods generally have ash contents below 5%, while some processed foods may exceed 12%.

Moisture content is another critical parameter that influences the shelf life of grains. Most millet varieties in this study exhibited moisture levels above the optimal range for long-term storage, which is typically less than 14% (Simonelli *et al.*, 2017). The Proso variety, with the lowest moisture content, therefore has the highest storage potential. The observed moisture levels fall within the ranges reported by Ibrahim *et al.* (2022) and Ikegwu *et al.* (2023), but are higher than those reported in earlier studies (Vandana, 2018). The variations in moisture content could be due to differences in drying methods and the duration of the drying process.

Lipid content, an important factor in determining the shelf life of food products, varied among the millet varieties. De-husked Proso millet, with 0% lipid content, is expected to have a longer shelf life compared to other varieties with higher lipid content, such as whole Foxtail millet, which would be more susceptible to oxidative rancidity due to the presence of unsaturated fatty acids (Iwe *et al.*, 2016). The lipid values obtained in this study were higher than those reported in previous studies (Dhliwayo *et al.*, 2023; Ibrahim *et al.*, 2022), suggesting that these millet varieties could be valuable in terms of their fat content, though this may impact their shelf life.

Protein content is a critical nutritional factor, and the studied millet varieties showed promising levels. The protein content did not differ significantly between whole and de-husked grains, except in Foxtail millet, where it increased upon de-husking. This finding highlights millet as a good source of protein. Proteins are essential building blocks for cell and tissue formation and also contribute to the nutritional value, texture, and sensory properties of food (Awuchi *et al.*, 2019).

The carbohydrate content was relatively high across all varieties, confirming that millets are good sources of energy. The Pearl millet variety, in particular, exhibited significantly higher carbohydrate levels, aligning with the values reported in the FAO's compendium of millet post-harvest (FAO, 2001). This makes millet an important dietary staple, particularly in regions where carbohydrates are a primary energy source. Our findings are consistent with the study of Ibrahim *et al.* (2022) on locally grown millet; however, they are higher than the values reported by Vandana (2018) and Aniket *et al.* (2020) in different cultivars grown in India.

#### *Phytochemical Composition*

Phytochemicals are non-nutritive substances found in plants that contribute significantly to their flavour and colour as well as enhancing the nutritional value and potential health benefits of grains (Pujari and Hoskeri, 2022; Rudzińska *et al.*, 2023). Phytochemicals, such as flavonoids, alkaloids, saponins, and tannins, were present in all millet varieties, with varying concentrations. The high levels of flavonoids and alkaloids are particularly noteworthy, as these compounds contribute significantly to the antioxidant properties of millets. Flavonoids, derived from phenylalanine and tyrosine, are potent antioxidants that help reduce oxidative stress by scavenging free radicals stress (Kumar, 2014). Alkaloids, known for their therapeutic properties, are used in medicine for their stimulant, relaxant, and antimicrobial effects (Owheru *et al.*, 2019; Szewczyk & Pęczek, 2023).

Saponins, present in moderate levels, are valued for their role as natural emulsifiers, foaming agents, and stabilisers in food applications. They also possess cholesterol-lowering, anticancer, anti-inflammatory, antimicrobial, antiviral, and antiparasitic properties, making them nutritionally and pharmaceutically valuable (Mounika *et al.*, 2022; Timilsena *et al.*, 2023). The significant reduction in

phytochemical levels after de-husking suggests that these bioactive compounds are concentrated in the bran layer, which is often discarded during processing. This finding emphasises the nutritional importance of whole grains, particularly in the context of functional foods and supplements.

#### *Amino Acid Profile*

Amino acids are indispensable and abundant biological molecules in living organisms, vital for numerous metabolic functions (Dandare *et al.*, 2021; Ezeonwumelu *et al.*, 2022), and the millet varieties studied showed a favourable amino acid profile. Glutamic acid was the most abundant amino acid, followed by leucine, alanine, proline, and arginine. The relatively high levels of essential amino acids indicate that millets can contribute significantly to dietary protein requirements. The lower levels of tryptophan observed in this study are consistent with previous findings, highlighting a common limitation in millet amino acid profiles (Gowda *et al.*, 2022). The higher amino acid content in whole grains further underscores the nutritional advantage of consuming millets in their whole form and also the use of whole grains for making functional supplements and nutraceuticals.

#### *Vitamin Content*

The study also assessed the vitamin content of the millet varieties, focusing on vitamins C, E, and A. Vitamin C was found in higher concentrations than vitamins A and E, particularly in the whole grain of Proso millet. De-husking resulted in a significant reduction in vitamin C levels across all varieties, which could impact the antioxidant capacity and overall nutritional value of the grains. Vitamin E levels, however, were higher in de-husked grains, suggesting that dehushing might enhance the content of certain fat-soluble vitamins. The impact of de-husking on vitamin A levels varied among the varieties, with increases observed in Foxtail and Finger millet, but a decrease in Proso millet. These findings align with the understanding that processing methods can influence vitamin content, with potential implications for dietary intake and health outcomes.

Kumar *et al.* (2021) reported that the disparities in the content and types of bioactive compounds are largely dependent on the species of millet, a finding that aligns with the results of our study. These variations can be attributed to several factors, including genotypic differences, growing locations, biological activities, and other environmental conditions, all of which significantly influence the composition of bioactive compounds in millet species. Furthermore, our results corroborate the findings of Kumari *et al.* (2017), who observed that antioxidant activities in millets vary depending on the specific variety, production location, and processing methods used. These observations underscore the complex interplay of genetic, environmental, and processing factors in determining the nutritional and functional properties of millets.

#### *Mineral Composition*

This study analyzed the levels of microelements—copper (Cu), iron (Fe), magnesium (Mg), selenium (Se), and zinc (Zn)—and macroelements—calcium (Ca), manganese (Mn), phosphorus (P), and potassium (K)—in four millet varieties cultivated in Northern Nigeria. Significantly higher levels of Se and Mg were observed across all samples compared to other elements.

Millets are important sources of minerals, although their mineral composition can be affected by the presence of anti-nutritional factors like phytates and polyphenols, which inhibit mineral absorption (Nassarawa, 2019). This may explain the trace levels of Fe, Cu, and Zn detected in all varieties analyzed. However, since these minerals are required in small quantities, millets can still serve as good sources of essential nutrients, deficiencies of which can lead to malnutrition. Consuming diets rich in essential nutrients is critical for preventing or eradicating micronutrient deficiencies among vulnerable populations (Abduljalil *et al.*, 2021).

Magnesium was the second most abundant mineral after Se, aligning with the findings of Nassarawa (2019), who reported significantly higher amounts of Mg in millet varieties, excluding Se. Our study also found an abundant amount of Ca compared to other macroelements, consistent with studies by Nassarawa (2019) and Nidhee and Purnima (2023). Several studies have reported higher

levels (almost 95%) of K, Mg, and Ca in millets, while other elements are present in trace amounts (Kent, 2006; Mobolanle et al., 2013; Hassan et al., 2021; Usman et al., 2021). These findings are in line with ours, although Se was the most abundant element in our study, followed by Ca, Mn, K, and P.

Rosentrater and Evers (2017) noted that millets contain all the mineral elements required by the body, which conforms with our findings. Minute quantities of Fe, Cu, Zn, and Mn were detected in all varieties analyzed. The mineral content in millets depends on factors such as soil nitrogen availability—especially due to fertilizer application—water supply, cultivation conditions, and environmental factors (Usman et al., 2021). Our findings also agree with Ramashia et al. (2021), who described millets as a good source of Mg, a critical element in reducing asthma severity, migraine frequency, lowering high blood pressure, and reducing the risk of heart attack.

De-husking significantly increased the level of Se across the varieties, while in some varieties, it decreased the levels of Ca, Mg, P, and K. This suggests that the impact of de-husking depends on both the millet variety and the specific mineral element. Some elements decreased in a particular millet variety and increased in another after de-husking, which is consistent with previous studies (Joseph et al., 2020; Ismaila et al., 2022). Ismaila et al. (2022) revealed that de-hulling positively affected the concentration of mineral elements (K, Mg, Ca, Zn, Fe, and Cu) in millet samples, whereas Joseph et al. (2020) reported a significant decrease in the levels of Mg, Fe, and Zn in de-hulled millet compared to dehulled millets.

Our findings highlight the complex effects of de-husking on the mineral composition of millet varieties. Understanding these effects is crucial for developing processing methods that preserve the nutritional quality of millets, thereby maximizing their health benefits for consumers.

#### *Antioxidant Activity*

The antioxidant activity of the millet varieties was assessed using various assays, including TBA, TPC, FRAP, and DPPH radical scavenging activities. Antioxidants play a vital role in preventing oxidative damage by mechanisms such as inhibiting chain initiation, decomposing peroxides, reducing capacity, and scavenging free radicals (Yildirim *et al.*, 2000). The reducing power of a compound is often correlated with its phenolic content, serving as a key marker of antioxidant potential (Dandare *et al.*, 2014).

Our results demonstrated that de-husking generally led to a decrease in antioxidant activity across the millet varieties. Specifically, TBA levels, which indicate lipid peroxidation, dropped significantly in the Foxtail and Proso millet varieties post-dehusking. Total phenolic content (TPC), a crucial indicator of antioxidant capacity, also decreased in most varieties following de-husking, with the exception of Proso millet, which showed a slight increase. These findings suggest that whole grains possess higher antioxidant activity, essential for protecting against oxidative stress and associated health complications.

The FRAP assay, which measures the electron-donating ability of antioxidants, showed reduced activity in all de-husked millet varieties, with Finger millet demonstrating the highest reducing power. Similarly, the DPPH radical scavenging activity, which reflects the ability to neutralize free radicals, significantly decreased after de-husking. This further underscores the superior antioxidant properties of whole grains compared to their de-husked counterparts.

Overall, the higher antioxidant activity observed in whole grains highlights their potential for health benefits, especially in preventing oxidative damage. De-husking appears to diminish these antioxidant properties, emphasizing the importance of consuming millets in their unprocessed form to maximize their protective effects.

## **5. Conclusions**

The findings of this study underscore the significant nutritional and functional potential of millet varieties cultivated in Nigeria, positioning them as valuable sources of exogenous antioxidants and essential nutrients. The four millet varieties analysed revealed appreciable levels of antioxidant nutrients, which are known to offer a range of health benefits, including the reduction of cancer risk, obesity, diabetes, cardiovascular diseases, and gastrointestinal complications. These antioxidant



properties make millets promising candidates for nutraceutical formulations or as functional foods for promoting human health.

However, it was observed that the nutritional and antioxidant composition of these millet varieties is highly variable, influenced by genetic disparities, environmental conditions, and processing methods. Notably, the de-husking process, which is commonly practiced in Nigerian communities during the preparation of millet-based foods, significantly impacts the antioxidant parameters. The superior fiber, protein, and antioxidant content of the whole grains revealed by this study compared to their de-husked counterparts, indicates a greater nutraceutical capacity and ability to scavenge free radicals and protect against oxidative damage. Thus, emphasising the importance of consuming millets without de-husking.

This finding highlights a critical nutritional consideration: while de-husking is a common practice, it results in a significant loss of bioactive antioxidants, particularly those concentrated in the grain's bran layer. In many urban communities, the bran is often regarded as valueless and discarded, yet this study demonstrates that it contains essential bioactive compounds that are crucial for maintaining good health. Therefore, there is a need to reassess the traditional de-husking methods and promote the consumption of whole grains to maximise the health benefits of millet. By preserving the bran layer, millet can be fully leveraged as a functional food with enhanced nutritional and antioxidant properties, contributing more effectively to nutritional strategies aimed at improving public health and global food security.

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