

SUPPLEMENTATION AND COOKING OF PEARL MILLET: CHANGES IN ANTI-NUTRIENTS, AND TOTAL MINERALS CONTENT AND EXTRACTABILITY

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Abstract: Then the effect of cooking on anti-nutritional factors and minerals content and extractability of pearl millet flour supplemented with moringa or fenugreek seeds flour was investigated. The results revealed that supplementation with 5, 10, and 15% moringa or fenugreek seeds flour significantly ($P < 0.05$) increased the phytic acid, total polyphenols, and total minerals. Whereas, tannin was slightly decreased after supplementation with moringa seeds flour, but supplementation with fenugreek seed flour had no effect. Cooking of both raw and supplemented flour decreased the anti-nutritional factors, and concurrently increased ($P < 0.05$) the total minerals (Ca, Fe and P) of all flour types. The reduction in anti-nutritional factors of both raw and supplemented flour after cooking was concomitant with an increase in total minerals content and extractability. These findings demonstrated the beneficial effect of supplementing millet flour with moringa seed flour and/or fenugreek seed flour that could likely improve the nutritional quality of this important food for peoples in developing countries and hence overcome the malnutrition problems in these countries.

Key words: anti-nutrients, cooking, fenugreek seeds flour, millet, moringa seeds flour, supplementation.

Introduction

Millet is widely grown in the semiarid tropics of Africa and Asia and constitutes a major source of carbohydrates and proteins for people living in these areas. Millet is one of the most important drought-resistant crops and the 6th cereal crop in terms of world agriculture production (Saleh *et al.*, 2013). Also,

millet has resistance to pests and diseases, short growing season, and productivity under drought conditions, compared to major cereals (Devi *et al.*, 2011). Therefore, millet grains are now receiving specific attention from these developing countries in terms of utilization as food as well as from some developed countries in terms of its good potential in

the manufacturing of bioethanol and biofilms (Li *et al.*, 2008).

In many African and Asian countries, millets serve as a major food component and various traditional foods and beverages, such as bread (fermented or unfermented), porridges, and snack foods are made of millet (Chandrasekara *et al.*, 2012). In addition to their nutritive value, several potential health benefits such as preventing cancer and cardiovascular diseases, reducing tumor incidence, lowering blood pressure, risk of heart disease, cholesterol and rate of fat absorption, delaying gastric emptying, and supplying gastrointestinal bulk have been reported for millet (Gupta *et al.*, 2012). Because of their important contribution to national food security and potential health benefits, millet grain is now receiving increasing interest from food scientists, technologists, and nutritionists (Saleh *et al.*, 2013). In Sudan, pearl millet (*Pennisetum glaucum* L.) is grown as multipurpose crop, providing food, feed, construction materials, and fuel in the rainfed areas principally in the western region (Darfur and Kordofan). In these regions, pearl millet is prepared in several types of meals such as *Balila* (whole cooked grain), non-fermented whole millet flour to make *Aceeda* (stiff porridge) and *Madidat atroon* (thin porridge with natron), fermented dough to make *Kisra* (unleavened bread), *Nasha* (thin porridge) and *Marisa* (opaque beer), and the extracted starch from fermented decorticated millet to make *Jir* or *Geeria* (Abdalla *et al.*, 2009; Mohamed *et al.*, 2010a). Like other cereals, the nutritive value of millet is inadequate due to its deficiency in essential amino acids (lysine and tryptophan), and the presence of antinutritional factors such as phytate, tannins and polyphenols (Ali *et al.*, 2009). These antinutritional factors chelate dietary minerals in the gastrointestinal tract reducing their bioaccessibility and bioavailability as reported for millet (Abdelrahman *et al.*, 2007), sorghum (Idris *et al.*, 2005) and corn (Sokrab *et al.*, 2011).

Minerals are involved in activation of intracellular and extracellular enzymes, in regulation of critical pH level in body fluids necessary for control of metabolic reactions and in osmotic balance between the cell and

the environment. A deficiency of any one of the essential minerals can result in severe metabolic disorders and compromise the health of the body (Sokrab *et al.*, 2012). In developing countries, the low bioavailability of minerals (especially iron and zinc) in cereal-based foods is a crucial problem for infants and young children. Because of the great importance of millet as a basic staple food for large population groups, particularly in developing countries, and its low nutritional value, mainly with respect to protein quality, many efforts have been made to improve the biological utilization of the nutrients it contains (Ali *et al.*, 2009; Mohamed *et al.*, 2010a, b, c; Mohamed *et al.*, 2011).

In this regard, several traditional household food processing and preparation methods were used to enhance the bioavailability of micronutrients in millet grains. These include cooking, dehuling, soaking, fermentation, supplementation, and germination/malting. Supplementation of millet grains with natural food products to enhance their nutritive value can be promising and with high cost-effectiveness compared with fortification by chemical synthetic nutrients (Saleh *et al.*, 2013). Grains are low in the amino acid lysine, which makes their protein content less useful than it would otherwise have been. Legumes tend to be low in the amino acids methionine and cysteine, but are high in the amino acid lysine, so eating the two together leveled the protein content of both (Ali *et al.*, 2009).

Legumes are largely replacing milk and other sources of animal protein, which are expensive and not readily available as suitable substitutes for high quality protein (Annan and Plahar, 1995). Moringa (*Moringa oleifera*) and fenugreek (*Trigonella foenum-graecum* L.) are legumes commonly grown in many parts of the world for both culinary purposes and health benefits. In one hand, moringa leaves are rich in protein source, which can be used by doctors, nutritionists and community health cautious persons to solve worldwide malnutrition or under nutrition problems (Thurber and Fahey, 2009).

Some articles and research studies have reported that the dry leaves of moringa contain 7 times more

RESEARCH ARTICLE

vitamin C than orange, 10 times vitamin A than carrot, 17 times calcium than milk, 15 times potassium than bananas, 25 times iron than spinach and 9 times proteins than yogurt (Fugile, 1999). On the other hand, the fenugreek seed is very bitter but does have interesting proximate composition. Protein content ranges between 23 and 43% of the seed, carbohydrate represents up to 58%, moisture make up about 10 – 13% of the seed, lipid represent 5 – 6 % and minerals make up less than 1% (Elnasri, 2006).

Research evaluating antinutritional factors and mineral profile of millet flour supplemented with either moringa or fenugreek has not been reported. Therefore, in this study we would like to investigate the effect supplementation with defatted seeds of moringa or fenugreek seeds flour and cooking on antinutritional factors and minerals bioavailability of millet flour. This information will help in improving the nutritional quality of millet grain and thus increase its uses in the preparation of several value-added and healthy food-products, which may then result in high demand from large urban populations and nontraditional millet users.

Materials and methods

Materials

The grains of pearl millet were obtained from the Department of Agronomy, Faculty of Agriculture, University of Khartoum, Shambat, Sudan. The grains were cleaned, freed from foreign seeds, broken, and shrunken ones, and then milled into fine flour using house blender and mortar to pass through a 0.4 mm screen. The flours were then stored in polyethylene bags at 4 °C for further analysis. Fenugreek seeds were brought from Omdurman local market, Omdurman, Sudan, then cleaned and freed from extraneous matter. Then milled into fine flour using house blender and mortar to pass through a 0.4 mm screen, defatted, and stored in polyethylene bags at 4 °C for further analysis. Defatted moringa seeds (cake) obtained from moringa farm, Khartoum North, Sudan. The cake was subjected to further extraction by hexane to remove the remaining oils, then washed with distilled

water and dried in a hot air oven at 60 °C for 3-4 hours. The defatted cake was then ground to fine flour using house blender and mortar to pass through a 0.4 mm and stored in polyethylene bags at 4 °C for further use. All chemicals used in this study were of reagent grade.

Supplementation

To increase the nutritive value of millet flour each of defatted moringa and fenugreek seeds flour were added using Pearson square at the supplementation rate of 5, 10, and 15% (Pearson, 1981). The number of composite flour samples after supplementation with moringa and fenugreek were six samples. Control sample consisting on raw millet flour without supplementation was treated in the same manner as composite flours.

Cooking

Cooking of the sample was performed by suspending the flour samples in distilled water in the ratio of 1:2 (flour: water, w/v) and the slurry was shaken to avoid lumps while boiling in a water bath for 20 min. Then the viscous mass was spread out thinly in a dish and oven dried at 60 °C. The dried flakes were milled into fine flour using house blender and mortar to pass through 0.4 mm screen and stored at 4 °C for further analysis.

Determination of phytic acid

Phytic acid content of the samples was determined by the method described by Wheeler and Ferrel (1971). Briefly, 2 g of raw millet flour and/or composite flour was extracted with 50 ml of 3 % trichloroacetic acid (TCA) for 3 h with shaking and precipitated as the ferric-phytate salt. Fifteen milliliters of 1.5 N NaOH was used to convert the ferric-phytate salt to ferric hydroxide. After acid hydrolysis of the precipitate with 3.2 N HNO₃, 2 ml of 1.5M KSCN (potassium thiocyanate) were added, and then immediately the iron content of the ferric hydroxide was determined at 480 nm (Hach DR3 spectrophotometer, Loveland, Colorado, USA). The phytate content calculated from this value assuming a constant 4Fe:6 P molecular ratio in the precipitate. The iron content in the unknown samples was read from the previously prepared

standard curve (different solution of ferric nitrate having varied concentration of Fe^{+++}). Phytic acid content was determined by multiplying the phytate phosphorus content by a constant factor of 3.55.

Determination of tannin content

Quantitative estimation of tannins was carried out using the modified vanillin-HCl method (Price and Bulter, 1977). Briefly, 200 mg sample was extracted using 10 ml of 1% (v/v) concentrated HCl in methanol for 20 min in capped rotating test tubes. Vanillin reagent (0.5%, 5 ml) was added to the extract (1 ml) and the absorbance of the colour developed after 20 min at 30 °C was read at 500 nm. A standard curve was prepared expressing the results as catechin equivalents, i.e. amount of catechin (mg/ml) which gives a colour intensity equivalent to that given by tannins after correcting for blank.

Determination of polyphenols content

Total polyphenols were determined according to the Prussian blue spectrophotometric method (Price and Bulter, 1977) with a minor modification. Sixty milligrams of ground sample was shaken manually for 1 min in 3.0 ml methanol. The mixture was filtered (Whatman No. 1). The filtrate was mixed with 50 ml distilled water and analyzed within an hour. About 3.0 ml of 0.1 M FeCl_3 in 0.1 M HCl was added to 1.0 ml filtrate, followed immediately by timed addition of 3.0 ml freshly prepared $\text{K}_3\text{Fe}(\text{CN})_6$. The absorbance was monitored on a spectrophotometer (Pye Unicam SP6-550 UV, London, UK) at 720 nm after 10 min from the addition of 3.0 ml of 0.1 M FeCl_3 and 3.0 ml of 0.008M $\text{K}_3\text{Fe}(\text{CN})_6$.

A standard curve was obtained, expressing the result as tannic acid equivalents; that is, the amount of tannic acid (mg/100 g) that gives a color intensity equivalent to that given by polyphenols after correction by blank sample.

Determination of total minerals

Minerals were determined by the dry ashing method described by Chapman and Pratt (1982). About 2.0 g of sample was acid digested with diacid mixture ($\text{HNO}_3:\text{HClO}_4$, 5:1, v/v) in a digestion chamber. The

digested samples were dissolved in double-distilled water and filtered (Whatman No. 41). The filtrate was made to 50 ml with double-distilled water and was used for determination of total minerals. Phosphorus was determined by the ammonium molybdate/ammonium vanadate method (Chapman and Pratt, 1982). Five millilitres of the aliquot extracted above was transferred into 50 ml volumetric flask, 10 ml ammonium molybdate–vanadate reagent was added and mixed again. After 30 min, the density of the color was read at 450 nm using Ultraviolet/Visible spectrophotometer (Jenway 6305, Essex, UK). Phosphorus concentration was determined from the standard curve. Calcium and iron were determined by atomic absorption spectrometer model 3110 (Perkin Elmer, Norwalk, CT, USA) following the methods of AOAC (1995).

Determination of HCl-extractability of minerals

Minerals in the samples were extracted by the method described by Chauhan and Mahjan (1988). 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 (Whatman No. 41). 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 described above. HCl extractability (%) was determined as follows:

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

Statistical analysis

All data were subjected to statistical analysis, each determination was carried out and analyzed in triplicate and figures were then averaged. Data was assessed by the analysis of Variance (ANOVA) Gomez and Gomez (1984).

Duncan Multiple Range Test (DMRT) was used to separate means. The mean values are presented together with standard deviation. Significance was accepted at $P = 0.05$.

RESEARCH ARTICLE

Results and Discussion

Effect of supplementation with moringa seeds flour (MSF) and cooking on phytic acid content of millet flour

Phytic acid in cereals is one of major concern as it chelates mineral cations and interacts with proteins

forming insoluble complexes which lead to reduced bio-availability of minerals and reduced digestibility of protein. The results on the effect of supplementation with MSF and cooking on phytic acid content of millet flour is shown in Table 1.

Table 1. Effect of cooking on anti- nutritional factors (mg/100g) of millet supplemented with different ratios of defatted moringa seeds

Supplementation levels (%)	Treatment	Phytic acid	Tannin	Total polyphenols
0	Raw	203.02 ^b (±7.25)	19.00 ^a (±0.03)	441.24 ^z (±5.23)
	Cooked	175.26 ^h (±2.51)	18.01 ^a (±0.02)	426.13 ^h (±2.05)
5	Raw	248.50 ^c (±5.08)	8.00 ^d (±0.01)	547.81 ^e (±8.57)
	Cooked	215.22 ^f (±5.08)	7.00 ^e (±0.00)	543.78 ^f (±4.24)
10	Raw	255.16 ^b (±0.96)	8.00 ^d (±0.01)	600.52 ^b (±6.38)
	Cooked	237.96 ^e (±4.40)	8.00 ^d (±0.01)	571.27 ^d (±3.39)
15	Raw	260.70 ^a (±4.19)	10.00 ^b (±0.01)	621.49 ^a (±2.61)
	Cooked	241.84 ^d (±2.54)	9.00 ^c (±0.01)	579.73 ^c (±2.96)
Lsd _{0,05}		3.5263 ^{**}	0.0087 [*]	5.4216 ^{**}
SE±		1.5247	0.00	2.0986

^{a-h} Mean ±SD values having same superscript within a column are insignificantly different (P 0.05) according to DMRT.

Phytic acid content of millet flour was found to be 203.02 mg/100g which was within the range 107-943 mg/100 g reported for many Sudanese millet cultivars (El Hag *et al.*, 2002; Mohamed *et al.*, 2010a; Mohamed *et al.*, 2011).

Supplementation of millet flour with 5, 10, and 15% MSF proportionally (P 0.05) increased the phytic acid content to 248.50, 255.16 and 260.70 mg/100g, respectively. The increase of the phytic acid content after millet flour supplementation with MSF can be explained through the high amounts of phytate present in defatted moringa seed flour (Anhwange *et al.*, 2004; Makkar and Becker 1997; Abiodun *et al.*, 2012). This phytate content is of a similar order of magnitude as observed for many other conventional protein supplements (soybean meal 320-380 mg/100g,

rapeseed meal 600-730 mg/100 g, sunflower 620-920 mg/100 g, groundnut meal 320-430 mg/100 g) (Pointillart, 1993).

Cooking of raw flour and that supplemented with 5, 10, and 15% MSF significantly (P 0.05) decreased the phytic acid to 175.26, 215.22, 237.96 and 241.84 mg/100g, respectively. Similar trend in phytic acid reduction after cooking was reported for fermented pearl millet flours (Abdelrahman *et al.*, 2005; Mohamed *et al.*, 2010a; Mohamed *et al.*, 2011) and sicklepod (*Cassia obtusifolia*) leaves (Osman *et al.*, 2010).

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 (Osman *et al.*, 2010). Degradation of phytic acid may take place either through endogenous phytases of grains or due to heat.

Effect of supplementation with moringa seeds flour (MSF) and cooking on tannin content of millet flour

Tannin content was significantly ($P = 0.05$) varied between raw and composite flours (Table 1). These variations in the tannin content between the raw and composite flours could be due to the fact that the substitution of millet flour (high tannin content) with moringa seed flour (low tannin content) might greatly affected the tannin content. These values were slightly higher than those reported previously for other millet cultivars (Mohamed *et al.*, 2010a). This variation could be attributed to the differences in genotypes, cultivation practices, and environmental and soil conditions. Supplementation significantly ($P = 0.05$) reduced the tannin content from 19 mg/100 g in raw flour to 10 mg/100 g in 15 % MSF composite flour.

It is well known that legumes contained lower concentration of tannin compared to that of cereals such as sorghum and millet. Particularly, different parts of moringa were reported to have negligible amounts of tannins (0.1-1.2 mg/100 g) and condensed tannins were not detectable (Makkar and Becker, 1997). Thus, this could explain the slight reduction in tannin content after supplementation with MSF.

Cooking of raw millet flour and composite flours supplemented with 5, 10, and 15% MSF insignificantly ($P = 0.05$) reduced the tannin content to 18, 7, 8 and 9 mg/100 g, respectively. Similarly, a reduction in tannin content of cooked millet flour compared to uncooked one has been reported (Mohamed *et al.*, 2010c). This may be due to the fact that heat treatment may likely denatured tannins.

Effect of supplementation with moringa seeds flour (MSF) and cooking on total polyphenol content of millet flour

The total polyphenols content of raw millet flour was 441.26 mg/100g (Table 1). This value is in general agreement with those reported for different millet

genotypes (Abdelrahman *et al.*, 2005; Mohamed *et al.*, 2011). However, after supplementation with 5, 10 and 15% MSF was significantly ($P = 0.05$) increased the polyphenols content of the composite flours to 547.18, 600.52 and 621.49 mg/100g, respectively. The increasing of polyphenols after supplementation with MSF could be attributed to the fact that moringa contained high amounts of polyphenols (Makkar and Becker, 1997) and upon supplementation this will lead to the increase in polyphenols contents of the composite flours.

However, cooking significantly ($P = 0.05$) decreased the total polyphenols of both raw millet flour and flours supplemented with 5, 10, and 15% MSF to 426.13, 543.78, 571.27 and 579.73 mg/100g, respectively. Similar observations on the reduction of polyphenols by heat treatment have also been reported by many investigators (Abdelrahman *et al.*, 2005; Mohamed *et al.*, 2011; Osman *et al.*, 2010). The reduction in polyphenols after cooking might be as a result of the fact that phenols react with protein during cooking forming poorly extractable protein-phenolic complexes (Osman *et al.*, 2010).

Overall, although the supplementation with MSF could possibly improve the protein quality of millet flour, but it could increase the antinutritional factors mainly phytic acid and polyphenols of the composite flour.

Thus it is recommended to either soak or ferment both millet flour and moringa seed flour before supplementation. These processes could efficiently reduce the antinutritional factors of the composite flours.

Effect of supplementation with fenugreek seeds flour (FSF) and cooking on phytic acid content of millet flour

Results on the effect of cooking and supplementation with FSF on the phytic acid content of millet and its composite flours are shown in Table 2. The phytic acid content of millet flour was significantly ($P = 0.05$) decreased with the increase in the supplementation level of FSF.

RESEARCH ARTICLE

Effect of supplementation with fenugreek seeds flour (FSF) and cooking on tannin content of millet flour

The reduction of phytic acid content of composite flour could be linked to its relatively lower concentration (164.0-168.1 mg/100 g) in fenugreek seeds (El-Shimi *et al.*, 1984; Abdel-Nabey and Damir, 1990) compared to that of millet flour.

Cooking significantly ($P = 0.05$) decreased the phytic acid content of millet flour and flours supplemented with 5, 10, and 15% FSF to 175.28, 145.88, 155.87 and 163.63 mg/100g, respectively. These results agreed with the findings of Abdelrahman *et al.* (2005) who investigated the destruction of phytate during heat processing, and their results showed that cooking reduced phytic acid content of two pearl millet cultivars by rates 6% and 10%.

According to DeBoland *et al.* (1975) the reduction in phytic acid contents during cooking could probably be explained on the basis that phytase activity at a temperature of 40 – 55 °C may degrade inositol hexaphosphate to the pentaphosphate or lower molecular weight forms.

The tannin content of both millet flour and composite flours was found to be 19 mg/100 g (Table 2).

Supplementation with FSF does not affect the tannin content of millet flour. After cooking the tannin content of millet flour and flour supplemented with 10% FSF insignificantly ($P = 0.05$) decreased to 18 and 17 mg/100 g, respectively. Whereas, it significantly ($P = 0.05$) decreased the tannin content of 5% FSF composite flour to 15 mg/100 g, but did not affect the tannin content of 15% FSF composite flour.

The reduction in tannin content of raw millet flour and composite flours after cooking may be due to the heat degradation of these molecules as well as changes in their chemical reactivity or the formation of insoluble complexes. A similar finding was reported by Fasoyiro *et al.* (2005) who reported that cooking significantly reduced the tannin content of pigeon pea seeds.

Table 2. Effect of cooking on anti-nutritional factors (mg/100g) of millet flour supplemented with different ratios of fenugreek seeds flour

Supplementation Levels (%)	Treatment	Phytic acid	Tannin	Total polyphenols
0	Raw	203.02 ^a (±7.25)	19.00 ^a (±0.001)	441.24 ^d (±5.23)
	Cooked	175.28 ^d (±2.54)	18.00 ^a (±0.02)	426.13 ^e (±2.05)
5	Raw	175.84 ^d (±6.72)	19.00 ^a (±0.03)	456.67 ^e (±5.42)
	Cooked	145.88 ^g (±0.96)	15.00 ^b (±0.00)	412.12 ^h (±0.37)
10	Raw	186.38 ^c (±1.66)	19.00 ^a (±0.03)	461.27 ^b (±0.58)
	Cooked	155.87 ^f (±0.95)	17.00 ^{ab} (±0.01)	421.08 ^g (±0.74)
15	Raw	194.69 ^b (±2.88)	19.00 ^a (±0.03)	475.96 ^a (±0.74)
	Cooked	163.63 ^e (±0.96)	19.00 ^a (±0.03)	423.32 ^f (±1.29)
Lsd _{0.05}		1.0322 ^{**}	0.1158 [*]	2.8569 ^{**}
SE±		0.7254	0.0209	0.9856

^{a-h} Mean ±SD values having same superscript within a column are insignificantly different ($P = 0.05$) according to DMRT.

Effect of supplementation with fenugreek seeds flour (FSF) and cooking on tannin content of millet flour

The tannin content of both millet flour and composite flours was found to be 19 mg/100 g (Table 2).

Supplementation with FSF does not affect the tannin content of millet flour. After cooking the tannin content of millet flour and flour supplemented with

10% FSF insignificantly ($P = 0.05$) decreased to 18 and 17 mg/100 g, respectively.

Whereas, it significantly ($P = 0.05$) decreased the tannin content of 5% FSF composite flour to 15 mg/100 g, but did not affect the tannin content of 15% FSF composite flour.

The reduction in tannin content of raw millet flour and composite flours after cooking may be due to the heat degradation of these molecules as well as changes in their chemical reactivity or the formation of insoluble complexes.

A similar finding was reported by Fasoyiro *et al.* (2005) who reported that cooking significantly reduced the tannin content of pigeon pea seeds.

Effect of supplementation with fenugreek seeds flour (FSF) and cooking on total polyphenol content of millet flour

Polyphenols content of millet flour was found to be 441.24 mg/100 g (Table 2). Supplementation with 5, 10, and 15% FSF significantly ($P < 0.05$) increased the polyphenols to 456.67, 461.27 and 475.96 mg/100g, respectively. Cooking of both raw millet flour and composite flours supplemented with 5, 10, and 15% FSF significantly ($P < 0.05$) decreased the total polyphenols to 426.13, 412.12, 421.08 and 423.32mg/100g, respectively. The loss in poly phenols during cooking might be due to the fact that phenols react with protein forming poorly extractable protein-phenolic complexes (Abdel Hady *et al.*, 2005; Osman *et al.*, 2010).

Generally, supplementation with FSF could possibly improve the nutritional quality of the millet composite flours.

However, supplementation was observed to increase the antinutritional factors mainly polyphenols of the composite flour, but when followed with cooking the content of polyphenols was significantly decreased compared to that of raw millet flour.

Effect of supplementation with moringa seeds flour (MSF) and cooking on total and extractability of minerals of millet flour

Results on the effect of supplementation with MSF followed by cooking on the minerals content and extractability of millet flour are shown in Table 3. Supplementation with 5, 10, and 15% MSF significantly ($P < 0.05$) increased the Ca content of

millet flour to 8.73, 11.22 and 12.73 mg/100g, respectively. A similar finding was reported by Awadelkareem *et al.* (2008) who reported that the Ca content of sorghum flour significantly ($P < 0.05$) was increased after supplementation with soybean protein concentrate. The proportional enhancement of Ca content after supplementation with MSF may likely be linked to extremely high Ca content (25.0-83.75 mg/100 g) in moringa cake flour (Abiodun *et al.*, 2012; Nzikou *et al.*, 2009). Supplementation with 5% MSF significantly ($P < 0.05$) increased the extractability of Ca to 79.51% but that of the flour supplemented with 10 and 15% was significantly ($P < 0.05$) decreased to 54.23 and 52.05%, respectively. Similarly, the extractability of Ca was also increased due to mixing cereals with legumes (Gahlawat and Sehgal, 1993).

Cooking significantly ($P < 0.05$) increased the calcium content of millet flour from 3.81 mg/100 g in uncooked raw millet flour to 4.29 mg/100g. Although cooking significantly ($P < 0.05$) decreased Ca content of composite flours supplemented with 5, 10, and 15% MSF to 7.84, 8.34 and 10.49 mg/100g, respectively, but their Ca content is still higher than that of un-supplemented flour.

Cooking significantly ($P < 0.05$) improved the extractability of Ca of composite flours supplemented with 5, 10, and 15 % MSF to 65.14%, 63.47 and 76.58%, respectively, compared to that of cooked raw millet flour.

Similar findings were reported by Mohamed *et al.* (2010b) who reported that cooking is increased Ca content of whole and dehulled pearl millet. The increase in Ca content may be attributed to the reduction in phytic acid content.

The iron content of raw millet flour was found to be 1.62 mg/100g (Table 3), which was lower than that reported previously for some millet cultivars (Abdelrahman *et al.*, 2005; Mohamed *et al.*, 2011).

RESEARCH ARTICLE

Table 3. Effect of cooking on the extractability (%) and total (mg/100g) minerals of millet flour supplemented with different ratios of defatted moringa seeds flour

Supplementation levels (%)	Treatment	Ca		Fe		P	
		Total	Extractability	Total	Extractability	Total	Extractability
0	Raw	3.81 ^e (±0.05)	67.20 ^c (±1.03)	1.62 ^e (±0.07)	31.48 ^b (±0.99)	312.67 ^g (±5.89)	42.43 ^g (±2.10)
	Cooked	4.29 ^d (±0.07)	39.19 ^b (±1.12)	3.55 ^d (±0.08)	42.50 ^e (±0.87)	352.87 ^f (±11.04)	58.52 ^e (±3.44)
5	Raw	8.73 ^{bc} (±0.03)	79.51 ^a (±0.98)	10.81 ^{cd} (±0.12)	65.58 ^a (±0.23)	549.40 ^e (±12.63)	96.08 ^a (±2.76)
	Cooked	7.84 ^{cd} (±0.02)	65.14 ^d (±1.19)	12.66 ^{bc} (±0.13)	56.46 ^d (±0.41)	549.40 ^e (±13.97)	53.72 ^f (±1.91)
10	Raw	11.22 ^{ab} (±0.05)	54.23 ^f (±0.86)	11.60 ^c (±0.14)	62.93 ^c (±0.46)	580.67 ^d (±10.23)	63.48 ^d (±2.05)
	Cooked	8.34 ^c (±0.04)	63.47 ^e (±1.07)	15.12 ^a (±0.09)	49.46 ^e (±0.49)	674.47 ^a (±9.87)	88.24 ^b (±1.54)
15	Raw	12.37 ^a (±0.90)	52.05 ^g (±1.22)	13.05 ^b (±0.16)	63.25 ^b (±0.51)	620.87 ^c (±12.63)	88.49 ^b (±2.03)
	Cooked	10.49 ^b (±0.13)	76.58 ^b (±3.46)	15.39 ^a (±0.10)	46.18 ^f (±0.26)	647.67 ^b (±13.41)	74.45 ^c (±1.41)
Lsd _{0.05}		1.0923 ^{**}	3.5247 ^{**}	2.6981 ^{**}	1.8560 ^{**}	5.6971 ^{**}	2.7862 ^{**}
SE±		0.2496	0.5086	0.4611	0.2387	0.8649	0.4722

^{a-h} Mean ± SD values having same superscript within a column are not significantly different (P 0.05) according to DMRT

These differences in Fe content between these studies could be attributed to the variation in genotypes, agronomical practices, soil fertility and environmental conditions. The Fe content was significantly (*P* 0.05) increased as the result of supplementation with 5, 10, and 15% MSF to 10.81, 11.60 and 13.05 mg/100g, respectively.

This enhancement of Fe content after supplementation with MSF may likely be linked to the high Fe content (12.32 mg/100 g) in moringa cake flour (Abiodun *et al.*, 2012). Extractability of Fe of raw millet flour was found to be 31.48%, and it was significantly (*P* 0.05) improved to 65.58, 62.93, and 63.25% after supplementation with s with 5, 10, and 15% MSF. This enhancement of Fe extractability after supplementation with MSF is positively correlated with phytate content that has also progressively increased with increasing MSF supplementation. Cooking of raw millet flour and composite flours supplemented with 5, 10, and 15% FSF significantly (*P* 0.05) increased the Fe content to 3.55, 12.66, 15.12 and 15.39mg/100g, respectively. Cooking significantly (*P* 0.05) improved the Fe extractability of raw millet flour to 42.50%, while it significantly (*P* 0.05) reduced that of the composite flours supplemented with 5, 10, and 15% MSF to 56.46, 49.46 and 46.18%, respectively. But the Fe extractability of cooked composite flours is still higher than that of raw millet flour. Thus the combination of

MSF supplementation and cooking could significantly improve the Fe content and extractability of millet flour.

Phytate, the major phosphorus-bearing compound in grains and legumes, chelated essential divalent cations such as copper, zinc, iron and calcium, forming insoluble complexes (Vohra *et al.*, 1965).

The decrease in phytic acid content, possibly through its destruction on heating may indicate that the divalent cations are free from the phytate mineral complex which may account for their increased content and extractability in cooked millet flours. Phosphorus content of pearl millet flour was found to be 312.67 mg/100g (Table 3) that was far below the concentration of P in some millet cultivars (Abdelrahman *et al.*, 2005; Mohamed *et al.*, 2011). Differences observed between results can be attributed to genotypes, geographical, soil composition, cultivation climate, ripening stage, the harvesting time of the seeds and the extraction method used. Phosphorus content was significantly (*P* 0.05) increased after supplementation with 5, 10, and 15% MSF to 549.40, 580.67 and 620.87 mg/100g, respectively. Phosphorus extractability of raw millet flour was found to be 42.43% and after supplementation with 5, 10, and 15% MSF it was significantly (*P* 0.05) improved to 96.08, 63.48, and 88.49%, respectively.

The enhancement of P extractability after supplementation with MSF is again positively correlated with phytate content. The elevation of both P content and extractability after supplementation is an advantage of using moringa seed flour to improve the nutritional quality of millet flour. For unknown reasons cooking significantly ($P = 0.05$) increased the P content of millet flour and flours supplemented with 10 and 15% MSF to 352.87, 674.47 and 647.67 mg/100g, respectively, while not affect the P content of flour supplemented with 5% MSF. After cooking the extractability of P of raw millet flour and 10% FSF composite flour significantly ($P = 0.05$)

improved to 58.52 and 88.24%, while that of the flours supplemented with 5 and 15% MSF significantly ($P = 0.05$) decreased to 53.72 and 74.45%, respectively. The improvement of P extractability after cooking might likely to be due to the fact that cooking had been reported to reduce the antinutritional factors of cereals (Idris *et al.*, 2007). Overall, supplementation with MSF was demonstrated to improve the minerals content and extractability of the millet composite flours. Moreover, MSF supplementation followed by cooking was revealed to enhance both minerals content and extractability of composite flours compared to that of raw millet flour.

Table 4. Effect of cooking on the extractability (%) and total (mg/100g) minerals of millet flour supplemented with different ratios of fenugreek seeds flour

Supplementation levels (%)	Treatment	Ca		Fe		P	
		Total	Extractability	Total	Extractability	Total	Extractability
0	Raw	3.81 ^d ±0.05	67.20 ^b ±1.03	1.62 ^g ±0.07	31.48 ^e ±0.99	312.67 ^d ±5.89	42.43 ^h ±2.10
	Cooked	4.29 ^{cd} ±0.31	39.13 ^c ±2.57	3.55 ^b ±0.04	42.50 ^d ±2.16	352.87 ^c ±2.08	58.52 ^d ±1.28
5	Raw	3.85 ^d ±0.04	32.17 ^f ±0.14	3.25 ^b ±0.03	48.63 ^c ±0.62	352.87 ^c ±2.08	81.11 ^c ±1.92
	Cooked	3.74 ^{de} ±0.58	85.31 ^a ±1.18	2.84 ^c ±0.33	47.11 ^c ±1.77	285.87 ^e ±11.47	47.41 ^g ±2.57
10	Raw	4.86 ^c ±0.01	86.08 ^a ±0.54	1.85 ^f ±0.05	26.49 ^g ±0.76	259.07 ^g ±12.47	86.43 ^b ±4.59
	Cooked	6.31 ^{ab} ±0.01	62.47 ^c ±0.87	2.48 ^d ±0.01	53.12 ^b ±0.26	393.07 ^a ±11.47	59.10 ^e ±0.48
15	Raw	5.32 ^b ±0.05	48.81 ^d ±0.88	2.20 ^e ±0.00	28.09 ^f ±0.29	276.93 ^f ±12.47	93.25 ^a ±2.99
	Cooked	7.52 ^a ±0.02	80.81 ^{ab} ±0.50	7.52 ^a ±0.02	84.33 ^a ±3.16	368.50 ^b ±11.60	56.38 ^f ±0.00
Lsd_{0.05}		0.6325 [*]	4.5241 ^{**}	0.3879 [*]	5.0974 ^{**}	6.8526 ^{**}	3.5261 ^{**}
SE±		0.2417	1.0952	0.0954	1.1662	1.7986	1.0863

^{a-h} Mean ± SD values having same superscript within a column are not significantly different ($P = 0.05$) according to DMRT.

Effect of supplementation with moringa seeds flour (FSF) followed by cooking on total and extractability of minerals of millet flour

As shown in Table 4 the calcium content of raw pearl millet flour was found to be 3.81 mg/100g that was lower than the range 10-80 mg/100 g reported for ten millet cultivars (Abdalla *et al.*, 1998; Abdelrahman *et al.*, 2005).

Supplementation with 5, 10, and 15% FSF significantly ($P = 0.05$) increased Ca content of the composite flours to 3.85, 4.86, and 5.32 mg/100g,

respectively. With exception to 5% FSF composite flour, cooking significantly ($P = 0.05$) increased Ca content of raw millet flour as well as composite flours. Similarly, cooking of maize and lentil flours significantly increased the contents of Ca contents as reported previously (Abdel Hadi *et al.*, 2005). Extractability of Ca of raw millet flour was found to be 67.20% (Table 4), it was significantly ($P = 0.05$) reduced to 32.17% after supplementation with 5% FSF, and significantly ($P = 0.05$) increased to 86.08% after supplementation with 10% FSF, and then again dropped to 48.81% after supplementation with 15% FSF. Extractability of Ca significantly ($P = 0.05$) reduced after cooking of raw millet flour and 10%

RESEARCH ARTICLE

FSF composite flour, whereas it is significantly ($P < 0.05$) increased of composite flours supplemented with 5 and 15% FSF. Khetarpaul and Chauhan (1991) reported similar results showed an increase in availability percentage of Ca when pearl millet was subjected to autoclaving. Ca is generally present in association with phytic acid; this may be responsible for its lower extractability. However, reduction in phytic acid as the result of soaking and cooking may explain higher extractability of Ca and other minerals (Duhan *et al.*, 2002).

Phytic acid is the primary source of inositol and storage phosphorus in plant seed. The abundance of phytic acid in cereal grains is a concern in the food industries because the phosphorus in this form is unavailable to monogastric animals due to a lack of endogenous phytases; enzymes specific for the dephosphorylation of phytic acid. In addition, the strong chelating characteristic of phytic acid reduces the bioavailability of other essential dietary nutrients, such as minerals, proteins and amino acids (Singh *et al.*, 2013). As shown in Table 4 the iron content of millet flour was found to be 1.62 mg/100g, supplementation with 5, 10, and 15% FSF significantly ($P < 0.05$) increased the Fe content to 3.25, 1.85, and 2.20 mg/100g, respectively.

This enhancement of Fe content after supplementation with FSF may likely comes from the fenugreek seed flour, because fenugreek is known to contain appreciable amounts of Fe. Cooking significantly improve the Fe content of raw millet flour and composite flours supplemented with 5, 10, and 15 % FSF 3.55, 2.48, 2.48, and 7.52mg/100g, respectively, compared to uncooked raw millet flour. Similar observation on the effect of cooking on the Fe content in some millet flours has been reported (Abdelrahman *et al.*, 2005; Mohamed *et al.*, 2011). Supplementation with 5% FSF significantly ($P < 0.05$) improved the extractability of Fe to 48.63, but that with 10 and 15% FSF significantly ($P < 0.05$) decreased Fe extractability to 26.49 and 28.09%, respectively compared to that of raw millet flour. Extractability of Fe of raw millet flour and composite flours supplemented with 5, 10, and 15% FSF was significantly ($P < 0.05$) increased after cooking to

42.50, 47.11, 53.12 and 84.33%, respectively, compared to that of uncooked raw millet flour. Jood and Kapoor (1997) reported that ordinary cooking of soaked seeds had a significant improvement of HCl-extractability (availability) of all minerals. The reduction in phytic acid as the result of cooking may explain higher extractability of minerals (Duhan *et al.*, 2002).

Table 4 shows the phosphorus content of millet flour was found to be 312.67mg/100g. With exception to 5% FSF composite flour, increasing the supplementation rate significantly ($P < 0.05$) decreased the phosphorus content to 276.93 mg/100g. Although the P content was decreased by supplementation, its extractability was however, greatly ($P < 0.05$) enhanced following the supplementation with 5, 10, and 15% FSF which recorded the values of 81.11, 86.43, and 93.25%, respectively. The reduction in P content of flours supplemented with 10 and 15% FSF could be due to the interaction between P and phytic acid in fenugreek seeds flour. With exception to 5% FSF composite flour, cooking of millet flour and flours supplemented with 10 and 15% FSF significantly ($P < 0.05$) increased the total P content to 352.87, 393.07, and 368.50 mg/100 g, respectively.

Cooking significantly decreased the P extractability of all composite flours, however, their values still higher than that of uncooked raw millet flour. The decrease in total phosphorus and its extractability may attributed to the interaction between phosphorus and phytic acid. Generally, FSF supplementation followed by cooking was found to enhance both minerals content and extractability of composite flours compared to that of raw millet flour.

Conclusion

Supplementation of millet flour with moringa or fenugreek seeds flour slightly increased the phytic acid, total polyphenol and majority of minerals (Ca, P and Fe) content. However, the antinutrients were significantly decreased after cooking with a concomitant increase in total and extractability of minerals. Thus, supplementation of millet flour with

either MSF or FSF followed by cooking could potentially enhance the nutritional quality of millet flours by improving both minerals content and extractability.

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