Effect of Supplementation Followed by Processing on Nutritional Quality of Protein, Ca, P and Fe of Millet Flour

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Abstract: The effect of processing methods on millet flour supplemented with different levels (5, 10 and 15%) of defatted seed flour of *Moringa* (DSFM) and fenugreek (DSFF) on antinutritional factors, protein content and digestibility and total and extractable Ca, P and Fe was investigated. The antinutritional factors were increased ($P \leq 0.05$) with an increase in supplementation levels. However, processing of both raw and supplemented flour decreased ($P \leq 0.05$) the antinutritional factors. The reduction in antinutrients was accompanied by an increase ($P \leq 0.05$) in protein content, IVPD, total and extractable Ca, P, and Fe of supplemented flour compared to that of raw samples. Fermentation increased ($P \leq 0.05$) the protein content, IVPD, total and extractable Ca, P and Fe contents of the samples with supplementation level. Cooking of fermented dough slightly increased the protein content and IVPD of the samples but lowered both total and extractable Ca, Fe and P. Higher antinutritional factors, protein and IVPD were observed in millet dough supplemented with DSFM compared to that of DSFF while the total and extractable minerals of the supplemented flours are varied. Results obtained revealed that addition of DSFM and DSFF followed by the cooking of the fermented dough is a useful method to improve the nutritional value of millet flour.
Keywords: millet; moringa; fenugreek; fermentation; supplementation; anti-nutrients; protein; minerals

1. Introduction

In developing countries, cereal grains serve as staple food for the majority of the people due to limited income and the high cost of animal foods[1]. Regarding world agricultural production, millet is the 6th cereal crop and one of the most significant drought resistant crops cultivated in the semiarid tropics of Africa and Asia. It serves as a source of carbohydrates and proteins for people living in these areas[2]. It is used in the production of various traditional foods and beverages such as bread, porridges and snack foods[3]. Like other cereals, millet is deficient in essential amino acids such as lysine and tryptophan but rich in minerals and antinutritional factors like phytate, tannins and polyphenols[4]. Because of the great use of millet as a basic staple food for majority of people in developing countries, and its low nutritional value, mainly with respect to protein quality and mineral bioavailability, many efforts (supplementation and processing) have been made to improve the biological utilization of millet flour[4,5,6]. The use of natural food products, particularly of plant sources, compared with chemical synthetic nutrients and animal sources in supplementation of millet grains can be promising in terms of high cost-effectiveness and lower health risk[2].

Moringa (*M. oleifera*) seeds have high protein content, which can be utilized in solving worldwide malnutrition or undernutrition problems by nutritionists and community health cautious persons[7]. The seeds are rich in essential amino acids, minerals, vitamins, oil, antioxidants[8]. Fenugreek is a rich source of protein, fiber, ash and carbohydrate and minerals[9]. Previous study has shown that addition of 10% of fenugreek flour to wheat flour increased protein content, fiber, total calcium and total iron[10]. Researchers have shown an increase in protein content and digestibility of millet flour after supplementation with moringa or fenugreek, but the values of antinutritional factors increased[11]. Millet has high antinutritional factors that lowered the bioaccessibility and bioavailability of some minerals by chelating dietary minerals[12]. Similar observations have been reported for other cereal crops like sorghum[13] and corn[14]. Antinutrients such as phytate have been reported to affect the bioavailability of major minerals like Ca and P and trace ones such as Zn, Fe, Cu and Mn. Other antinutrients such as tannins and polyphenols found in pearl millet are known to limit the utilization of it as food or feed[15].

Reduction of phytic acid is very advantageous, due to its influence on nutrition; therefore, interest has grown to reduce its antinutritional effects[16].

Research has shown that through processing (cooking, radiation, fermentation or germination) the levels of phytates and tannins have been reduced significantly by exogenous and endogenous enzymes formed during processing[13,17-19]. Fermentation is one of the processes that lower the values of antinutritional factors in food grains and raise minerals extractability[20], *in vitro* protein digestibility and nutritive value of pearl millet[21]. Also, the levels of antinutrients in pearl millet have been decreased and minerals availability increased through the cooking process[15]. Therefore, for efficient utilization of millet flour as human food, information on the combined effects of supplementation as well as fermentation and cooking on improving the nutritional quality of the flour and elimination of antinutrients is required. Thus, the aim of the study was to investigate the effects of supplementation of millet flour with defatted moringa or fenugreek flour followed by fermentation and cooking on antinutrients and total and available protein, Ca, P and Fe.

2. Materials and methods

2.1 Sample preparation

Grain sample of pearl millet seeds (*Pennisetum glaucum* L.) was obtained from Department of Agronomy, Faculty of Agriculture, University of Khartoum. The seeds were cleaned, freed from foreign, broken and shrunken seeds, milled into fine flour using house blender and mortar to pass through a 0.4 mm screen and then stored in polyethylene bags at 4°C for further analysis. Moringa (*Moringa oliefera*) and fenugreek seeds (*Trigonella foenum-graecum* L.) were brought from a local farm, cleaned, freed from extraneous matter; the seeds were soaked in water for 12 hours with changing water every two hours. After that, the seeds were dried in a freeze drier (12525, Virtis Company, Gardner, New York), milled into fine powder, defatted and stored in polyethylene bags at 4°C for further analysis. Both defatted *Moringa* and fenugreek seeds flours were added individually to millet flour using Pearson square to increase the nutritive value of millet flour by 5, 10 and 15%. All chemicals used in this study were of reagent grade.

2.2 Processing methods

2.2.1 Fermentation

Natural fermentation of millet flour and composite flours was carried out by mixing the flour with distilled water (1:2 w/v). About 250 g of each sample were mixed with 500 ml distilled water in 750 ml beaker and incubated (Gallenkamp, England) at 37°C for periods 0, 8 and 16 h. After the incubation periods, the samples were mixed using a glass rod and transferred to aluminum dishes (30 cm diameter), and dried in a freeze dryer (12525, Virtis Company, Gardner, New York). Dried samples were ground to pass a 0.4 mm screen and stored at 4°C for further analysis.

2.2.2 Cooking of fermented dough

A slurry of untreated and fermented dough of each sample was cooked for 10 minutes, cooled and dried in a freeze dryer (12525, Virtis Company, Gardner, New York). The dried flakes were milled into fine flour and stored at 4℃ for further analysis.

2.3 Determination of protein content and digestibility

The protein content of the samples was determined according to standard method[22]. The *in vitro* protein digestibility of the samples was measured according to literature procedure[23], using pepsin and pancreatin digestion method. The digested protein was analyzed for nitrogen using micro-Kjeldahl procedure[22] and expressed as a percent of the total N.

2.4. Determination of antinutritional factors

2.4.1 Phytic acid content

The phytic acid content was determined by the literature procedure[24] using 2.0g dried sample. A standard curve was prepared to express the results as $Fe(NO_3)_3$ equivalent. Phytate phosphorus was calculated from the standard curve assuming a 4:6 iron to phosphorus molar ratio.

2.4.2 Tannin content

Vanillin-HCl method was used to assay tannin content according to literature procedure[25]. Catechin was used as reference standard.

2.4.3 Polyphenols content

Total phenols were determined according to the Prussian blue spectrophotometric method[25] with a minor modification.

2.5 Mineral analysis

2.5.1 Total minerals content

Minerals were extracted from the samples by dry ashing method[26]. About 2.0 g of sample was acid-digested with diacid mixture (HNO_3:$HCLO_4$, 5:1, v/v) in a digested chamber. The digested samples were dissolved in double-distilled water and were used for determination of total calcium, phosphorous and iron. Calcium was determined by a titration method. Iron was determined by atomic absorption spectrophotometer (Perkin-Elmer 2380). Phosphorus was determined spectrophotometrically by using molybdovanadate method.

2.5.2 HCl extractability of minerals (*In vitro bio*availability)

Minerals in the samples were extracted by literature procedure[27]. About 1.0 g of the sample was shaken with 10 ml of 0.03 m HCl for 3 h at 37 °C and then filtered. The clear extract obtained was oven-dried at 100 °C and then acid-digested. The amount of the extractable minerals was determined by the methods above. HCl-extractability (%) was determined as follows:

$$Mineral\ extractability\ (\%) = \frac{Mineral\ extractable\ in\ 0.03\ N\ HCl\ (mg/100g)}{Total\ minerals\ (mg/100g)} \times 100$$

2.6 Statistical Analysis

All data were subjected to statistical analysis, and each determination was carried out and analyzed in triplicate and figures were then averaged. Data was assessed by the analysis of Variance (ANOVA)[28]. Duncan Multiple Range Test (DMRT) was used to separate means. Significance was accepted with at $P \leq 0.05$.

3. Results and discussion

3.1 Antinutritional factor and total phenolic contents

Table 1 shows the effect of supplementation and processing on antinutritional factors contents of MF supplemented with DSFM and DSFF. Phytic acid, tannin and polyphenols contents of raw MF were 203.02, 190.04 and 441.24 mg/100g, respectively. Supplementation of the dough with 5, 10 and 15 % DSFM and DSFF significantly ($P \leq 0.05$) increased the phytic acid, tannin and polyphenols contents of the flour. The meal supplemented with *Moringa* seeds flour having higher values of phytic acid and polyphenols. This could be due to higher phytic acid and polyphenols contents of raw *Moringa* seeds powder compared to fenugreek flour. The phytic acid of the dough obtained in this study was lower than that reported for pearl millet cultivars, but the polyphenol content was higher[15]. Fermentation of raw and supplemented samples significantly ($P \leq 0.05$) decreased the antinutritional factors, and further decrease was observed with fermentation time. Fermentation of the dough for 16 h significantly ($P \leq 0.05$) lowered the phytic acid to 143.11, 190.26 and 200.47mg/100g after supplementing with 5, 10 and 15% DSFM, respectively, while dough supplemented with 5, 10 and 15% DSFF had phytic acid of 143.19, 178.13 and 185.35%, respectively. The decrease in phytic acid during fermentation might be due to the activity of the enzyme phytase naturally present in cereal and legumes that released by microorganisms in the dough[13]. For both MF supplemented with DSFM and DSFF at different levels, their tannin and total phenols contents followed a trend similar to that obtained for phytic acid. The decrease in polyphenols agreed with previous findings[29] where a decrease in polyphenols content of pearl millet with increasing fermentation time was reported. Moreover, cooking of the dough after fermentation was observed to be more efficient in reducing antinutritional factors levels than fermentation alone. Further reduction in phytic acid, tannin and polyphenols contents was observed when the supplemented MF was fermented for 16 h and then cooked. Similar observations of the reduction in phytic acid, tannin and total polyphenols contents of supplemented pearl millet flour by cooking have also been reported[11]. The apparent decrease in phytate content during cooking may be as a result of the formation of insoluble complexes between phytate and other components such as phytate–protein and phytate–mineral complexes and accordingly the amount of free phytate was reduced. The reduction in tannin agreed with previous findings[30] where decrease in total tannin content during cooking of legumes due to heat degradation of these molecules and changes in their chemical reactivity or the formation of insoluble complexes were reported. The loss in polyphenols may be ascribed to the presence of polyphenoloxidase that hydrolyzed it during fermentation and the heat destruction during cooking. In addition, the reduction in polyphenols after cooking might be as a result of the fact that polyphenols react with protein during cooking forming poorly extractable protein phenolic complexes[17]. The results revealed that supplementation of MF with DSFM and DSFF increased the level of antinutritional factors like phytic acid, tannin and total polyphenols but fermentation of the composite flour alleviates the effect of supplementation. Therefore, fermentation followed by cooking could be employed to reduce significantly the harmful effect of the antinutritional factor of the dough.

3.2 *In-vitro* protein digestibility (IVPD)

The protein content of raw MF was 12.29%. Supplementation of the dough with 5, 10 and 15% DSFM significantly ($P \leq 0.05$) increased the protein content to 16.91, 19.39 and 22.02% while that of DSFF significantly ($P \leq 0.05$) rose to 15.35, 15.76 and 15.76%, respectively (Table 2). The rise in protein contents after supplementation with DSFM and DSFF could be attributed to the high protein content of raw *Moringa* (63.41%) and fenugreek (30.33%) seeds used as supplements. Fermentation increased the protein contents of MF with an increase in fermentation time. The increment in protein content during fermentation is also significant increase attributed to the utilization of carbohydrates by microorganisms as well as solubilization of insoluble proteins during fermentation. Cooking of the fermented dough slightly increased the protein content of the flour. Fermentation of the flour followed by cooking showed no significant effect on the protein content of the flour. Cooking of the supplemented flour after fermentation increased the protein content to 23.93% and 20.895, respectively for DSFM and DSFF supplements. The results obtained agree with those found in beans where an increase in the protein quality and quantity after cooking of beans in water with or without pressure was reported[31].

The IVPD of raw MF was 45.75% and significantly ($P \leq 0.05$) increased with supplementation level for both DSFM and DSFF supplements (Table 2). Similarly, increase in protein digestibility of sorghum flour was observed after supplementation with different levels of chickpea flour[32]. Fermentation of both DSFM and DSFF supplements significantly ($P \leq 0.05$) increased the IVPD with a maximum value of 79.42% obtained for 15% *Moringa* supplement fermented for 16 h. The increase in protein digestibility by enzymatic breakdown after fermentation can be attributed to the partial degradation of complex storage proteins into more simple and soluble products as well as the reduction in antinutrients. The results obtained for IVPD in the present study agrees with previous study[33] where it was reported that natural fermentation of sorghum dough caused a highly significant improvement in *in vitro* protein and starch digestibility. Moreover, fermentation of the dough followed by cooking showed further significant ($P \leq 0.05$) improvement in the IVPD of the dough. This result contradicts earlier findings for sorghum[34] and millet[11] where it was reported that cooking lowered protein digestibility. The improvement in protein digestibility observed in this study after fermentation and cooking could be attributed to the decrease in antinutrients such as phytic acid and tannins and polyphenols which interact with protein to form complexes.

3.3 Total Ca, P and Fe contents

As shown in Table 3, the total Ca, P and Fe of raw MF were 3.81, 1.62 and 312.67 mg/100g, respectively and were significantly ($P \leq 0.05$) increased with supplementation level for both DSFM and DSFF. The increment could be attributed to the high total Ca, Fe, and P exhibited by raw *Moringa* and fenugreek seeds. The total mineral contents of the flour significantly ($P \leq 0.05$) increased with fermentation time especially P content which showed further increases after 16 h fermentation. Cooking of the flour after fermentation further increased the P content but lowered the Ca and Fe contents. Previous study has shown that divalent cations such as Ca and Fe ions may be present as mineral-phytate chelates in untreated grains, and this may be responsible for the low total Ca and Fe content compared to P[35]. Degradation of phytic acid during fermentation probably releases some of the complex divalent metal ions resulting in increase in their contents.

Fermentation decreased the Ca content of DSFM supplement but increased that of DSFF supplement. The lower phytic acid content of DSFF supplement especially after fermentation may be responsible for its high Ca content since phytic acid is known to chelate the metals. Increase in fermentation time lowered the Ca content of both DSFM and DSFF supplements. Cooking after fermentation further decreased Ca content of DSFM and DSFF supplements at all supplementation levels except at 10% DSFM. The reduction may be attributed to heat treatment, which may affect the quantitative determination of such minerals.

Supplementation with 15% DSFM significantly ($P \leq 0.05$) increases the Fe content of the flour compared to the control. Maximum Fe content value of 55.95mg/100g was obtained after 16 h fermentation of flour supplemented with 15% DSFM. However, cooking of the flour supplemented with 15% DSFM significantly ($P \leq 0.05$) lowered the Fe content to 16.79% while it enhanced the Fe content of those supplemented with 5 and 10% DSFM. The total Fe content of DSFF supplement increased with the fermentation and supplementation levels, but the values were lower than that of the control. At 10 and 15% DSFF supplementation levels, cooking after fermentation (16 h) significantly ($P \leq 0.05$) increased the total Fe content of the dough, and this could be due to synergistic effect of long fermentation period and heat treatment. Similar findings have been reported [36] and they attributed the increment in total Fe to the decrease in phytic acid content and other antinutritional factors. Moreover cooking expected to concentrate Fe therefore, its amount supposed to increase.

Table 1. Effect of supplementation followed by processing on anti-nutrients and total phenolic contents (mg/100g) of millet flour

Supplementation level (%)	Fermentation time (h)	*Moringa* seed flour			Fenugreek seed flour		
		Phytic acid	Tannin	Total phenols	Phytic acid	Tannin	Total phenols
0	Fermented dough						
	0	203.02 ± 7.25^{d}	190.04 ± 0.93^{c}	441.24 ± 5.23^{o}	203.02 ± 7.25^{d}	190.04 ± 0.93^{d}	441.24 ± 5.23^{d}
	8	160.30 ± 2.54^{m}	180.32 ± 1.02^{e}	382.26 ± 2.99^{p}	160.30 ± 2.54^{j}	180.32 ± 1.02^{e}	382.26 ± 2.99^{k}
	16	140.33 ± 0.96^{p}	150.12 ± 0.81^{f}	377.16 ± 0.57^{q}	140.33 ± 0.96^{k}	150.12 ± 0.81^{g}	377.16 ± 1.57^{n}
	Fermented/cooked						
	8	149.21 ± 4.19^{n}	130.13 ± 0. 61^{g}	380.10 ± 0.76^{p}	139.21 ± 1.19^{m}	130.13 ± 0. 56^{j}	380.10 ± 0.76^{l}
	16	129.80 ± 3.33^{r}	80.78 ± 0.67^{m}	373.92 ± 0.57^{r}	129.80 ± 3.33^{o}	60.78 ± 0.47^{bc}	373.92 ± 0.57^{o}
5%	Fermented dough						
	0	218.50 ± 5.08^{c}	188.09 ± 0.61^{d}	547.81 ± 8.57^{c}	208.84 ± 6.72^{c}	197.01 ± 0.83^{c}	456.67 ± 5.42^{c}
	8	161.41 ± 1.66^{l}	110.11 ± 0.57^{i}	527.85 ± 1.34^{f}	182.57 ± 3.46^{h}	130.13 ± 0.78^{j}	393.58 ± 2.85^{j}
	16	143.11 ± 1.66^{o}	92.46 ± 0.00^{j}	500.10 ± 3.47^{i}	143.19 ± 1.68^{l}	110.25 ± 0.00^{m}	381.47 ± 1.67^{l}
	Fermented/cooked						
	8	150.87 ± 4.18^{n}	78.21 ± 0.88^{m}	443.11 ± 0.74^{n}	133.83 ± 2.51^{n}	80.05 ± 0.87^{n}	380.38 ± 0.64^{l}
	16	135.95 ± 2.82^{q}	60.04 ± 0.45^{p}	440.62 ± 4.48^{op}	120.94 ± 1.92^{p}	70.14 ± 0.50^{o}	379.39 ± 0.57^{m}
10%	Fermented dough						
	0	233.16 ± 0.96^{b}	192.00 ± 0.71^{b}	600.52 ± 6.38^{b}	215.38 ± 1.66^{b}	205.00 ± 0.86^{b}	461.27 ± 0.58^{b}
	8	199.71 ± 6.66^{g}	114.43 ± 0.12^{h}	529.71 ± 0.37^{e}	192.00 ± 0.96^{f}	160.08 ± 0.65^{f}	408.64 ± 0.43^{f}
	16	190.26 ± 3.46^{h}	84.24 ± 0.64^{l}	504.83 ± 5.71^{h}	178.13 ± 7.25^{i}	140.01 ± 0.91^{h}	404.84 ± 1.68^{fg}
	Fermented/cooked						
	8	176.39 ± 3.33^{j}	79.08 ± 0.21^{m}	475.71 ± 6.90^{k}	159.24 ± 2.54^{j}	132.00 ± 0.79^{i}	395.51 ± 2.80^{i}
	16	164.74 ± 1.66^{k}	68.31 ± 0.65^{o}	450.84 ± 1.06^{lm}	145.14 ± 0.37^{k}	120.07 ± 0.88^{l}	380.39 ± 0.37^{l}
15%	Fermented dough						
	0	253.70 ± 4.19^{a}	199.04 ± 0.12^{a}	621.49 ± 2.61^{a}	223.69 ± 2.88^{a}	209.09 ± 0.73^{a}	475.96 ± 0.94^{a}
	8	209.18 ± 1.02^{e}	109.06 ± 0.45^{i}	532.95 ± 1.68^{d}	201.42 ± 1.66^{e}	180.13 ± 0.98^{e}	411.00 ± 1.74^{e}
	16	200.47 ± 0.79^{f}	90.17 ± 0.52^{k}	512.66 ± 12.39^{g}	185.35 ± 0.95^{g}	160.00 ± 0.81^{f}	403.53 ± 2.37^{h}
	Fermented/cooked						
	8	191.92 ± 1.92^{h}	80.02 ± 0.73^{m}	480.56 ± 4.31^{j}	177.55 ± 2.54^{i}	140.12 ± 1.04^{h}	405.40 ± 3.06^{g}
	16	184.26 ± 2.58^{i}	70.14 ± 0.54^{n}	451.69 ± 0.37^{l}	143.14 ± 1.66^{l}	122.05 ± 0.98^{k}	391.59 ± 1.67^{k}
Raw Moringa/Fenugreek		346.68 ± 4.19	100.00 ± 1.08	635.10 ± 3.21	140.89 ± 1.58	130.00 ± 0.58	444.23 ± 3.18

Values are Mean ±SD. Values having different superscript within a column are significantly different ($P \leq 0.05$) according to DMRT

Table 2. Percent protein content and digestibility (IVPD) of supplemented, fermented and cooked millet flour.

Supplementation level (%)	Fermentation time (h)	*Moringa* seed flour		Fenugreek seed flour	
		Protein	IVPD	Protein	IVPD
0	Fermented dough				
	0	12.29 ± 0.10^{i}	45.75 ± 0.04^{l}	12.29 ± 0.10^{cd}	45.75 ± 0.04^{m}
	8	14.83 ± 0.05^{h}	58.84 ± 0.03^{j}	14.83 ± 0.05^{cd}	58.84 ± 0.03^{k}
	16	15.32 ± 0.33^{gh}	64.38 ± 0.06^{i}	15.32 ± 0.33^{c}	64.38 ± 0.06^{i}
	Fermented/cooked				
	8	15.38 ± 0.06^{gh}	68.30 ± 0.07^{f}	15.38 ± 0.06^{c}	68.30 ± 0.07^{h}
	16	15.67 ± 0.50^{g}	78.60 ± 0.02^{c}	15.67 ± 0.29^{c}	78.60 ± 0.02^{b}
5%	Fermented dough				
	0	16.91 ± 0.16^{f}	52.62 ± 0.02^{k}	15.35 ± 0.04^{c}	46.29 ± 0.04^{l}
	8	17.50 ± 0.11^{ef}	68.79 ± 3.30^{f}	15.36 ± 0.16^{c}	46.37 ± 0.07^{l}
	16	18.18 ± 0.24^{e}	66.04 ± 0.32^{g}	17.06 ± 0.18^{bc}	63.21 ± 0.01^{j}
	Fermented/cooked				
	8	19.25 ± 0.25^{d}	71.65 ± 0.01^{e}	18.27 ± 0.48^{b}	65.08 ± 0.19^{h}
	16	19.39 ± 0.09^{d}	79.89 ± 0.29^{bc}	17.50 ± 0.00^{bc}	69.18 ± 3.34^{g}
10%	Fermented dough				
	0	19.39 ± 0.18^{d}	65.89 ± 0.01^{h}	15.76 ± 0.08^{c}	63.49 ± 0.58^{j}
	8	19.79 ± 0.16^{d}	71.31 ± 0.33^{e}	15.77 ± 0.31^{c}	72.43 ± 0.19^{f}
	16	20.75 ± 0.06^{c}	78.41 ± 0.02^{c}	17.08 ± 0.07^{bc}	75.07 ± 0.13^{e}
	Fermented/cooked				
	8	20.85 ± 0.11^{c}	80.17 ± 0.65^{b}	18.15 ± 0.36^{b}	75.23 ± 0.02^{e}
	16	22.02 ± 0.15^{b}	80.60 ± 0.14^{b}	20.17 ± 0.24^{a}	78.98 ± 0.10^{b}
15%	Fermented dough				
	0	22.02 ± 0.25^{b}	66.50 ± 0.55^{g}	15.76 ± 0.29^{c}	64.12 ± 0.21^{i}
	8	22.46 ± 0.13^{b}	73.80 ± 0.37^{d}	15.78 ± 0.11^{c}	69.15 ± 0.03^{g}
	16	23.61 ± 0.18^{a}	79.42 ± 0.01^{c}	19.83 ± 0.14^{ab}	76.84 ± 0.31^{d}
	Fermented/cooked				
	8	23.63 ± 0.16^{a}	81.35 ± 0.56^{b}	19.33 ± 0.37^{ab}	77.17 ± 0.17^{c}
	16	23.93 ± 0.24^{a}	92.31 ± 0.02^{a}	20.89 ± 0.08^{a}	80.29 ± 0.15^{a}
Raw Moringa/Fenugreek		63.41± 0.40	86.10 ± 0.11	30.33 ± 0.58	84.53 ± 0.65

Values are Mean ±SD. Values having different superscript within a column are significantly different ($P \leq 0.05$) according to DMRT

Fermentation increased the P content of DSFM and DSFF supplements with an increase in fermentation and supplementation level. This could be attributed to the reduction in antinutritional factors such as phytate of the supplements by phytase enzymes which synthesize and release P during fermentation as reported for bread fruit (*Trecula africana*) seed flour[37] and lentil[38]. However cooking lowered the P content of DSFM supplemented dough but increased that of DSFF supplemented dough. The increase in P content of DSFF supplemented dough agrees with previous findings[39] where cooking of fermented flour of finger millet was found to significantly reduced the antinutritional factors which interfere with minerals such as P.

3.4 HCl extractable Ca, P and Fe contents

The effect of supplementation, fermentation and cooking on extractable Ca, P and Fe of MF are shown in Table 4**.** The data indicated that Ca (67.20%) was the most available minerals in raw MF while Fe (31.48%) was the least available. The addition of DSFM and DSFF significantly ($P \leq 0.05$) enhanced the extractable Ca, Fe and P with supplementation level compared to the control flour which could be attributed to the high availability of minerals exhibited by raw *Moringa* and fenugreek seeds used as supplements. Fermentation alone increased the availability of all minerals with P being the most available. The increment in minerals availability after fermentation could be due to a reduction in antinutritional factors with some phytic acid still forming complexes with divalent metals such as Fe and Ca thereby reducing their availability compared to P. Values obtained in this study was higher than that of malted and fermented pearl millet flour[15]. Fermentation of DSFM and DSFF supplements increased ($P \leq 0.05$) the extractable minerals with supplementation and fermentation levels. In a similar study[14], increase in Ca, Fe and P extractability of high and low phytate corn genotype was observed after fermentation for 14 h. Although the extractability of P was higher in DSFM supplement compared to DSFF but the later had higher content of P. In addition, despite the low phytic acid content of the DSFF supplement but phytic acid had a negative correlation with extractability of P. This variation may be due to the chemical nature

of phytate and P of raw *Moringa* and fenugreek seeds used as supplements. Cooking after fermentation significantly ($P \leq 0.05$) reduced the extractable minerals. The present finding disagreed with the previous findings[15] in which they observed an increase in extractable minerals after cooking of pearl millet flour. The reduction may be attributed to heat treatment, which may have inactivated the enzymes such as phytase obtained during fermentation. However, increasing the fermentation period to 16 h followed by cooking significantly increased the extractable mineral contents but the values were lower compared to fermentation alone. The increment in Ca, Fe and P extractability (%) of supplemented millet dough is likely due to the reduction of antinutrients by fermentation. The difference between total and extractable Ca, Fe and P may be attributed to the differences in the level of antinutrients of the supplements before and after treatments.

Table 3. Effect of supplementation followed by processing on total Ca, P and Fe (mg/100g) of millet flour

Supplemen-tation level (%)	Fermentation time (h)	*Moringa* seed flour			Fenugreek seed flour		
		Ca	Fe	P	Ca	Fe	P
0	Fermented dough						
	0	3.81 ± 0.05^{jk}	1.62 ± 0.07^{l}	312.67 ± 3.47^{q}	3.81 ± 0.05^{g}	1.62 ± 0.07^{g}	312.67 ± 1.47^{p}
	8	6.25 ± 0.13^{ef}	12.68 ± 0.35^{gh}	446.67 ± 3.11^{p}	6.25 ± 0.54^{e}	12.67 ± 0.25^{b}	446.67 ± 2.55^{j}
	16	5.95 ± 0.14^{h}	12.06 ± 0.29^{h}	571.73 ± 1.04^{i}	5.95 ± 0.16^{f}	12.06 ± 0.26^{b}	571.73 ± 1.84^{h}
	Fermented/cooked						
	8	7.67 ± 0.25^{d}	7.38 ± 0.31^{kl}	701.27 ± 5.68^{a}	7.67 ± 0.23^{d}	7.38 ± 0.15^{de}	701.27 ± 4.08^{e}
	16	4.57 ± 0.11^{i}	8.39 ± 0.41^{jk}	683.40 ± 2.46^{c}	4.57 ± 0.19^{fg}	8.39 ± 0.13^{cd}	683.40 ± 3.25^{f}
5%	Fermented dough						
	0	8.73 ± 0.12^{c}	10.81 ± 0.25^{j}	500.40 ± 2.84^{k}	3.85 ± 0.13^{g}	3.25 ± 0.08^{f}	352.87 ± 2.08^{o}
	8	4.31 ± 0.08^{ij}	12.86 ± 0.51^{g}	549.27 ± 4.86^{n}	8.19 ± 0.17^{cd}	7.96 ± 0.12^{d}	393.00 ± 4.52^{m}
	16	3.18 ± 0.14^{kl}	7.72 ± 0.54^{k}	692.33 ± 4.68^{b}	9.69 ± 0.31^{ab}	11.89 ± 0.16^{c}	647.67 ± 3.59^{g}
	Fermented/cooked						
	8	2.12 ± 0.06^{n}	17.17 ± 0.68^{d}	491.13 ± 2.32^{m}	8.66 ± 0.25^{c}	5.67 ± 0.13^{e}	701.27 ± 2.62^{e}
	16	2.26 ± 0.09^{mn}	14.22 ± 1.03^{ef}	$518.33 \pm 5.41o$	6.91 ± 0.29^{de}	9.48 ± 0.18^{cd}	683.40 ± 1.25^{f}
10%	Fermented dough						
	0	11.22 ± 0.13^{b}	11.60 ± 2.68^{i}	560.27 ± 2.43^{h}	4.86 ± 0.15^{f}	3.86 ± 0.12^{f}	380.07 ± 1.09^{n}
	8	2.86 ± 0.31^{l}	11.03 ± 1.52^{ij}	572.27 ± 5.07^{n}	9.83 ± 0.17^{a}	6.72 ± 0.15^{de}	419.87 ± 3.39^{k}
	16	2.33 ± 0.27^{m}	15.67 ± 2.55^{e}	584.27 ± 2.87^{n}	9.03 ± 0.19^{b}	9.15 ± 0.20^{cd}	710.00 ± 1.09^{d}
	Fermented/cooked						
	8	3.52 ± 0.25^{k}	12.86 ± 3.08^{g}	536.80 ± 4.33^{j}	8.22 ± 0.26^{cd}	6.69 ± 0.13^{de}	710.20 ± 1.54^{d}
	16	3.91 ± 0.15^{j}	23.21 ± 1.38^{b}	562.27 ± 3.31^{l}	8.68 ± 0.18^{c}	14.68 ± 0.15^{a}	808.47 ± 2.68^{a}
15%	Fermented dough						
	0	12.37 ± 0.19^{a}	13.05 ± 1.54^{f}	620.87 ± 5.74^{f}	5.32 ± 0.28^{ef}	4.19 ± 0.16^{f}	415.93 ± 2.54^{l}
	8	6.86 ± 0.24^{e}	23.04 ± 2.06^{b}	647.67 ± 3.37^{e}	9.77 ± 0.37^{ab}	10.16 ± 0.17^{c}	464.53 ± 1.93^{i}
	16	5.43 ± 0.21^{g}	55.95 ± 1.52^{a}	678.30 ± 6.63^{d}	8.70 ± 0.41^{c}	10.25 ± 0.14^{c}	728.07 ± 2.05^{c}
	Fermented/cooked						
	8	4.83 ± 0.23^{hi}	16.79 ± 1.63^{de}	576.20 ± 2.06^{i}	7.07 ± 0.42^{de}	8.01 ± 0.19^{cd}	808.47 ± 3.27^{a}
	16	5.12 ± 0.26^{gh}	20.48 ± 0.94^{c}	594.07 ± 5.44^{g}	9.83 ± 0.29^{a}	11.83 ± 0.12^{c}	763.80 ± 4.33^{b}
.aw Moringa/Fenugreek		41.18 ± 0.12	98.95 ± 1.50	1326.60 ± 2.16	45.49 ± 1.03	10.87 ± 0.06	701.27 ± 3.47

Values are Mean ±SD. Values having different superscript within a column are significantly different ($P \leq 0.05$) according to DMRT

Table 4. Effect supplementation followed by processing on extractable (%) Ca, P and Fe of millet flour.

Supplemen-tation level (%)	Fermentation time (h)	*Moringa* seed flour			Fenugreek seed flour		
		Ca	Fe	P	Ca	Fe	P
0	Fermented dough						
	0	67.20 ± 1.03^{h}	31.48 ± 0.99^{n}	42.43 ± 2.10^{g}	67.20 ± 1.03^{g}	31.48 ± 0.99^{k}	42.43 ± 2.10^{j}
	8	73.99 ± 1.02^{ef}	36.18 ± 2.03^{l}	78.19 ± 1.62^{e}	70.99 ± 0.18^{f}	36.18 ± 0.26^{i}	78.19 ± 0.68^{f}
	16	76.40 ± 0.25^{m}	59.79 ± 1.51^{i}	82.15 ± 1.33^{d}	71.40 ± 0.19^{f}	59.79 ± 0.38^{h}	80.15 ± 0.55^{e}
	Fermented/cooked						
	8	70.96 ± 0.67^{o}	34.81 ± 5.63^{m}	76.39 ± 2.05^{f}	66.96 ± 0.22^{g}	34.18 ± 0.31^{j}	74.39 ± 0.57^{h}
	16	73.63 ± 0.64^{f}	53.48 ± 3.08^{k}	79.90 ± 2.04^{e}	70.63 ± 0.10^{h}	53.18 ± 0.24^{b}	76.90 ± 0.41^{g}
5%	Fermented dough						
	0	73.51 ± 2.05^{f}	65.58 ± 2.56^{f}	84.08 ± 1.55^{e}	69.17 ± 0.09^{f}	58.63 ± 0.31^{h}	80.11 ± 0.00^{e}
	8	79.97 ± 1.03^{d}	66.77 ± 3.46^{f}	89.49 ± 0.96^{cd}	73.72 ± 0.19^{e}	61.48 ± 0.25^{g}	83.00 ± 0.00^{d}
	16	87.07 ± 3.46^{b}	75.48 ± 1.99^{d}	90.99 ± 1.47^{c}	75.02 ± 0.14^{d}	73.14 ± 0.26^{c}	87.35 ± 0.00^{b}
	Fermented/cooked						
	8	76.03 ± 2.55^{c}	61.35 ± 2.47^{i}	78.46 ± 1.25^{j}	70.97 ± 0.25^{f}	56.20 ± 0.41^{g}	76.66 ± 0.00^{g}
	16	83.56 ± 2.04^{a}	69.46 ± 2.01^{e}	85.02 ± 2.09^{e}	71.89 ± 0.23^{f}	59.11 ± 0.37^{h}	82.16 ± 0.00^{ab}

Table 4. (*Continued*)

10%	Fermented dough						
	0	74.23 ± 1.69^{e}	62.93 ± 1.32^{h}	86.48 ± 1.33^{e}	80.08 ± 0.19^{c}	63.74 ± 0.24^{f}	83.22 ± 0.00^{d}
	8	80.89 ± 2.41^{d}	64.92 ± 2.04^{g}	89.49± 1.54^{cd}	82.52 ± 0.19^{b}	67.75 ± 0.19^{e}	85.02 ± 0.00^{c}
	16	88.67 ± 1.14^{b}	82.41 ± 1.62^{b}	91.49 ± 1.64^{b}	84.23 ± 0.14^{b}	70.06 ± 0.37^{d}	87.60 ± 0.00^{b}
	Fermented/cooked						
	8	75.54 ± 3.69^{e}	57.64 ± 3.25^{j}	84.44 ± 3.07^{l}	80.28 ± 0.26^{c}	51.96 ± 0.26^{k}	72.63 ± 0.00^{i}
	16	78.12 ± 1.93^{d}	77.34 ± 1.25^{c}	89.30 ± 1.47^{cd}	81.07 ± 0.16^{c}	67.42 ± 0.40^{e}	78.59 ± 0.00^{f}
15%	Fermented dough						
	0	76.05 ± 1.54^{e}	63.25 ± 1.64^{h}	88.49 ± 2.05^{d}	81.18 ± 0.13^{c}	68.09± 0.28^{e}	85.49 ± 0.00^{c}
	8	78.88 ± 2.06^{d}	65.18 ± 1.24^{f}	90.13 ± 1.1^{bc}	83.22 ± 0.14^{b}	72.17± 0.19^{c}	87.23 ± 0.00^{b}
	16	90.17 ± 1.52^{a}	84.58 ± 0.77^{a}	94.46 ± 1.76^{a}	86.28 ± 0.18^{a}	76.62 ± 0.37^{b}	90.06 ± 0.00^{a}
	Fermented/cooked						
	8	71.36 ± 1.78^{g}	54.78 ± 0.98^{k}	87.91 ± 2.68^{d}	80.87 ± 0.16^{c}	67.65 ± 0.27^{e}	77.93 ± 0.00^{f}
	16	83.25 ± 1.37^{c}	74.39 ± 1.61^{d}	89.08 ± 2.33^{cd}	82.61 ± 0.11^{b}	82.53 ± 0.30^{a}	85.52 ± 0.00^{c}
Raw Moringa/Fenugreek		98.95 ± 1.50	37.67 ± 1.92	99.60 ± 1.15	20.44 ± 0.93	45.28 ± 0.26	92.53 ± 1.17

Values are Mean ±SD. Values having different superscript within a column are significantly different ($P \leq 0.05$) according to DMRT

4. Conclusion

The results of this study indicate that supplementation of millet flour with DSFM and DSFF resulted in a significant increase in protein content, IVPD, total and extractable Ca, Fe and P. Also, fermentation of the supplemented flours decreased their phytate, tannin and polyphenols with further reduction obtained after cooking. Fermentation alone was more effective in increasing the total and extractable Ca, Fe and P.

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