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Mineral and anti-nutritional properties of pearl millet and pumpkin leaf flour as affected by fermentation

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Abstract. This study was aimed at determining the mineral and anti-nutritional properties of naturally fermented millet and pumpkin leaf flour blends. The millet grains were allowed to ferment spontaneously for 24 hrs and 48 hrs and were processed into flour. Dried pumpkin leaves were blended into flour and substituted using D-optimal mixture design, which resulted in ten experimental runs. The mineral content and the anti-nutritional properties of the flour blend formulation were analysed. Duncan's multiple range test was used to evaluate the mean at $p < 0.05$ with SPSS version 21.0. Significant differences were observed in the mineral and anti-nutritional composition of the fermented millet and pumpkin leaf flour blends at 24 hrs and 48 hrs of fermentation time respectively. Calcium, potassium, and iron content increased significantly ($p < 0.05$) with increasing the amount of pumpkin leaf flour in the flour blends. The values for tannins and total phenolic composition ranged from 0.089 to 0.162% and from 0.075 to 0.120% for 24 hrs and from 0.080 to 0.141% and from 0.060 to 0.120% for 48 hrs of fermentation time respectively. Results showed that fermentation technique could be used to enrich the nutritional and bioactive potential of millet.

Keywords and phrases: tannin, phytate, trypsin inhibitor, phenol, D-optimal mixture

1. Introduction

Millet is a seeded grass that is widely grown for human and animal nutrition all over the world (*Ranasalva & Visvanathan*, 2014). It is an important crop in semi-arid tropics of Africa and Asia, particularly in Nigeria, due to its ability to grow under adverse weather conditions such as little rainfall, no fertilizer availability, or various other scarcities; therefore, they are mostly recommended for farmers having difficult circumstances (*Soumya et al.*, 2016). The most common types of cultivated millet are foxtail millet (*Setaria itallica*), finger millet (*Eleusine coracona*), pearl millet (*Pennisetum typhoideum*), and proso millet (*Panicum miliaceum*) (*Thilagavathi et al.*, 2015). Pearl millet provides higher energy compared to other cereal grains such as rice and wheat and is considered a significant source of nutrients such as calcium, potassium, thiamine, niacin, and riboflavin (*Shweta*, 2015). Millet can be used in producing food and beverage products such as papad, muruku, bread, hot kolukattai, milk, malt beverage, and alcoholic beverage (*Singh et al.*, 2021; *Mahajan et al.*, 2021). Due to the presence of tannins, polyphenolics, and phytic acid, the bioavailability of these nutrients is low. They are typically considered anti-nutrients and have been associated with inhibitory effect on protein and starch digestibility and mineral bioavailability. Studies have shown that these phytochemicals can be reduced substantially by food processing operations such as dehulling, malting, fermentation, and heat treatment (*Marston et al.*, 2016).

Fermentation is one of the oldest and widely used methods for processing millet, especially in Africa and other developing countries where modern food preservation methods are rarely available. It is a process involving the transformation of substrates (millets) into new products through the action of certain microorganisms (lactic acid bacteria and yeasts). This is regarded as an economical traditional processing technique adopted to yield large amounts of products. During fermentation, enzymes are activated through the action of microorganisms leading to changes in the pH; this is in addition to other biochemical changes that occur during this process leading to the modification of the substrate (*Adebiyi et al.*, 2018; *Srivastava et al.*, 2020). These biochemical changes contribute to preservative properties, improved flavours, and significant increase in nutritional properties (*Obilana et al.*, 2014). Fermentation also ensures the safety of the food by suppressing the growth and survival of undesirable microflora. Fermentation has been reported to effectively improve the nutritional quality of millet by increasing protein content, in-vitro protein digestibility (IVPD), and mineral extractability (*Ranasalva & Visvanathan*, 2014; *Adebiyi et al.*, 2018). Other significant roles of fermentation in millet processing include the development of a wide variety of flavours, aromas, and textures, detoxification, and a decrease in cooking time (*Jay et al.*, 2005; *Adebiyi et al.*, 2018).

Other sources of nutrients, such as legumes and leaves, could be used to supplement cereal flours in complementary porridge and bakery products to

improve the nutritional and sensory qualities of such products (*Chikondi et al.*, 2018). The consumption of vegetables, such as pumpkin leaves, has a significant impact on human health: they typically protect against chronic diseases and contain large amounts of iron, folic acid, vitamin A and C. It is a drought-tolerant plant; the young shoots and leaves are used in cooking soups, yam and vegetable sauces and also for medicinal purposes (*Mashiane et al.*, 2021). Deficiency of iron (anaemia) is a major problem in underdeveloped and developing countries (*Llanos et al.*, 2016). The causes of anaemia can be classified into blood loss, reduced production and increased destruction of red blood cells (*Thilagavathi et al.*, 2015). *Debasmita* and *Binata* (2017) reported that the major cause of anaemia is the inadequate nutrient intake of iron, vitamins A, B_{12}, C, folic acid, niacin and pantothenic acid, which are also responsible for maintaining the level of haemoglobin in the blood. To prevent the growing rate of anaemia, dietary improvement, fortification, and supplementation are beneficial ways for the entire population or for certain groups of people. The nutritional potentials of millet and fluted pumpkin leaves as composite flour in food products would enhance food security and improve the overall health status of consumers of such formulated food products (*Adeyeye*, 2016). Many researchers (*Onuoha et al.*, 2017; *Srivastava et al.*, 2020; *Devi & Rajendran*, 2021; *Azeez et al.*, 2022) have worked on the fermentation of pearl millet with other legumes, however, not in combination with pumpkin leaves. Therefore, the objective of this work is to determine the effect of fermentation on the minerals and anti-nutritional properties of flours from fermented pearl millet and fluted pumpkin leaves.

2. Materials and methods

Millet grains and fluted pumpkin leaves were procured in 2022 at the Odo-Eran market, Abeokuta Ogun State, Nigeria.

Fermented millet flour production

Fermented millet flour was produced according to the method described by *Adebayo-Oyetoro et al.* (2017). The millet grains were cleaned and soaked in potable water for 24 hrs. They were wet milled and sieved through a fine-mesh sieve (muslin cloth). The millet slurry was allowed to ferment naturally in clean plastic bucket for 24 hrs and 48 hrs. The fermented meal was then pressed using a fine-mesh sieve to produce the fermented cake. The cake was dried using a cabinet drier (LEEC Limited, Serial No 3114, United Kingdom) at 60°C for 10 hrs and milled with a milling machine (Fritsch, D-55743, Idaroberstein-Germany) to produce the fermented millet flour and was packaged in polyethylene bags.

Fluted pumpkin leaf flour production

Fluted pumpkin leaf flour was produced using the modified method of *Lawal et al.* (2021). Pumpkin leaves were washed thoroughly with tap water to remove dirt and sorted to remove stalks. The green leaves were drained using a plastic sieve, then sliced and dried at 60°C for 6 hrs using cabinet drier (LEEC Limited, Serial No 3114, United Kingdom). Dried pumpkin leaves were milled using blender, cooled and stored in high-density polyethylene bags.

Formulation of fermented pearl millet and fluted pumpkin leaf flour blends

Using D-optimal design, different blends of composite flour samples were prepared by combining fermented pearl millet and fluted pumpkin leaf flour in the following ratios: 93.75:6.25, 91.25:8.75, 90.00:10.00, 90.00:10.00, 95.00:5.00, 92.50:7.50, 92.50:7.50, 90.00:10.00, 95.00:5.00, and 100.00:0.00.

Mineral analysis of fermented pearl millet and fluted pumpkin leaf flour blends

The mineral content of the samples was assessed using the procedure of *AOAC* (2000). Calcium, iron, sodium, and potassium were measured using atomic absorption spectrophotometer (Thermo scientific S Series Model GE 712354) after they have been digested with perchloric-nitric acid mixture. Prior to digestion, 0.50 g of samples were weighed into a 125-ml Erlenmeyer flask, over which 4 ml of concentrated perchloric-nitric acid, 25 ml of concentrated nitric acid, and 2 ml of concentrated sulphuric acid were added under a fume hood. The contents were mixed, heated gently in a digester (Buchi Digestion unit K-424) with low to medium heat on a hot plate under perchloric acid fume hood, and heating was continued for about 30 s until the appearance of dense white fume. Then it was allowed to cool followed by addition of 50 ml distilled water. The solution was allowed to cool and filtered completely with a wash bottle into a Pyrex volumetric flask and then made up with distilled water. The solution was then read on atomic absorption spectrophotometer.

Determination of tannins

The determination of tannin content was done using the method of *Swain* (1979). Ground sample (0.2 g) was measured into a beaker (50 ml), 20 ml of 50% methanol was added, covered, and placed in 80°C water bath for about 1 h and was stirred to avoid clumping. Double-layered Whatman No. 1 filter paper was used in the filtration of solution into a volumetric flask (100 ml), and 50% methanol was used for rinsing. It was made up to the mark with distilled water and mixed;

an extract of 1 ml was pipetted into a volumetric flask (50 ml); 20 ml of distilled water and 10 ml of 17% Na_2C0_3 and Folin–Denis reagent were thoroughly mixed. This was made up with distilled water and allowed to stand for about 20 min until a bluish-green colour was visible. Standard tannic acid solutions in the range of 0–10 (µg/L) were treated similarly to the 1 ml of the sample. The absorbance of tannic acid standard solutions as well as the sample were read on the Spectronic 21D Spectrophotometer at a wavelength of $\lambda = 760$ nm.

Determination of trypsin inhibitor activity

Determining trypsin inhibitor activity was done by the method described by *Kakade et al.* (1974). Flour sample of 1 g was extracted with 50 ml of 0.01 M of NaOH for 1 h, and the pH of the resulting slurry was adjusted to between 9.4 and 9.6 with 1 M NaOH or 1 M HCl. At this stage, the slurry was shaken and left at 4°C overnight; but occasionally it was more convenient to stir at ambient temperature for 3 hrs or to macerate continuously (Ultra-Turrax) for 2 min. After extraction, the suspension was shaken and diluted with water, so an amount of 1 ml produced trypsin inhibition of between 40% and 60%. Trypsin solution of 2 ml was added to the test tube; the tubes were placed in 37°C water bath and were allowed to stay there for 10 min. Then 1 ml of 30% acetic acid was added and the contents were thoroughly mixed and filtered with Whatman No. 3. The absorbance was measured at $\lambda = 410$ nm against a blank reagent.

Determination of total phenolic content

The method described by *Turturică et al.* (2016) was used in the determination of total phenolic content. 1 g of sample was ground with pestle and mortar with the addition of 10 ml 80% ethanol. The mixture was centrifuged at 10,000 rpm for 20 min. The supernatant was kept, and the residue was further extracted with 80% ethanol, centrifuged, and exposed for evaporation to take place. The residue was to dissolve in 5 ml of distilled water. The sample was measured into the test tubes, and the volume of each was made up with distilled water to 3 ml. 0.5 ml of Folin–Ciocâlteu reagent was added to the test tubes. After about 3 min, 2 ml sodium carbonate (20%) solution was added to each tube. The contents of the test tubes were mixed thoroughly, and the tubes were placed in a water bath for 1 min. It was allowed to cool, and absorbance was read at $\lambda = 650$ nm against a blank reagent.

Determination of phytate

The method described by *Maga* (1982) was used in the determination of phytate. 2 g of sample was soaked in 20 ml of 0.2 M of HCl for 3 hrs and filtered. The filtrate (0.5 ml) was mixed with 1 ml of 70% ferric-ammonium-sulphate solution in a test

tube, boiled for 30 min in the water bath (100°C), cooled in ice, and centrifuged at 3,000 rpm for 15 min. 1 ml of the supernatant was mixed thoroughly with 0.1 M of 2,2-pyridine solution (1.5 ml), and the absorbance was read at λ = 519 nm using spectrophotometer.

Statistical analysis

Each analysis was carried out in triplicate. Mean values of the three replicates' results were subjected to one-way analysis of variance (ANOVA) to determine the significant difference, and the means were separated using Duncan's multiple range test at 95% confidence level ($p < 0.05$).

3. Results and discussions

The mean values for the mineral composition of fermented millet grains (FM) and pumpkin leaf flour (PF) at 24 hrs and 48 hrs of fermentation time are shown in *figures 1–2*. Significant ($p < 0.05$) difference was observed in the mineral composition of 24 hrs and 48 hrs of fermentation time. The mean values of calcium ranged from 49.49 mg/100 g to 206.39 mg/100 g and from 51.86 mg/100 g to 202.90 mg/100 g for 24 hrs and 48 hrs of fermentation time respectively. Calcium increased significantly ($p < 0.05$) with increase in pumpkin leaf flour in the flour blends.

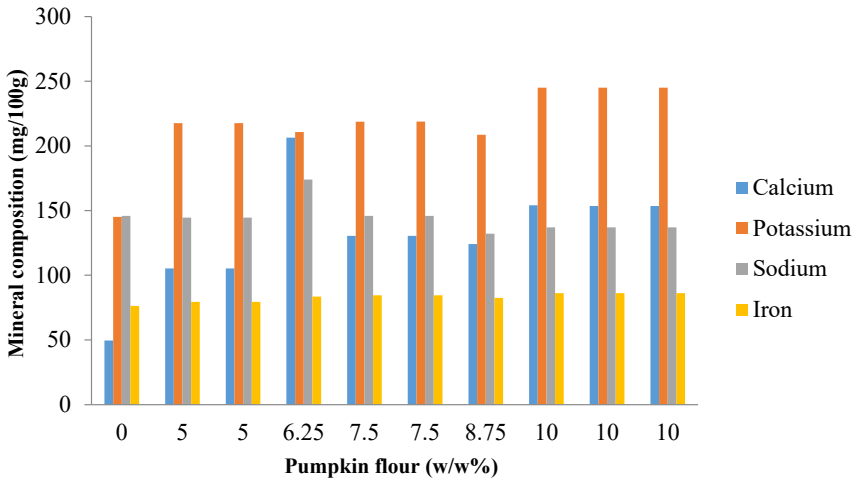

Figure 1. Mineral composition of 24 hrs fermented millet and pumpkin leaf flour

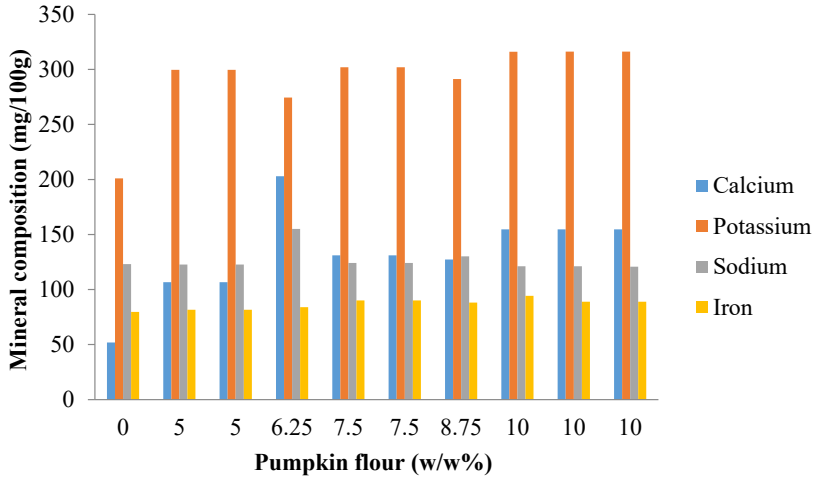

Figure 2. Mineral composition of 48 hrs fermented millet and pumpkin leaf flour

The regression coefficient of the flours at 24 hrs and 48 hrs of fermentation time are shown in *tables 1–2*. Their linear and interaction effect was not significant ($p > 0.05$) on the calcium content of fermented millet flour and fluted pumpkin leaves for 24 hrs and 48 hrs. The coefficients of determination (R^2) values were 0.51 and 0.70 for the flour blends of 24 hrs and 48 hrs of fermentation time respectively.

Potassium content for the fermented millet and pumpkin leaf flour at 24 hrs and 48 hrs ranged from 145.15 mg/100 g to 245.02 mg/100 g and from 200.92 mg/100 g to 316.11 mg/100 g respectively. Potassium increased significantly ($p < 0.05$) with increase in pumpkin leaf flour in the flour blends. The least value at both the 24 hrs and 48 hrs millet fermentation period was observed in 0% PF formulation blends, while the maximum values were observed at 10% PF formulation blends. In addition, the interaction effect of 24 hrs fermented millet flour and fluted pumpkin leaf had significant effect ($p < 0.05$) on the potassium content of the formulation blend. However, the linear effect of 48 hrs fermented millet flour and fluted pumpkin leaves, as well as the interaction effect had no significant effect ($p > 0.05$) on the potassium content of formulation blends. The coefficients of determination (R^2) values were 0.84 and 0.62 respectively for the flour blends of 24 hrs and 48 hrs fermented time. This indicates that the equation is a good fit in predicting the potassium content of the flour blend. There was at first a decrease and then later an increase in the potassium content of the formulation blends in both fermentation periods as the fluted pumpkin leaf flour decreased.

Table 1. Regression coefficient of mineral composition of fermented millet and pumpkin leaf flour at 24 hrs

Parameter	Calcium	Potassium	Sodium	Iron
A	120.03	218.41	149.49	79.81
B	147.57	243.17	134.56	85.80
AB	51.84	-71.33*	31.03	4.70
F-value	0.53	15.42	1.82	14.20
R^2	0.509	0.8371	0.5779	0.8256

Notes: * Significant at ($p < 0.05$); A: fermented millet flour, B: fluted pumpkin leaf flour, AB: interaction effects of fermented millet and pumpkin leaf flour, R^2: coefficient of determination.

Table 2. Regression coefficient of mineral composition of fermented millet and pumpkin leaf flour at 48 hrs

Parameter	Calcium	Potassium	Sodium	Iron
A	120.58	297.02	126.93	81.40
B	148.92	315.65	120.22	90.51
AB	48.96	-56.47	37.92	11.00
F-value	0.61	4.88	1.02	10.85
R^2	0.701	0.620	0.541	0.783

Notes: * Significant at ($p < 0.05$); A: fermented millet flour, B: pumpkin leaf flour, AB: interaction effects of fermented millet and pumpkin leaf flour, R^2: coefficient of determination.

The sodium content of fermented millet grains and pumpkin leaf flour ranged from 132.13 mg/100 g to 173.98 mg/100 g and from 120.70 mg/100 g to 155.10 mg/100 g for 24 hrs and 48 hrs of fermentation time respectively. Sodium decreased significantly ($p < 0.05$) with increase in pumpkin leaf flour in the blends. The least values were observed in 8.75% PF formulation blends, while the maximum values were observed in 6.25% PF formulation blends. The maximum values were observed in 93.75% FM and 6.25% PF formulation blends, whereas the least values were observed in 90% FM and 10% PF formulation blends. The main and the interaction effect showed no significant ($p > 0.05$) effect on the sodium content of formulation blend for 24 hrs and 48 hrs of fermentation time for millet flour and fluted pumpkin leaves. The coefficients of determination were 0.58 and 0.54 for the flour blends of 24 hrs and 48 hrs respectively.

The mean values of the iron content of the fermented millet grains and pumpkin leaf flour blends ranged from 76.30 mg/100 g to 86.19 mg/100 g and from 79.63 mg/100 g to 94.34 mg/100 g for 24 hrs and 48 hrs of fermentation

time respectively. Iron content increased significantly ($p < 0.05$) with increase in pumpkin leaf flour in the flour blends. The linear and interactive effect of 24 hrs and 48 hrs of fermentation time for millet flour and fluted pumpkin leaves does not show a significant level ($p > 0.05$) regarding the iron content of the formulation blends. The coefficients of determination (R^2) values were 0.83 and 0.78, respectively, for the flour blends of 24 hrs and 48 hrs of fermentation time for millet flour and fluted pumpkin leaves. This indicates that the equation is a good fit in predicting the iron content of the flour blend. There was a decrease in the iron content of the formulation blends in both fermentation periods as the fluted pumpkin leaf flour decreased.

The mean values for the anti-nutritional composition of the formulated blends of 24 hrs and 48 hrs fermentation time for millet grains and pumpkin leaf flour are shown in *tables 3–4*. Tannin content ranged from 0.089 to 0.162% and from 0.080 to 0.141% respectively.

Table 3. Anti-nutritional composition of fermented millet and pumpkin leaf flour at 24 hrs

PF (w/w%)	Tannin (%)	Trypsin inhibitor (%)	Total phenolic (%)	Phytate (%)
6.25	0.135 ± 0.00^{b}	0.024 ± 0.00^{a}	0.120 ± 0.00^{k}	0.004 ± 0.01^{bc}
8.75	0.134 ± 0.00^{h}	0.040 ± 0.00^{a}	0.106 ± 0.01^{g}	0.004 ± 0.01^{bc}
10.00	0.141 ± 0.11^{lm}	0.027 ± 0.00^{a}	0.111 ± 0.00^{i}	0.004 ± 0.01^{bc}
10.00	0.142 ± 0.00^{mn}	0.026 ± 0.00^{a}	0.112 ± 0.00^{ij}	0.004 ± 0.01^{bc}
5.00	0.138 ± 0.00^{i}	0.024 ± 0.00^{a}	0.107 ± 0.00^{gh}	0.004 ± 0.01^{bc}
7.50	0.161 ± 0.00^{o}	0.029 ± 0.01^{a}	0.119 ± 0.01^{k}	0.004 ± 0.00^{bc}
7.50	0.162 ± 0.00^{o}	0.029 ± 0.01^{a}	0.119 ± 0.01^{k}	0.006 ± 0.01^{bc}
10.00	0.143 ± 0.00^{n}	0.029 ± 0.00^{a}	0.113 ± 0.00^{j}	0.006 ± 0.01^{e}
5.00	0.139 ± 0.00^{ij}	0.025 ± 0.00^{a}	0.108 ± 0.00^{bc}	0.005 ± 0.00^{de}
0.00	0.089 ± 0.00^{a}	0.063 ± 0.00^{a}	0.075 ± 0.00^{a}	0.001 ± 0.00^{a}

Notes: Mean values with different superscripts within the same column are significantly different ($p < 0.05$); PF: pumpkin flour.

As shown in *Table 5*, the linear effect of 24 hrs fermentation time for millet and fluted pumpkin leaves, as well as the interaction had no significant ($p > 0.05$) effect on the tannin content of the blend formulation. However, the interaction of 48 hrs fermentation time for millet flour and fluted pumpkin leaves had a significant ($p < 0.05$) effect on the tannin content of formulation blend, as shown in *Table 6*.

Table 4. Anti-nutritional composition of fermented millet and pumpkin leaf flour at 48 hrs

PF (w/w%)	Tannin (%)	Trypsin inhibitor (%)	Total phenolic (%)	Phytate (%)
6.25	0.119 ± 0.00^{f}	0.021 ± 0.00^{a}	0.093 ± 0.00^{e}	0.003 ± 0.01^{a}
8.75	0.127 ± 0.00^{g}	0.032 ± 0.15^{a}	0.103 ± 0.00^{f}	0.003 ± 0.01^{a}
10.00	0.139 ± 0.01^{ij}	0.027 ± 0.00^{a}	0.076 ± 0.00^{bc}	0.003 ± 0.01^{a}
10.00	0.140 ± 0.01^{jk}	0.028 ± 0.00^{a}	0.077 ± 0.00^{cd}	0.004 ± 0.00^{bc}
5.00	0.109 ± 0.00^{c}	0.021 ± 0.01^{a}	0.106 ± 0.00^{g}	0.003 ± 0.01^{a}
7.50	0.111 ± 0.01^{d}	0.021 ± 0.01^{a}	0.119 ± 0.00^{k}	0.003 ± 0.01^{a}
7.50	0.113 ± 0.01^{e}	0.022 ± 0.01^{a}	0.120 ± 0.00^{k}	0.003 ± 0.01^{ab}
10.00	0.141 ± 0.00^{kl}	0.029 ± 0.00^{a}	0.076 ± 0.00^{d}	0.005 ± 0.00^{de}
5.00	0.110 ± 0.00^{c}	0.022 ± 0.01^{a}	0.107 ± 0.00^{gh}	0.004 ± 0.00^{bc}
0.00	0.080 ± 0.00^{a}	0.014 ± 0.00^{a}	0.060 ± 0.00^{a}	0.003 ± 0.01^{a}

Notes: Mean values with different superscripts within the same column are significantly different ($p < 0.05$); PF: pumpkin flour.

Table 5. Regression coefficient of anti-nutritional composition of fermented millet and pumpkin leaf flour at 24 hrs

Parameter	Tannin	Trypsin inhibitor	Total phenolic	Phytate
A	0.14	8.2985×10^{-3}	0.11	4.440×10^{-3}
B	0.14	0.049	0.11	4.612×10^{-3}
AB	0.049	0.20	0.030	2.019×10^{-4}
F-value	1.35	0.50	2.05	0.020
R^2	0.4102	0.7438	0.60	0.76

Notes: * Significant at $p < 0.05$); A: fermented millet flour, B: pumpkin leaf flour, AB: interaction effects of fermented millet and pumpkin leaf flour, R^2: coefficient of determination.

The coefficients of determination (R^2) values were 0.41 and 0.93, respectively, for the flour blends of 24 hrs and 48 hrs fermentation time for millet flour and fluted pumpkin leaves. This is an indication that the equation is a poor fit in predicting the tannin content of the flour blend of 24 hrs fermentation time for millet flour and fluted pumpkin leaves, but it can accurately predict the tannin content of the flour blends of 48 hrs fermentation time for millet flour and fluted pumpkin leaves. Tannin intake has been implicated to cause depletion in the digestive enzymes which are responsible for the secretion and production of endogenous protein,

the malfunctioning of the digestive tract, and the toxic effect resulting from the metabolites (*Jan et al.*, 2022).

Table 6. Regression coefficient of anti-nutritional composition of fermented millet and pumpkin leaf flour at 48 hrs

Parameter	Tannin	Trypsin inhibitor	Total phenolic	Phytate
A	0.11	0.014	0.10	3.514×10^{-3}
B	0.14	0.039	0.077	3.971×10^{-3}
AB	-0.039*	0.084	0.091*	-3.326×10^{-3}
F-value	40.34	0.48	10.87	1.92
R^2	0.9308	0.84	0.7838	0.5906

Notes: * Significant at $p < 0.05$); A: fermented millet flour, B: pumpkin leaves flour, AB: interaction effects of fermented millet and pumpkin leaves flour, R^2: coefficient of determination.

The value of the trypsin inhibitor for the formulated blends of 24 hrs fermentation time for millet and pumpkin leaf flour ranged from 0.024% to 0.063% resp. On the other hand, the mean values of trypsin inhibitor for the formulated blends of 48 hrs fermentation time for millet and pumpkin leaf flour ranged from 0.014% to 0.032%, resp., as shown in *Table 6*. The main effect of the 24 hrs and 48 hrs fermentation time for millet and fluted pumpkin leaf flour, as well as the interaction, had no significant ($p > 0.05$) effect on the trypsin inhibitor of flour blends. In addition, the R^2 values were 0.74 and 0.84 for both the flour blends at 24 hrs and 48 hrs respectively. This indicates that the equation is a good fit in predicting the trypsin inhibitor content of the flour blend. There was first an increase and then later a decrease in the trypsin inhibitor content of the formulation blends at 24 hrs and 48 hrs as the fermented millet flour increased. However, the fact that the trypsin inhibitors are heat-labile suggests that they can be destroyed through processing such as grinding and cooking (*Venter & van Eyssen*, 2001; *Adane et al.*, 2013).

The values of the total phenolic content for the formulated blends at 24 hrs fermentation time for millet grains and pumpkin leaf flour ranged from 0.075 to 0.120%. The least values were observed in 100% FM and 0% PF formulation blends, while the maximum values were observed in 93.75% FM and 6.25% PF formulation blends. The mean values of the total phenolic content for the formulated blends of 48 hrs fermentation time for millet grains and pumpkin leaf flour ranged from 0.060 to 0.120%. The maximum values were observed in 92.50% FM and 7.50% PF formulation blends, whereas the least values were observed in 100% FM and 0% PF formulation blends. The linear effect of 24 hrs fermentation time for millet flour and fluted pumpkin leaves, as well as the interaction, had

no significant ($p > 0.05$) effect on the total phenolic content of the formulation blend. However, the interaction of 48 hrs fermentation time for millet flour and fluted pumpkin leaves had a significant ($p < 0.05$) effect on the total phenolic content of flour blends. The coefficients of determination (R^2) values were 0.60 and 0.78 for the flour blends of 24 hrs and 48 hrs fermentation time for millet and fluted pumpkin leaf flour respectively. The total phenolic content was observed to increase gradually upon the substitution levels of fermented millet and thereafter decrease at higher substitution levels notwithstanding the varying fermentation periods. During fermentation, the factors' conditions (temperature, time, and pH), the microorganism species present as well as the grain type all have significant effect on phenolic compounds (*Jan et al.*, 2022).

The values for the phytate content of the flour blends of 24 hrs fermentation time for millet grains and pumpkin leaf flour ranged from 0.004 to 0.006%. However, the mean values for the phytate content of the formulated blends of 48 hrs fermentation time for millet grains and pumpkin leaf flour ranged from 0.003% to 0.005%. The linear effect of 24 hrs and 48 hrs fermentation time for millet flour and fluted pumpkin leaves, as well as the interaction, had no significant ($p > 0.05$) effect on the phytate content of formulation blend. Also, the coefficients of determination (R^2) values were 0.76 and 0.59, respectively, for the flour blends of 24 hrs and 48 hrs fermentation time for millet flour and fluted pumpkin leaves. There was a slight decrease in the phytate content of the formulation blends at 24 hrs as fermented millet flour increased and fluted pumpkin leaf flour decreased. However, the phytate content of the formulation blends at 48 hrs fermentation time for millet flour increased as the fluted pumpkin leaf flour decreased – it was observed to initially decrease and then later increase at higher levels of fluted pumpkin leaves.

Conclusions

The study shows the effect of fermentation time on minerals and anti-nutritional factors of flour blends from pearl millet and pumpkin leaves. The calcium, potassium, sodium, and iron content of the flour blends from fermented millet and pumpkin leaf flour increased significantly at 24 hrs and 48 hrs fermentation time, while tannin, total phenolic, and phytate decreased significantly at both 24 hrs and 48 hrs fermentation time. Calcium, potassium, and iron were maximized, while sodium tannin, trypsin inhibitor, total phenolic, and phytate were set to "none". However, the optimized solutions from the D-optimal design were fermented millet flour of 90.81% and pumpkin leaf flour of 9.19% at 24 hrs and fermented millet flour of 90.52% and pumpkin leaf flour of 9.48% at 48 hrs fermentation time respectively.

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