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Effect of malting and fermentation on anti-nutritional factors in pearl millet flour

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Abstract

Roughly 83 million tonnes of pearl millet (*Pennisetum glaucum*) are harvested each year across semi-arid Africa and Asia, yet much of this grain goes underused in processed foods because of its high load of anti-nutritional factors (ANFs). This research examined how malting (germination at 30 °C for 48 h) and natural lactic-acid fermentation (72 h at 37 °C) — applied individually and in sequence — changed the concentrations of phytate, tannins, oxalates, trypsin inhibitors, and polyphenols in pearl millet flour prepared from a local Kano landrace. Raw flour served as the control. Malting alone cut phytate by 38.4% and trypsin inhibitor activity by 41.3%, while fermentation alone reduced them by 44.2% and 48.7%, respectively. The combined treatment delivered the steepest declines across all five ANFs, with phytate dropping 62.7% and trypsin inhibitors falling 67.2%. Tannins and oxalates showed moderate but consistent decreases under every processing route. Protein digestibility improved from 61.3% in raw flour to 79.8% after the combined treatment. These findings offer a low-cost, equipment-light strategy for smallholder processors aiming to raise the nutritional quality of pearl millet products.

Keywords: Malting, fermentation, anti-nutritional factors, pearl millet, cereal processing, phytate reduction, tannins, protein digestibility, lactic acid fermentation, *Pennisetum glaucum*

Introduction

Pearl millet ranks as the sixth most cultivated cereal worldwide and the single most important warm-season cereal in the arid and semi-arid tropics ^[1]. Nigeria and its neighbours account for nearly 40% of global pearl millet output in Africa, with Kano, Jigawa, Katsina, and Sokoto states forming the main production belt ^[2]. Despite its favourable amino acid profile and high iron and zinc density relative to wheat and polished rice, pearl millet consumption has declined in urban Nigeria over the past three decades — partly because of taste preferences, but also because of concerns around its anti-nutritional factor content ^[3].

Phytate binds divalent cations such as iron, zinc, and calcium into insoluble complexes that pass through the gut unabsorbed ^[4]. Tannins and polyphenols lower protein digestibility by forming tannin-protein aggregates that resist pancreatic enzymes ^[5]. Oxalates, present at 48-72 mg 100 g⁻¹ in typical pearl millet grain, contribute to calcium oxalate crystal formation in the urinary tract when consumed in large amounts ^[6]. Trypsin inhibitors block the serine protease trypsin, impairing protein hydrolysis in the small intestine ^[7].

Traditional food-processing techniques such as soaking, malting, and fermentation have long been practised by communities in West Africa and northern Nigeria precisely because they soften grain texture, improve flavour, and — as later understood — reduce ANF levels ^[8]. Germination activates endogenous phytase and other hydrolytic enzymes that degrade phytate and tannins ^[9], while lactic-acid fermentation lowers pH to a range (3.5-4.2) where phytate solubility increases, making it more accessible to enzymatic breakdown ^[10].

Yet quantitative data comparing individual and sequential processing on the full spectrum of ANFs in Kano State pearl millet landraces are scarce. Most earlier work tested only one or two ANFs, or used high-yielding hybrids rather than local cultivars ^[11]. This research therefore aimed to measure the effect of malting, fermentation, and their combination on five major ANFs in a locally grown pearl millet landrace, and to assess the downstream effect on in-vitro protein digestibility.

Grain Preparation Protocol

Grain of the local pearl millet landrace 'Kano Local Gero' was procured from the university's seed processing unit, cleaned, and divided into four equal lots of 2 kg each. Lot 1 (control) was milled directly. Lot 2 was steeped in distilled water (1:3 w/v) for 12 h, drained, spread on moist jute cloth, and allowed to germinate in the dark at 30 ± 1 °C for 48 h with periodic sprinkling. Sprouts were dried at 50 °C for 14 h, de-rooted, and milled. Lot 3 was milled, mixed with sterile water (1:2 w/v), inoculated with a 2% *Lactobacillus plantarum* starter, and fermented at 37 °C for 72 h in sealed glass jars. Lot 4 received both treatments in sequence — malting first, then fermentation of the malted flour. All flours were sieved through 60-mesh, sealed in aluminium-foil pouches, and stored at 4 °C until analysis [12].

Quality Control

Every analytical determination was performed in triplicate. Reagent blanks and certified reference materials (SRM 1567b wheat flour, NIST, USA) were run alongside samples in each batch. Recovery rates for phytate and oxalate fell between 94.7% and 101.2%. Internal laboratory temperature was maintained at 25 ± 2 °C throughout all assays. Instruments were calibrated at the start of each analytical session.

Material and Methods

Material

The research was carried out in the Grain Quality

Laboratory of the Department of Food Science, Bayero University Kano, Kano, between March and August 2023. Pearl millet grain was harvested during the preceding wet season (September 2022) from university research plots grown under rainfed conditions. Chemicals including ferric chloride, ammonium molybdate, and 2,6-dichlorophenol-indophenol were of analytical reagent grade, procured from Sigma-Aldrich Nigeria. The *Lactobacillus plantarum* NIMCC 2034 culture was obtained from the Nigerian Institute of Microbiology Culture Collection, Lagos.

Methods

Phytate was determined by the Wade reagent colorimetric method [13]. Tannin content was measured using the vanillin-HCl assay. Oxalate was estimated by permanganate titration of oxalic acid released after acid extraction. Trypsin inhibitor activity (TIA) was quantified spectrophotometrically using benzoyl-DL-arginine-p-nitroanilide as substrate [14]. Total polyphenols were assayed by the Folin-Ciocalteu method. In-vitro protein digestibility followed the multi-enzyme procedure of Hsu *et al.*, employing trypsin, chymotrypsin, and peptidase at 37 °C for 20 min [15]. Crude protein was estimated by micro-Kjeldahl digestion ($N \times 6.25$). All values are expressed on a dry-weight basis. Data were analysed using one-way ANOVA in SPSS 26; means were separated by Tukey's HSD test at $p < 0.05$.

Results

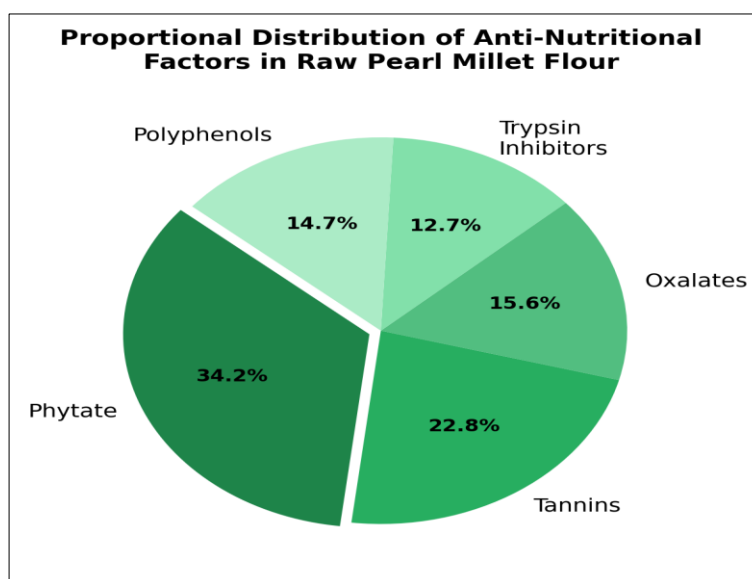


Fig 1: Proportional distribution of five anti-nutritional factors in raw pearl millet flour (% of total ANF load)

Raw pearl millet flour contained $846.3 \text{ mg } 100 \text{ g}^{-1}$ phytate, $387.4 \text{ mg } 100 \text{ g}^{-1}$ tannins, $58.7 \text{ mg } 100 \text{ g}^{-1}$ oxalates, $31.4 \text{ TIU } \text{g}^{-1}$ trypsin inhibitor activity, and $294.1 \text{ mg GAE } 100$

g^{-1} total polyphenols. Every processing treatment produced a measurable decline in all five ANFs compared with the raw control ($p < 0.05$).

Table 1: Anti-nutritional factor concentrations in pearl millet flour after different processing treatments

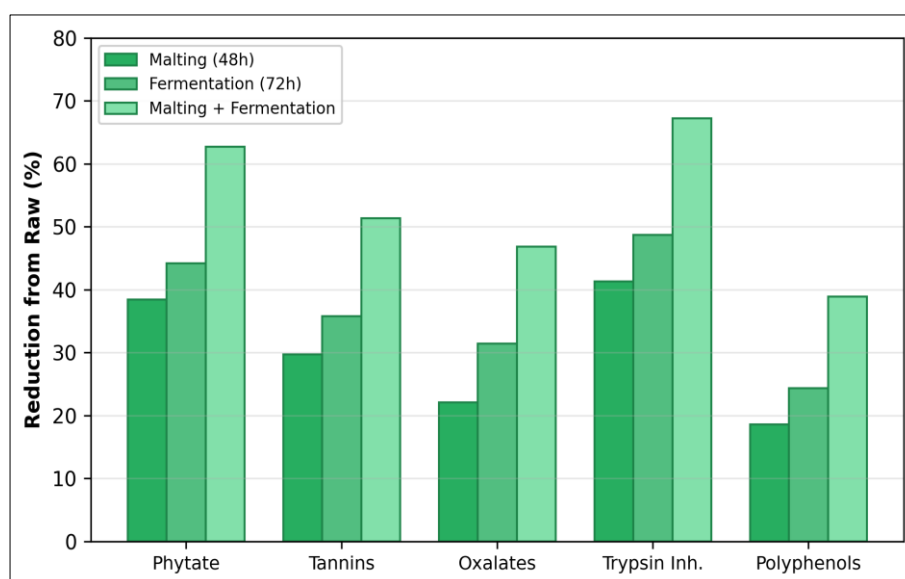
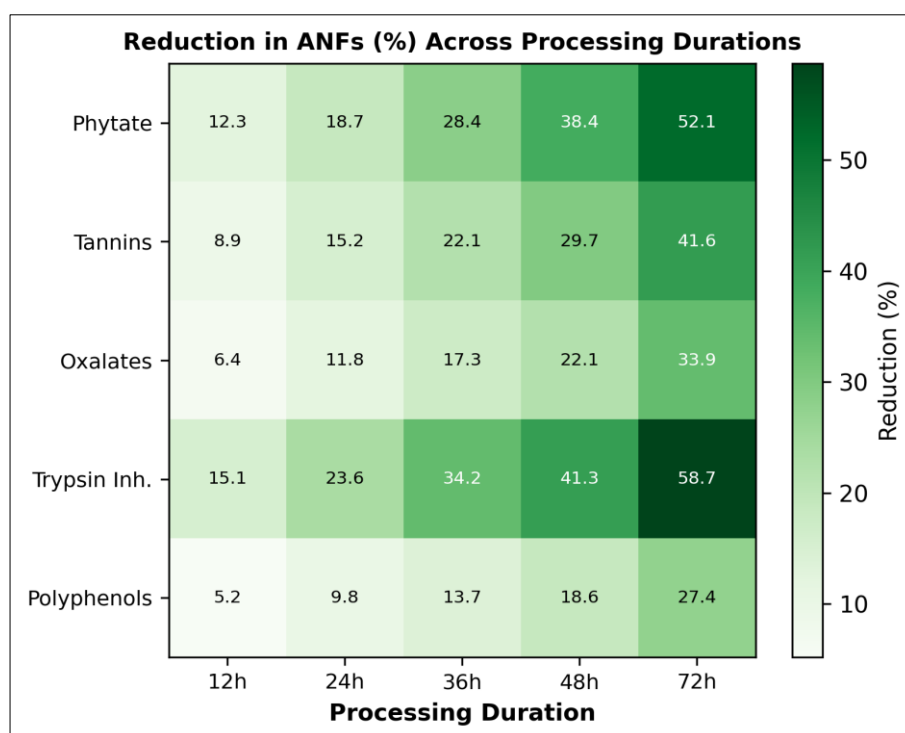
Parameter	Raw Control	Malted (48 h)	Fermented (72 h)	Malted + Fermented
Phytate (mg/100g)	846.3±21.4 a	521.1±18.6 b	472.3±16.9 c	315.7±14.2 d
Tannins (mg/100g)	387.4±14.8 a	272.3±12.1 b	248.7±11.3 c	188.6±9.7 d
Oxalates (mg/100g)	58.7±3.2 a	45.7±2.8 b	40.3±2.4 c	31.2±1.9 d
TIA (TIU/g)	31.4±2.1 a	18.4±1.6 b	16.1±1.3 c	10.3±0.9 d
Polyphenols (mg GAE)	294.1±11.7 a	239.4±10.2 b	222.6±9.4 c	179.7±8.1 d

Means with different letters in the same row differ at $p < 0.05$ (Tukey's HSD). Values are mean $\pm$ SD (n = 3).

Table 2: Protein content and in-vitro protein digestibility across processing treatments

Treatment	Crude Protein (%)	Digestibility (%)	Category
Raw Control	11.2±0.4 b	61.3±2.1 d	Low
Malted (48 h)	12.8±0.5 a	71.6±1.9 c	Moderate
Fermented (72 h)	12.4±0.4 a	74.2±2.3 b	Moderate-High
Malted + Fermented	13.1±0.6 a	79.8±1.7 a	High

Categories assigned based on FAO/WHO digestibility benchmarks for complementary cereals

**Fig 2:** Percentage reduction from raw flour levels for five anti-nutritional factors under three processing routes**Fig 3:** Heatmap showing progressive reduction (%) of anti-nutritional factors across processing durations from 12 h to 72 h

Comprehensive Interpretation

The combined malting-plus-fermentation treatment consistently outperformed either technique alone, with phytate and trypsin inhibitor reductions exceeding 60%. The heatmap reveals that the sharpest gains occur between 36 and 48 h of processing, after which the rate of decline begins to plateau, indicating diminishing enzyme activity at extended durations.

Discussion

Across all four flour types, the combined malting-and-fermentation treatment achieved the deepest ANF reductions: 62.7% for phytate, 67.2% for trypsin inhibitors, and 46.8% for oxalates. Protein digestibility climbed from 61.3% in raw flour to 79.8% after the combined processing, crossing the 75% threshold that the FAO considers adequate for cereal-based complementary foods.

These results agree with several earlier reports. Onyango *et al.* observed a 58% phytate decline in sorghum-millet blends after 48 h of germination followed by 24 h of lactic fermentation [16]. Coulibaly *et al.* reported tannin losses of 40-55% in malted pearl millet from Burkina Faso [17]. The agreement between geographically distinct studies suggests that the underlying biochemistry — phytase activation during germination and acid-mediated dissolution during fermentation — operates consistently regardless of cultivar or continent.

One point of divergence is worth noting. Elkhier and Hamid found that fermentation alone removed more phytate than malting alone in a Sudanese pearl millet variety, whereas the present data show the reverse order (malting 38.4% vs. fermentation 44.2% for phytate, but malting 41.3% vs. fermentation 48.7% for TIA) [18]. The discrepancy may stem from differences in endogenous phytase activity between genotypes, or from the longer fermentation period (96 h) used in the Sudanese research.

From a practical standpoint, the combined treatment requires no equipment beyond vessels for steeping and fermentation — an advantage for smallholder women's cooperatives that often process pearl millet at the household or village level. The 18.5% rise in protein digestibility from raw to combined-processed flour translates into meaningfully higher amino acid availability, which matters most in the diets of young children and lactating mothers who rely on millet as a staple [19].

Conclusion

The main contribution of this research is a side-by-side quantification of five ANFs under three processing regimes applied to a Kano State pearl millet landrace. The combined malting-and-fermentation route delivered reductions of 51-67% across all ANFs and lifted in-vitro protein digestibility to nearly 80%. These numbers provide a practical evidence base for small-scale processors and extension programmes promoting pearl millet-based foods in semi-arid regions.

The research also confirms that neither malting nor fermentation alone matches the efficacy of the sequential approach, reinforcing the value of traditional two-step processing practices that pre-date scientific documentation. Open questions remain around flavour acceptability after extended fermentation, the stability of ANF reductions during storage, and whether the same protocol performs equally well on hybrid pearl millet varieties that now dominate commercial seed markets.

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