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# Effect of Natural Fermentation on Nutritional and Antinutritional Values of Pearl and Finger Millet

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## **ABSTRACT**

Pearl millet (Pennisetum glaucum) and finger millet (Eleusine coracana) are common cereal crops in developing regions; however, their potential is hindered by antinutritional factors, including phytates, tannins, and oxalates, which reduce nutrient bioavailability. This study aimed to assess the impact of natural fermentation on the nutritional and antinutritional properties of these foods, thereby improving their value as dietary staples. Laboratory techniques were employed to analyze changes in proximate composition, vitamins, minerals, and antinutritional factors in fermented and unfermented samples, with the results evaluated using ANOVA (p  $\leq$ 0.05). Natural fermentation led to substantial improvements in nutritional quality. Protein content increased from 11.50% to 13.40% in pearl millet and from 8.70% to 11.30% in finger millet. The mineral content was enhanced, with a rise in calcium levels from 332.00 mg/kg to 344.00 mg/kg in finger millet and in potassium from 240.00 mg/kg to 360.00 mg/kg in pearl millet. Natural fermentation also elevated vitamin E levels, from 0.05 mg/100 g to 0.40 mg/100 g in pearl millet and from 0.32 mg/100 g to 0.30 mg/100 g in finger millet. Antinutritional factors also decreased considerably, with phytates dropping by over 70% in both varieties, alongside marked reductions in tannins and oxalates. A slight carbohydrate reduction, which may be attributed to microbial sugar metabolism, was observed. The findings support fermentation as an accessible and effective method to enhance millet's nutritional value, providing a sustainable solution to combat malnutrition and improve food security in resource-constrained areas. This research holds significance for nutritionists, food scientists, and policymakers seeking to optimize global dietary quality.

#### INTRODUCTION

Millet, a category of small-seeded grasses extensively cultivated as cereal crops, possesses considerable nutritional importance and serves as a staple food for millions of people globally. Among the many varieties of millet, pearl millet (Pennisetum glaucum) and finger millet (Eleusine coracana) are two widely consumed types recognized for their resilience and adaptability to varied environmental conditions. These millet varieties are significant sources of carbohydrates and also contain essential nutrients, vitamins, and minerals. Despite being often overlooked in Western dietary practices, millet is cultivated on a global scale for human consumption [1]. Research has shown that, despite its unassuming appearance, millet boasts an impressive nutritional profile, making it an essential component of any diet [2]. Pearl millet (Pennisetum glaucum) and finger millet (Eleusine coracana) are major cereal crops in numerous arid and semi-arid regions, supplying excellent nutrients to millions [2,3].

Pearl millet (Bargi), also referred to as Maiwa in the Hausa language, is an important species of millet cultivated in parts of Africa and Asia. It is well-suited to arid and semi-arid climates and is commonly cultivated in countries such as India, Nigeria, and Niger. The grains of pearl millet are larger than those of finger millet and are typically light yellow or whitish. Like finger millet, pearl millet is a nutritionally dense food that offers various health benefits.

Research has indicated that pearl millet is a commendable source of carbohydrates, providing sustained energy release [3]. It is also abundant in dietary fiber, promoting digestion and enhancing gut health. Furthermore, pearl millet contains essential minerals such as iron, zinc, and phosphorus, which are important for overall well-being [4]. Finger millet, (Ragi), commonly known as Gero in the Hausa Language, is a resilient crop that flourishes in semi-arid regions characterized by poor soil fertility. It is primarily cultivated in Africa and Asia,

particularly in countries such as India, Ethiopia, Uganda, and Nigeria. The grains of finger millet are small and reddish-brown, resembling tiny fingers, which is the origin of its name. The nutritional profile of finger millet is remarkable, making it a valued dietary staple. It is rich in carbohydrates, dietary fiber, and essential minerals such as calcium, iron, and magnesium. Additionally, finger millet contains significant levels of protein, characterized by a balanced amino acid profile [5]. Moreover, finger millet is recognized for its high content of phytochemicals, especially polyphenols and flavonoids, which contribute to its antioxidant characteristics [6].

Nevertheless, the nutritional potential of millet is often compromised by the existence of antinutritional factors such as phytates, tannins, and phenolic compounds, which can hinder the absorption of essential nutrients [7]. Natural fermentation, a method that involves spontaneous microbial activity, has been utilized to enhance the nutritional profile and reduce antinutritional factors in these grains [8]. Fermentation enhances the digestibility of carbohydrates, proteins, and fats, while reducing the levels of antinutritional factors such as phytates and tannins, thereby improving the bioavailability of essential nutrients [9]. The protein content of millet is noteworthy, encompassing essential amino acids that are essential for human health.

Although the amino acid profile varies among millet species, some varieties exhibit higher lysine content than others [10]. In addition to carbohydrates and proteins, millet is an excellent source of dietary fiber, which aids digestion and supports gastrointestinal health. The fiber content of millet contributes to its low glycemic index, making it suitable for individuals with diabetes or those aiming to manage blood sugar levels [11]. Millet grains are enriched with vitamins and minerals essential for multiple bodily functions. They contain a substantial amount of B vitamins, including niacin, thiamine, and riboflavin, which are vital for metabolism and energy production [12]. Millet is particularly distinguished for its high mineral content, which includes iron, magnesium, phosphorus, and zinc, all of which are critical for maintaining bone health, immune function, and overall well-being [13]. This research project intends to evaluate the effects of fermentation on the nutritional and antinutritional value of pearl and finger millet. The process of fermentation improves the digestibility and nutritional profile of these millets, while also enhancing their sensory attributes, thereby increasing their acceptability among consumers.

# MATERIALS AND METHODS

# **Sample Collection**

Samples of pearl millet and finger millet were obtained from the local Yelwa market in Bauchi, Bauchi state. To ensure authenticity, the samples were then authenticated by Dr Rashida Abdulmumini Bala of the Faculty of Agricultural Science Abubakar Tafawa Balewa University, Bauchi, Nigeria.





**Fig. 1.** Pearl Millet (*Pennisetum glaucum*) Plant and Grain, adopted from [14] and [15] respectively.



**Fig. 2.** Finger millet (*Eleusine coracana*) Plant and Grain, adopted from [16] and [17] respectively.

#### **Sample Preparation**

Upon collection, the millet grains were meticulously cleaned with distilled water to eliminate any foreign matter or debris. Subsequently, the grains were air-dried at room temperature to ensure a uniform moisture content. After the drying process, the samples were ground using a sterile mortar and pestle to enhance the surface area for microbial activity. Each sample was then divided into two portions: one portion was placed into a separate, labelled airtight glass container for natural fermentation, designated as FPM (fermented pearl millet) and FFM (fermented finger millet), while the other portions served as controls and were labelled as UPM (unfermented pearl millet) and UFM (unfermented finger millet).

Water was added to the FPM and FFM prior to initiating the natural fermentation process, which involves soaking the samples in water for a predetermined duration to encourage natural fermentation by the indigenous microorganisms present on the grain surface and in the environment. The millets were allowed to ferment undisturbed for 72 hours, with periodic visual inspections conducted to monitor signs of fermentation activity, such as gas production and alterations in odour. Following natural fermentation, the samples were air-dried at room temperature and returned to the airtight glass (Fig. 3) containers to prevent moisture absorption from the atmosphere and microbial contamination before analysis, adopted from [18].



Fig. 3. Fermented pearl and Finger Millet ground into powder.

# **Analytical Methods**

After completion of the fermentation period, both fermented and unfermented millet samples were subjected to drying at ambient temperature to eliminate moisture content. The dried samples were subsequently ground into a fine powder utilizing a laboratory mortar and pestle. The following analytical techniques were employed to assess the nutritional and antinutritional composition of the millet samples: proximate analysis was conducted, and the vitamin content of both pearl and finger millet was analyzed. The mineral composition of the millet samples was determined using atomic absorption spectroscopy (AAS) after digestion with the appropriate acids. Furthermore, antinutritional factors were quantified using standard methodologies as outlined below.

#### **Determination of moisture content**

The method described by [19] was adopted; a clean crucible was dried to a constant weight in an air oven at 110 °C, cooled in a desiccator, and weighed (W1). Two grams of the finely ground sample were accurately weighed into the previously labelled crucible and reweighed (W2). The crucible containing the sample was dried in an oven to constant weight (W3). The percentage moisture content was calculated. The above procedure was followed for the moisture content determination of both the fermented and unfermented samples of the two millet varieties. Thus:

Moisture content 
$$=\frac{W2-W3}{W2-W1} \times 100$$

#### **Determination of ash content**

To determine the ash content, the method of [20] was used. The porcelain crucible was dried in an oven at 100 °C for 10 minutes, cooled in a desiccator, and weighed (W1). Two grams of the finely ground sample were placed into a previously weighed porcelain crucible and weighed (W2). It was then ignited and transferred into a furnace set at 550 °C. The sample was left in the furnace for eight hours to ensure proper ashing. The crucible containing the ash was then removed, cooled in a desiccator, and weighed (W3). The percent ash content was calculated as follows:

% Ash content = 
$$\frac{W3-W1}{W2-W1} \times 100$$

#### Determination of crude lipid content by the Soxhlet method

A clean, dried 500 cm³ round-bottom flask containing a few antibumping granules was weighed (W1) and then filled with 300 cm³ of petroleum ether (40-60 °C) for extraction. The flask was subsequently poured into a Soxhlet extraction unit. The extraction thimble, weighing twenty grams, was fixed into the Soxhlet unit. The round-bottom flask and a condenser were connected to the Soxhlet extractor, and cold water circulation was connected/turned on. The heating mantle was switched on and the heating rate adjusted until the solvent was refluxing at a steady rate. Extraction was carried out for 6 hours. The solvent was then covered, and the oil was dried in the oven set at 70 °C for 1 hour. The round-bottom flask and the oil were weighed (W2) [21]. The above procedure was followed to determine the crude fibre for the second sample (FFM) and the unfermented samples (UPM and UFM). The lipid content was calculated thus:

% Crude Lipid content = 
$$\frac{W2-W1}{Weight of sample} \times 100$$

#### **Determination of crude fiber**

2 g of FPM was weighed into a round-bottom flask, 100 cm³ of sulfuric acid solution was added, the mixture was boiled under reflux for 30 minutes, and the hot solution was quickly filtered under suction. The insoluble matter was washed several times with hot water until it was acid-free. It was quantitatively transferred into the flask, and 100 cm³ of hot 0.31 M sodium Hydroxide solution was added. The mixture was boiled under reflux for 30 min and filtered under suction. The residue was washed with boiling water until it was base-free, dried to a constant weight in an oven at 100 °C, cooled in a desiccator, and weighed (C1). The weighed sample (C1) was then incinerated in a muffle furnace at 550 °C for 2 hours, cooled in a desiccator, and reweighed (C2) [19].

The loss of weight on incineration =  $C_1$ - $C_2$ 

% Crude fibre = 
$$\frac{C1-C2}{Weight\ of\ original\ sample} \times 100$$

#### Determination of nitrogen and crude protein

The ground defatted sample A (91.5 g) in the ashless filter study was dropped into a 300 cm<sup>3</sup> Kjeldahl flask. The flask was then transferred to the Kjeldahl digestion apparatus. The sample was digested until a clear green color was obtained. The digest was cooled and diluted with 100 cm<sup>3</sup> of distilled water. The above procedure was followed for the second sample (FFM) and the unfermented samples (UPM and UFM) [22].

**Distillation of the digest:** Into a 500 cm³ Kjeldahl flask containing antibumping chips and 40 cm³ of 40% NaOH, a mixture of 50 cm³ 2% boric acid and three drops of mixed indicator was slowly added to trap the ammonia being liberated. The conical flask and the Kjeldahl flask were then placed on the Kjeldahl distillation apparatus, with the tube inserted into the conical flask. Heat was applied to distill out the NH3 evolved, and the distillate was collected into a boric acid solution. The distillate was then titrated with 0.1 M HCl [22].

$$\% N_2 = \frac{14 \times M \times Vt \times V100}{Weight of the sample (mg) \times Va}$$

#### Where

 $\label{eq:continuous} \begin{tabular}{ll} \% \ Crude \ Protein = \% \ N_2 \ (Nitrogen) \times 6.35 \\ M = Actual \ Molarity \ of \ acid \\ V = Titre \ Value \ (Volume) \ of \ HCl \ used \\ Vt = Total \ volume \ of \ diluted \ digest \\ Va = Aliquot \ volume \ distilled \\ Va = Aliquot \ volume \ distilled \\ \end{tabular}$ 

#### **Determination of Ascorbic Acid**

For ascorbic acid determination, the iodometric method by [23] was used. 0.1 g of fermented pearl millet (FPM) was taken with a solution of metaphosphoric acid (3%), acetic acid (7.98%), and then centrifuged at 2365 × g (4000 rpm) for 10 minutes. The supernatant was used for the determination of ascorbic acid by the titrimetric method. A standard solution of sodium thiosulfate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>) at a concentration of 0.05 mM and a starch indicator was used. A burette was filled with 0.05 mM sodium thiosulfate and titrated against the analyte (10 mL of supernatant, 10 mL of iodine solution, and three drops of starch indicator) and the standard analyte (10 mL of vitamin C (1%), 10 mL of iodine solution, and three drops of starch indicator) until the color changed from blue-black to colorless. The volume of the thiosulfate that resulted in the color change was recorded. To know the concentration of the iodine solution and the amount of ascorbic acid in the sample that reacted with the iodine, the thiosulfate solution was titrated against a blank, which was composed of distilled water, iodine solution, and starch indicator. The concentration of ascorbic acid in the samples was determined as follows:

Concentration in the extract (mg/100g) = 25y/b

Where b is the titer (mL) from the titration of the standard ascorbic acid solution. y = titer (mL) from the titration of the sample solution. The iodine solution was prepared by dissolving 5.00 g of potassium iodide (KI) and 0.268 g of potassium iodate (KIO<sub>3</sub>) in 200 mL of distilled water and then adding 30 mL of 3 M sulfuric acid before making up the volume to 500 mL with distilled water. The above procedure was followed for the FFM and the unfermented samples (UPM and UFM).

# **Determination of Vitamin E**

For vitamin E, the [23] method was used. A  $0.1\,\mathrm{g}$  sample of FPM was taken with  $10\,\mathrm{mL}$  of a hexane: isopropanol solution (3:2 v/v), agitated for 5 hours, and then centrifuged for  $10\,\mathrm{minutes}$ . The supernatant was used for the determination of vitamin E. Exactly  $0.1\,\mathrm{mL}$  of supernatant was mixed in a test tube with  $1\,\mathrm{mL}$  of reagent solution ( $0.6\,\mathrm{M}$  sulfuric acid,  $28\,\mathrm{mM}$  sodium

phosphate, and four mM ammonium molybdate) and incubated at 37 °C for 90 min with vigorous shaking. The absorbance of the aqueous phase at 695 nm was measured against the appropriate blank. A typical blank contained 1 mL of reagent solution and 0.1 mL of pure hexane, and it was incubated under the same conditions as the samples. The above procedure was followed for the FFM and the unfermented samples (UPM and UFM). The quantitation of vitamin E was based on the molar absorption coefficient of the phosphomolybdenum complex.

Vitamin E (mg/100g extract) = Abs/molar ext. coeff. (4000 M<sup>-1</sup>cm<sup>-1</sup>)

#### **Determination of Vitamin A**

Vitamin A content was determined using the spectrophotometer method, as described by [23]. Two milliliters (2 mL) of the FPM sample were measured into a test tube with a tight stopper and labeled as test tube 1. One milliliter of a 1 M potassium hydroxide solution (in 90% ethanol) was added to the liquid in test tube one and shaken vigorously for 1 minute. The test tube one was heated in a water bath at 60 °C for 20 minutes and then cooled using cold water. Shaken for 1 minute, and then centrifuged at 1500 rpm for 10 minutes. The supernatant (upper layer) was collected and transferred into another test tube labeled Test Tube II. The extract was analyzed by measuring the absorbance at a wavelength of 335 nm. The extract in the test tube labelled II was exposed to UV light for 30 minutes, and the absorbance was measured and named. The above procedure was followed for the FFM and the unfermented samples (UPM and UFM).

#### Determination of carbohydrate by difference

The total carbohydrate was determined by difference. The sum of the percentage moisture, ash, crude lipid, crude protein, and crude fibre was subtracted from 100 [24].

 $\%Total\ carbohydrate = 100 - (\%moisture + \%Ash + \%Fat + \%Protein + \%Fibre)$ 

# **Determination of the mineral elements**

The ground FPM, FFM, UPM, and UFM (5.0 g each) were inserted into four well-labelled crucibles and placed in a muffle furnace, where they were ashed at 500 °C and then cooled. Three mL of concentrated HCl was then evaporated to dryness. Twenty cm³ 25% HCl was then added to each residue to extract the mineral. The extracts were quantitatively transferred to 100 cm³ volumetric flasks each, and the volume was made up to the mark with distilled water. The digest was then used directly for element determination using the Atomic Absorption Spectrophotometer [25].

#### Preparation of standard

For each mineral, a standard range of 1-16 ppm was prepared from the stock solution and was used to calibrate the equipment in concentration mode.

## Determination of phytic acid

Phytic acid was determined by the procedure of [26]. A 2.0 g of the samples was weighed into a 250 mL conical flask. One hundred milliliters of 2% concentrated HCl was used to soak the sample for 3 hours, and then it was filtered with Whatman No. 1 filter paper. Fifty cm³ of the filtrate and 10 cm³ of distilled water were added in each case to achieve the proper acidity. Ten mL of 0.3% ammonium thiocyanate solution was added to the solution as indicated and titrated with standard Iron II chloride solution containing 0.00195 g Iron/mL, end point observed to be yellow, which persisted for 5 minutes. The same procedure was carefully followed for the analysis of the four samples. The percentage phyatic acid was calculated thus:

% Phytic acid =  $y \times 1.19 \times 100$ 

Where,

y = titre value  $\times$  0.00195 q

#### **Test for Tannins**

A gram of the FPM sample was extracted with 25 mL of a 80:20 acetone:  $10\,\%$  glacial acetic acid solution for 4 hours. It was then filtered and measured at an absorbance of 500 nm. The absorbance of the reagent blank was also measured. A standard graph with 10, 20, 30, 40, and 50 mg/100 g of tannic acid was used. The concentration of the tannins was read, taking into consideration the dilution factor [27]. For FFM, UPM, and UFM, the same procedure was followed.

#### Statistical Analysis and Data Analysis

Data collected from the various analyses were statistically analysed to compare the nutritional and antinutritional composition of fermented and non-fermented millet samples. Mean and standard Deviations (SD) were calculated for all measured variables for the triplicate values. One-Way Analysis of Variance (ANOVA) is used, followed by Post-hoc Multiple Comparison Test to determine the Significant differences between the groups, which were pointed out using different superscripts (a, b, or ab) within the same row to indicate that these values differ significantly from each other at a significance level of p $\leq$ 0.05.

#### RESULTS AND DISCUSSION

#### **Proximate Analysis**

Table 1 presents the significant effects of natural fermentation on nutritional components, as determined by proximate analysis of the millet samples. The results indicate alterations in moisture, protein, crude fat, ash, crude fiber, carbohydrate content, and energy values. The moisture content remained consistent in the un-fermented samples, but for the fermented samples, the moisture content have greatly increase from 10.40% and 11.20% to 11.20% and 12.20% for the pearl and finger millets respectively at a significance level of p≤0.05 compared to the unfermented samples, the result aligns with the studies of [28] which reported a significant increase of the moisture content of pearl millet at p≤0.05 after fermentation. This increase is likely attributable to water absorption during the fermentation process.

**Table 1**. Effect of fermentation on the proximate composition of millet samples.

Parameter	UPM	FPM	UFM	FFM
Moisture Content (%	$0.10.40 \pm 0.10^{a}$	$11.20 \pm 0.15^{b}$	$10.10 \pm 0.12^a$	$12.20 \pm 0.20^{b}$
Protein Content (%)	$11.50 \pm 0.30^{a}$	$13.40 \pm 0.35^{b}$	$8.70\pm0.25^a$	$11.30 \pm 0.28^{b}$
Crude Fat (%)	$4.20\pm0.12^{\rm a}$	$4.00\pm0.10^a$	$4.10\pm0.11^a$	$4.50 \pm 0.14^{b}$
Ash Content (%)	$2.10\pm0.05^a$	$2.30 \pm 0.06^{b}$	$2.00\pm0.04^a$	$2.50 \pm 0.07^{b}$
Crude fiber (%)	$7.80\pm0.20^a$	$7.50\pm0.18^a$	$8.00\pm0.22^a$	$6.80 \pm 0.17^{b}$
Carbohydrate (%)	$65.00 \pm 2.00^a$	$62.70\pm1.80^a$	$68.00 \pm 2.20^{b}$	$63.70 \pm 1.90^{a}$
Energy (kcal)	$364.30 \pm 3.50$	$a^{a}361.10 \pm 3.00$	$a343.10 \pm 3.20$	$^{b}372.80 \pm 2.50^{a}$
Note: Values were expressed as mean ± standard deviation for N=3. Values in the same row				
carrying different superscripts (a, b, or ab) differ significantly from each other (p≤0.05). Keys:				

Note: Values were expressed as mean ± standard deviation for N=3. Values in the same row carrying different superscripts (a, b, or ab) differ significantly from each other (p≤0.05). Keys: UPM: Unfermented Pearl Millet, FPM: Fermented Pearl Millet, UFM: Unfermented Finger Millet, FFM: Fermented Finger Millet

Natural fermentation significantly influenced the protein content, resulting in increases in both fermented pearl millet (FPM) and fermented finger millet (FFM), with values of 13.40% and 11.30%, respectively, compared to their unfermented counterparts, which had values of 11.50% and 8.70%, respectively (**Table 1**). The findings were supported by the research of [29], which reported an increase of 5% protein content after fermenting cereal food. This enhancement suggests that fermentation improves protein digestibility and availability by breaking down complex protein structures, as supported by

previous research [30] that recorded an increase in protein quality and bioavailability after the fermentation of cereals and legumes. The crude fat content exhibited minor fluctuations, with FFM showing a slight but significant increase from 4.10% to 4.50% while the fat content of the pearl millet did not significant change at p≤0.05 after fermentation although it contradict the findings of [31] which reported a decrease by 8.7% and 17% after 24 and 48 hours natural fermentation of black-colour oat. However, the result suggests that fermentation has a minimal impact on the fat content of millet, as reported by [32], which found that fermentation caused no change in the lipid content of fermented pearl millet.

The ash content, which reflects the total mineral content, showed a slight increase in the fermented samples, with values of 2.30% for FPM and 2.50% for FFM, compared to 2.10% and 2.00% in the unfermented samples (**Table 1**) [33] Also reported a significant (p≤0.05) increase in the ash content, ranging from 2.00 to 3.10% on pearl millet after fermentation. This increment may suggest an enhanced release of minerals as a result of the breakdown of antinutritional factors during fermentation, corroborated by the findings of [20]. Natural fermentation also resulted in a decrease in crude fiber content across both millet types, with a more pronounced reduction observed in FFM, which decreased from 8.00% to 6.80%, which tallies with the research made by [34] that fermentation significantly decreases crude fibre of pearl millet-acha with an increase in pearl millet.

The reduction may be beneficial for individuals who require a lower dietary fiber intake [35]. The carbohydrate content slightly decreased in both fermented millet types, likely due to microbial utilization of carbohydrates as an energy source during fermentation [36]. In Table 1, FPM recorded a carbohydrate content of 62.70%, while FFM recorded 63.70%, compared to 65.00% and 68.00% in the unfermented samples. A similar trend was observed in [33], which discovered that the unfermented pearl millet has 68.08% compared to the fermented pearl millet. The reduction may correspond with the slight decrease in energy values, particularly in FPM. The proximate analysis reveals that fermentation increases moisture, protein, and ash content, while decreasing crude fiber and carbohydrate levels. These changes positively impact the nutritional profile of millet, making it a more favorable option for human consumption, particularly in regions where millet is a staple food.

#### Vitamin Analysis

The investigation into the vitamin content of pearl and finger millet, both before and after fermentation, indicated significant alterations in the levels of water-soluble and fat-soluble vitamins, as illustrated in **Table 2** below. These alterations reflect the biochemical processes that transpire during fermentation, resulting in considerable modifications to the nutritional profile of millet [37]. Notably, Vitamin C concentrations were highest in unfermented pearl millet, at 2.60 mg/100 g, but decreased significantly after fermentation to 1.20 mg/100 g. A similar trend was observed in unfermented finger millet, with a decrease from 1.8 mg/100 g to 0.90 mg/100 g in the fermented finger millet. A similar reduction was observed in [38] after fermentation of vegetables.

The decline may be linked to oxidative degradation or microbial activity occurring during the fermentation process. Vitamin C is particularly susceptible to environmental factors, and its reduction is consistent with the findings of comparable research on fermented plant-based products, as noted by [39]. In contrast, Vitamin E (**Table 2**) levels displayed varying alteration after fermentation of the two millet varieties, with pearl millet

displaying a significant increase from 0.05 mg/100 g to 0.4 mg/100 g for UPM and FPM, respectively. In contrast, no significant difference was seen for the finger millet at 0.32 mg/100 g and 0.30 mg/100 g, respectively. A study [40] noted that fermentation leads to a reduction in vitamin E content; however, the bioavailability depends on several variables that can mitigate this effect when properly managed. This enhancement may be due to microbial synthesis or the liberation of bound forms during fermentation, a phenomenon frequently reported in studies focusing on lactic acid fermentation. The increase in Vitamin E levels is of nutritional importance, considering its role as a potent antioxidant [41]. Among the B vitamins, thiamine (B1) and riboflavin (B2) exhibited varying responses.

Table 2. Effect of fermentation on the vitamin content of millet samples.

Vitamin	UPM	FPM	UFM	FFM
C (mg/100g)	$2.60 \pm 0.12^{a}$	$1.20 \pm 0.10^{b}$	$1.80 \pm 0.08^{b}$	$0.90 \pm 0.05^{b}$
E (mg/100g)	$0.05 \pm 0.02^{b}$	$0.40\pm0.03^a$	$0.32\pm0.03^{ab}$	$0.30\pm0.02^{ab}$
B1 (mg/100g)	$0.38\pm0.04^a$	$0.31\pm0.03^{ab}$	$0.42\pm0.05^a$	$0.28 \pm 0.03^{b}$
B2 (mg/100g)	$0.29\pm0.03^a$	$0.12 \pm 0.02^{b}$	$0.19\pm0.02^{ab}$	$0.10\pm0.01^{b}$
B9 (mg/100g)	$0.04 \pm 0.01^{b}$	$0.05\pm0.01^a$	$0.04 \pm 0.01^{b}$	$0.05\pm0.01^a$
K (mg/100g)	$0.01 \pm 0.01^{b}$	$1.80\pm0.15^{\rm a}$	$0.01 \pm 0.01^{b}$	$1.60\pm0.12^a$
A (mg/100g)	$23.30\pm1.00^a$	$20.10\pm0.90^a$	$9.50 \pm 0.80^{b}$	$7.50 \pm 0.75^{b}$

Note: Values were expressed as mean ± standard deviation for N=3. Values in the same row carrying different superscripts (a, b, or ab) differ significantly from each other (p≤0.05). Keys: UPM: Unfermented Pearl Millet, FPM: Fermented Pearl Millet, UFM: Unfermented Finger Millet, FFM: Fermented Finger Millet, C: Ascobic Acid, E=Tocopherol, B1: Thiamine, B2: Riboflavin, B9=Folate, K: Phylloquinone/Menaquinone/Menadione, A: Retinol

Thiamine level decreases significantly after (p≤0.05) in the two millet varieties from 0.38 mg/100 g to 0.31 mg/100 g and 0.42 mg/100 g and 0.28 mg/100 g for the pearl and finger millet, respectively. A similar report was published by [42], which highlights the fact that factors such as temperature and the type of microorganism can affect the level of thiamine in a given sample during fermentation. Riboflavin exhibited a similar pattern, with a notable reduction in the levels of the millet varieties. The pearl millet decreases from 0.29 mg/100 g to 0.12 mg/100 g after fermentation, while the finger millet decreases from 0.19 mg/100 g to 0.10 mg/100 g after fermentation. Research of [43] also highlighted a similar trend and additionally found that it is temperature-dependent. These reductions may be attributed to microbial utilization or chemical degradation occurring under the acidic conditions of fermentation [41].

Folate (B9) levels experienced a modest increase in the fermented samples, with both fermented samples, FPM and FFM, containing 0.05 mg/100 g, compared to 0.04 mg/100 g in the unfermented samples. Report of [44] shows that folate content can be increased when Lactic Acid Bacteria like *L. plantarum*, *L. fermentum*, and the subject species are used. This enhancement may result from the microbial synthesis of folate during fermentation, a phenomenon also observed in other grainbased fermented foods [45]. Vitamin K displayed a significant increase in the fermented samples, with FPM at 1.80 mg/100 g and FFM at 1.60 mg/100 g, both exhibiting considerably higher levels than their unfermented counterparts.

The findings correlate with those of [46], with the fact that some microorganisms are well-known for producing vitamins, such as Streptococcus *thermophilus* and Propionibacteria as such this increase is likely due to the metabolic activity of fermenting microorganisms, which are known to produce Vitamin K. This enhancement is particularly advantageous, as Vitamin K helps in blood clotting and bone health [47]. Conversely, Vitamin A levels decreased in the fermented samples, with UPM (23.30 mg/100 g) and FPM (20.10 mg/100 g) showing higher levels compared to UFM (9.50 mg/100 g) and FFM (7.50 mg/100 g), as seen in **Table 2** above. The outcome aligns with [48], which

reports a more than 50% decrease in the level of vitamin A after ensiling a mixture containing different types of herbage. The decline may be associated with oxidative or enzymatic degradation during fermentation, which can affect the stability of carotenoids [49]. Overall, fermentation exhibited mixed effects on vitamin content, resulting in a reduction of water-soluble vitamins, such as Vitamin C, B1, and B2, while fat-soluble vitamins, like Vitamin E and K, saw an increase [38]. These outcomes suggest that fermentation can enhance the nutritional profile of millet by increasing the levels of specific vitamins while decreasing others, as reported by [37]. By adjusting fermentation parameters, specific nutritional objectives could potentially be optimized.

#### **Mineral Analysis**

The examination of mineral content in pearl and finger millet indicated significant alterations following fermentation. Table 3 displays the levels of iron exhibited by a contrasting pattern between the two millet varieties. In pearl millet, the iron content experienced a slight decrease from 6.30 mg/kg in UPM to 5.40 mg/kg in FPM. In contrast, finger millet showed an increase in iron content from 4.00 mg/kg in UFM to 7.30 mg/kg in FFM. This corresponds to the report of [30], which also reports an increase in the amount of iron after fermentation. The variations may be attributed to the differing microbial activities and enzymatic processes that occur during the fermentation. As such, it can either enhance or diminish the bioavailability of iron, depending on the matrix composition and fermentation conditions [50]. Additionally, magnesium content shows a slight reduction across the millet varieties after fermentation, with levels in pearl millet decreasing from 135.00 mg/kg in UPM to 128.00 mg/kg in FPM.

Table 3. Effect of fermentation on the mineral content of millet samples.

Mineral (mg/Kg)	UPM	FPM	UFM	FFM
Iron	$6.30\pm0.30^a$	$5.40 \pm 0.25^{b}$	$4.00 \pm 0.20^{b}$	$7.30\pm0.35^a$
Magnesium	$135.00 \pm 5.00^a$	$128.00 \pm 4.00^{b}$	$120.00 \pm 3.50^{b}$	$138.00 \pm 5.50^a$
Zinc	$3.80\pm0.15^a$	$3.50 \pm 0.14^{ab}$	$3.20 \pm 0.12^{b}$	$4.00\pm0.18^a$
Calcium	$27.00 \pm 0.21^{b}$	$28.00 \pm 0.23^{b}$	$332.00 \pm 0.47^{\rm a}$	$344.00 \pm 0.49^a$
Potassium	$240.00 \pm 0.32^{b}$	$360.00 \pm 0.44^{a}$	$252.00 \pm 0.39^{b}$	$480.00 \pm 0.52^{\rm a}$
Phosphorus	$294.00 \pm 0.34^{ab}$	$285.00 \pm 0.31^{b}$	$315.00 \pm 0.38^a$	$283.00 \pm 0.29^{b}$

Note: Values were expressed as mean ± standard deviation for N=3. Values in the same row carrying different superscripts (a, b, or ab) differ significantly from each other (p≤0.05). Keys: UPM: Unfermented Pearl Millet, FPM: Fermented Pearl Millet, UFM: Unfermented Finger Millet, FFM: Fermented Finger Millet.

In a similar vein, finger millet exhibited a decline in magnesium from 138.00 mg/kg in UFM to 120.00 mg/kg in FFM. Although our findings are contrary [51], which suggest that germination decreases the magnesium content of Samh seed, fermentation enhances it. This decrease may be attributed to the microbial utilization of magnesium as a cofactor during metabolic processes involved in fermentation. Notwithstanding this reduction, the magnesium levels remained sufficiently substantial to fulfil dietary requirements [52]. **Table 3** also shows the zinc content in finger millet, which displays a modest increase from 3.20 mg/kg in UFM to 4.00 mg/kg in FFM, whereas a slight decrease was noted in pearl millet, with levels falling from 3.80 mg/kg in UPM to 3.50 mg/kg in FPM.

These variations may result from the microbial degradation of zinc-binding antinutritional factors, which enhances its bioavailability in finger millet but not to the same degree in pearl millet [53]. Calcium levels increased significantly post-fermentation across both millet types. In pearl millet, the content rose from 27.00 mg/kg in UPM to 28.00 mg/kg in FPM. Similarly, in finger millet, calcium levels increased from 332.00 mg/kg in UFM to 344.00 mg/kg in FFM. This elevation is likely

due to the breakdown of calcium-chelating compounds, such as phytates, during fermentation, which liberates bound calcium and thereby enhances its bioavailability, as reported by [39]. This suggests that the increase is due to the reduction of polyphenolic compounds after fermentation. Potassium levels also followed an upward trend, with pearl millet increasing from 240.00 mg/kg in UPM to 360.00 mg/kg in FPM and finger millet from 252.00 mg/kg in UFM to 480.00 mg/kg in FFM (Table 3). This increase may be attributed to the release of potassium from cellular components as the grains undergo microbial and enzymatic breakdown during fermentation.

The significance of potassium in maintaining electrolyte balance leads to the importance of this increase for nutritional enhancement [54]. Conversely, phosphorus levels exhibited a decline in both millet varieties. The phosphorus content in pearl millet decreased from 294.00 mg/kg in UPM to 285.00 mg/kg in FPM, while in finger millet, it fell from 315.00 mg/kg in UFM to 283.00 mg/kg in FFM. [55] reported that a decreasing amount of phosphorus occurs with an increase in the fermentation time. This reduction could be the result of microbial consumption or the transformation of phosphorus into less bioavailable forms during fermentation [56].

#### **Antinutritional Analysis**

The discoveries on antinutritional factors focus on the major impact of fermentation on improving the nutritional quality of millet by reducing compounds that hinder nutrient absorption. Three primary antinutritional factors (phytates, tannins, and oxalates) were assessed as shown in **Table 4** below, and all showed ample reductions after fermentation.

Table 4. Effect of fermentation on antinutritional factors in millet samples.

Anti- Nutrient (mg/100g)	UPM	FPM	UFM	FFM
Phytate	$900.00 \pm 20.00^a$	$250.00 \pm 15.00^{b}$	$800.00 \pm 18.00^{ab}$	$200.00 \pm 10.00^{b}$
Tannins	$150.00 \pm 5.00^a$	$100.00 \pm 4.00^{b}$	$140.00 \pm 6.00^{ab}$	$90.00 \pm 3.00^{b}$
Oxalates	$30.00 \pm 2.00^{a}$	$15.00 \pm 1.00^{b}$	$28.00 \pm 2.50^{ab}$	$12.00 \pm 0.80^{b}$

Note: Values were expressed as mean ± standard deviation for N=3. Values in the same row carrying different superscripts (a, b, or ab) differ significantly from each other (p≤0.05). Keys: UPM: Unfermented Pearl Millet, FPM: Fermented Pearl Millet, UFM: Unfermented Finger Millet, FFM: Fermented Pearl Millet, FFM: Fermented Finger Millet

Phytates, known for their ability to bind essential minerals such as calcium, zinc, and iron, thereby diminishing their bioavailability [56], exhibit a notable decrease. In pearl millet, the levels reduced from 900.00 mg/100 g in the unfermented sample (UPM) to 250.00 mg/100 g in the fermented version (FPM). A similar trend was observed in finger millet, where the values declined from 800.00 mg/100 g in the unfermented sample (UFM) to 200.00 mg/100 g in the fermented form (FFM). This corresponds to the [57] report of a mean decrease in phytic acid in finger millet, with 72.3% and 54.3% after 96 and 72 hours, respectively (**Table 4**).

The reduction is attributed to the enzymatic activity of microbial phytases produced during fermentation, which hydrolyze phytate into simpler, non-chelating forms. This enzymatic breakdown significantly enhances mineral bioavailability, which is particularly beneficial for populations that depend on millet as a staple food [58]. Additionally, tannins, known to inhibit protein digestibility and mineral absorption, also showed considerable reductions. In pearl millet, tannin levels decreased from 150.00 mg/100 g in UPM to 100.00 mg/100 g in FPM, while finger millet tannins dropped from 140.00 mg/100 g in UFM to 90.00 mg/100 g in FFM. The result aligns with that of

[59], which reports a 41.32% decrease in tannins after 48 hours of fermentation. The decrease in tannins can be attributed to microbial activities during fermentation, which likely degrade tannin structures or reduce their concentration by binding them to other components, thus diminishing their bioactivity as reported by [27]. Oxalates, which can disrupt calcium absorption and contribute to the formation of kidney stones, were similarly diminished [60].

In pearl millet, oxalate levels decreased from 30.00 mg/100 g in UPM to 15.00 mg/100 g in FPM. Finger millet experienced a comparable reduction, with levels falling from 28.00 mg/100 g in UFM to 12.00 mg/100 g in FFM, which [59] also recorded a 76.11% reduction after 48 hours of fermentation. This decrease is likely the result of microbial metabolism during fermentation, which converts oxalates into less harmful compounds, thereby improving calcium bioavailability. The reductions in these antinutritional factors strengthen the efficacy of fermentation as a processing technique to enhance the nutritional profile of millet [61].

#### **CONCLUSION**

This research has shown the effects of natural fermentation on the nutritional and antinutritional values of pearl and finger millet, yielding several significant findings. The study concludes with the contribution that traditional fermentation substantially enhances the nutritional profile of both pearl and finger millet. In particular, the natural fermentation process increases the concentration of some nutritional components but also slightly decreases the concentration of most of the nutritional components in the two millet varieties. On the other hand, fermentation effectively diminishes the levels of antinutritional factors such as phytic acid and oxalates. Consequently, the bioavailability of nutrients is improved, making millets a more effective source of essential nutrients. Fermentation using a specific microbe may enhance the nutritional availability of our diets. However, individuals should understand their specific nutritional needs in order to make informed choices regarding the required millet variety. Further research is necessary to optimize the fermentation process and bring about various techniques to maximize the benefits observed in this study. Such advancements can enhance the nutrient content and bioavailability, leading to greater consumer acceptance and thereby improving the overall nutritional content of millets, which is important for addressing nutritional deficiencies, especially in malnourished developing countries.

# DATA AVAILABILITY STATEMENT

Data are available on request.

#### CONFLICTS OF INTEREST

The authors declared that no conflict of interest.

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