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Effect of Supplementation and Cooking on *in vitro* Protein Digestibility and Anti-Nutrients of Pearl Millet Flour

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Abstract

Millet flour was supplemented with different levels (5, 10 and 15%) of Moringa seeds flour (MSF) or fenugreek defatted seeds flour (FSF). The effect of supplementation and cooking on anti-nutrients and in vitro protein digestibility was investigated. Supplementation of millet flour with 5, 10 and 15% MSF or FSF significantly ($P \leq 0.05$) increased the anti-nutritional factors (phytic acid and total polyphenols) and *in vitro* protein digestibility of millet flour. Cooking of millet flour with or without MSF or FSF decreased the anti-nutritional factors, protein content and *in vitro* protein digestibility.

Keywords

Digestibility, Supplementation, Anti-Nutritional Factors, Phytic Acid, Tannin, Polyphenols, Cooking, Moringa, and Fenugreek

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1. Introduction

Pearl millet is a staple food for a large section of the population in Asian and African countries. Besides supplying calories and proteins in the diet, pearl millet is a good source of essential minerals (Abdalla *et al*., 1998). Pearl millet like other cereal grains, the abundance of anti-nutrients such as phytic acid and polyphenols inhibits proteolytic and amylolytic enzymes, limits protein and starch digestibility and makes poor human bioavailability of minerals. In Sudan, millet is a staple diet of the people in the Western region (Darfur) and is consumed as thick porridge (Aseeda), a thin porridge (Nasha and kisra) (unleavened bread) from fermented or unfermented dough.

Protein digestibility varied among milled fractions of the grain and was reported to be inversely related to the proportion of coarse bran removed during milling (Hulse *et al*., 1980). The *invitro* protein digestibility of three pearl millet cultivars was found to be 47.80, 50.62 and 45.22% (Eldogasabi, 2009), while Elyas (1999), findings was 60.5% and 61.9% of *in vitro* protein digestibility of two cultivars of pearl millet. Abdalla, (2003) reported 69.0 and 76.9% *in vitro* protein digestibility for two pearl millet cultivars, while Ali *et al*, (2003) reported 68.1% and 75.9% for two pearl millet cultivars.

The *invitro* protein digestibility of the millet flour was found to be 78% (Monawar, 1983). Khetarpaul and Chauhan (1991) reported a value of 51% for raw pearl millet which is

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considered less than of Wheat (85.8%), Maize (85.3%), and Rice (83.8%). Cereals and legumes are rich in minerals but the bioavailability of these minerals is usually low due to the presence of anti-nutritional factors such as phytate and polyphenols (Bergman *et al*, 1999). 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. Pearl millet like other cereals grains the abundance of anti-nutrients such as phytic acid and polyphenols inhibit protolytic and amylolytic enzymes, limit protein and starch digestibility, and make poor human bioavailability of minerals. Some articles and research studies have reported that the dry leaves of *M. oleifera* contain 7 times more 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).

Fenugreek is a good source of protein, fiber, ash and carbohydrate; it is rich in minerals such as Calcium, Sodium and Copper (Nikman *et al*, 2003; Patil *et al*, 1997). Cooking improves the nutritive value of cereals, but has a minor effect on phytate contents (Marfo *et al*., 1990). Abdelrahman et al., (2005) reported that cooking of pearl millet slightly reduced anti-nutritional factors and increased minerals availability. This study was conducted to investigate the effect of supplementation with Moringa or fenugreek seeds flour followed by cooking on anti-nutritional factors *and in vitro* protein digestibility.

2. Materials and Methods

2.1. 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 ^{o}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 ^{o}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 ^{o}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 ^{o}C for further use. All chemicals used in this study were of reagent grade.

2.2. Supplementation

To increase the nutritive value of millet flour each of defatted Moringa seeds and fenugreek seeds flour were added using Pearson square at the supplementation rate of 5, 10, and 15% (Pearson, 1981). A control sample consisting of raw millet flour was treated in the same manner as composite flours.

2.3. Cooking

Cooking of the samples 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 ^{o}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 ^{o}C for further analysis.

2.4. 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 ml 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 HNO_3, 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 ratios 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^{+3}). Phytic acid content was determined by multiplying the phytate phosphorus content by a constant factor of 3.55.

2.5. Determination of Tannin Content

Quantitative estimation of tannins was carried out using the modified vanillin-HCl method (Price and Bulter, 1978). 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.

2.6. Determination of Total 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 K_3Fe (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 $K_3Fe(CN)^6$. A standard curve was obtained, expressing the result as tannic acid equivalents; that is, the amount of tannic acid (mg/100g) that gives a color intensity equivalent to that given by polyphenols after correction by blank sample.

2.7. *In vitro* Protein Digestibility

In vitro protein digestibility of the samples was measured according to the method described by Manjula & Johon (1991), in which a pepsin digestion method was used in the determinations. The digestible protein was analyzed for nitrogen using micro Kjeldahl procedure (AOAC, 1995) and expressed as a percent of the total Nitrogen.

3. 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. Significance was accepted with $P \leq 0.05$.

4. Results & Discussion

4.1. Effect of Supplementation with MSF or FSF and Cooking on Phytic Acid Content of Millet Flour

The phytic acid content of millet flour was found to be 203.02mg/100g (Table 1). Supplementation of millet flour with 5, 10 and15% MSF significantly ($P \leq 0.05$) increased the phytic acid content to 248.50, 255.16 and 260.70mg/100g, respectively. Cooking of millet and composite flour supplemented with 5, 10 and 15% MSF significantly ($P \leq 0.05$) decreased phytic acid content to 175.26, 215.22, 237.96 and 241.84mg/100g, respectively. As shown in Table 2, the phytic acid content of millet flour was found to be 203.02mg/100g. Supplementation with 5% FSF significantly ($P \leq 0.05$) decreased phytic acid content of millet flour to 175.28 mg/100g, but when the flour was supplemented with 10 or 15% FSF phytic acid content was significantly ($P \leq 0.05$) dropped to 186.38 and 194.69mg/100g, respectively which higher than that of 5% supplement. The results indicated that both Moringa and fenugreek contributed significantly to the level of phytic acid in millet flour.

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^{g} (±7.25)	0.19^{a} (±0.03)	441.24^{g} (±5.23)
	Cooked	175.26^{h} (±2.51)	0.18^{a} (±0.02)	426.13^{h} (±2.05)
5	Raw	248.50^{c} (±5.08)	0.08^{ab} (±0.01)	547.81^{e} (±8.57)
	Cooked	215.22^{f} (±5.08)	0.07^{ab} (±0.00)	543.78^{f} (±4.24)
10	Raw	255.16^{b} (±0.96)	0.08^{ab} (±0.01)	600.52^{b} (±6.38)
	Cooked	237.96^{e} (±4.40)	0.08^{ab} (±0.01)	571.27^{d} (±3.39)
15	Raw	260.70^{a} (±4.19)	0.10^{ab} (±0.01)	621.49^{a} (±2.61)
	Cooked	241.84^{d} (±2.54)	0.09^{ab} (±0.01)	579.73^{c} (±2.96)
$Lsd_{0.05}$		**3.5263****	**0.0087***	**5.4216****
SE±		**1.5247**	**0.00**	**2.0986**

Mean ±SD values having same superscript within a column are insignificantly different ($P \geq 0.05$) according to DMRT.

Cooking was observed to decrease significantly ($P \leq 0.05$) the phytic acid content of millet flour and composite flours supplemented with 5, 10 and 15% FSF to 175.28, 145.88, 155.87 and 163.63mg/100g, respectively. The results agree with the findings of Abdelrahman (2004), who reported that, cooking reduced phytic acid content of two pearl millet cultivars by 6% and10%. According to Mashier et al. (2007), the reduction in phytic acid content during cooking could probably be explained on the basis that phytase activity at a temperature of 40-550 ℃ may degrade inositol hexaphosphate to the pentaphosphate or lower molecular weight forms.

4.2. Effect of Supplementation with MSF or FSF and Cooking on Tannin Content of Millet Flour

Tannin content of millet flour was found to be 0.19%.

Supplementation of millet flour with 5, 10 and 15% MSF insignificantly ($P \geq 0.05$) decreased the tannin content to 0.18, 0.18 and 0.10%, respectively. Cooking of millet flour and flours supplemented with 5, 10 and 15% MSF also insignificantly ($P \geq 0.05$) decreased the tannin content to 0.18, 0.07, 0.08 and 0.09%, respectively (Table 1).

Supplementation with FSF does not affect the tannin content of millet flour (Table 2). After cooking of millet flour and flour supplemented with 10% FSF, the tannin content slightly decreased to 0.18 and 0.17%, respectively. Whereas, it significantly ($P \leq 0.05$) decreased when the flour was supplemented with 5% FSF. However, supplementation with 15% FSF did not affect tannin content of the flour. The reduction in tannin content of the flour and supplements after cooking may be due to the heat degradation of the molecule 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	203.02a (±7.25)	0.19a (±0.001)	441.24d (±5.23)
	Cooked	175.28d (±2.54)	0.18a (±0.02)	426.13e (±2.05)
5	Raw	175.84d (±6.72)	0.19a (±0.03)	456.67c (±5.42)
	Cooked	145.88g (±0.96)	0.15b (±0.00)	412.12h (±0.37)
10	Raw	186.38c (±1.66)	0.19a (±0.03)	461.27b (±0.58)
	Cooked	155.87f (±0.95)	0.17ab (±0.01)	421.08g (±0.74)
15	Raw	194.69b (±2.88)	0.19a (±0.03)	475.96a (±0.74)
	Cooked	163.63e (±0.96)	0.19a (±0.03)	423.32f (±1.29)
Lsd0.05		**1.0322****	**0.1158***	**2.8569****
SE±		**0.7254**	**0.0209**	**0.9856**

Mean ±SD values having same superscript within a column are insignificantly different ($P \geq 0.05$) according to DMRT.

4.3. Effect of Supplementation with MSF or FSF Cooking on Total Polyphenol Content of Millet Flour

The total polyphenols content of millet flour was found to be 441.26 mg/100g (Table1). This value is in general agreement with those reported for different millet genotypes (Abdelrahman et al., 2005; Mohamed et al., 2011). Supplementation with 5, 10 and 15% MSF significantly ($P \leq 0.05$) increased the total polyphenols content of composite flours to547.18, 600.52 and 621.49 mg/100g, respectively (Table 1). The increasing in total polyphenols after supplementation with MSF could be attributed to the fact that Moringa contained high amounts of total polyphenols (Makkar and Becker, 1997) which lead to the increase the total polyphenols content of the composite flours. However, also cooking significantly ($P \leq 0.05$) increased the total polyphenols of flours supplemented with 5, 10, and 15% MSF to 543.78, 571.27 and 579.73 mg/100g, respectively but decreased that of raw flour. Similar observations of the reduction in total 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 total polyphenols after cooking might be as a result of the fact that polyphenols 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 anti-nutritional factors mainly phytic acid and total polyphenols of the composite flour. To avoid an increment in ant nutritional factors during supplementation and cooking, we recommend soaking or fermentation of ingredients before mixing.

Supplementation of millet flour with 5, 10, and 15% FSF significantly ($P \leq 0.05$) increased the total polyphenols to 456.67, 461.27 and 475.96 mg/100g, respectively (Table 2). Cooking of both raw millet and composite flours supplemented with 5, 10, and 15% FSF, significantly ($P \leq 0.05$) decreased the total polyphones to 426.13, 412.12, 421.08 and423.32mg/100g, respectively. The loss in total polyphenols during cooking might be due to the fact that phenols react with protein forming poorly extractable protein phenolic complexes (Abed Elhady 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 anti-nutritional factors mainly total polyphones of the composite flour, but when followed by cooking the content of total polyphenols was decreased significantly compared to that of raw flour.

4.4. Effect of Supplementation with MSF or FSF and Cooking on Protein Digestibility of Pearl Millet Flour

Table 3 shows the protein digestibility of millet flour with or without supplements. The protein digestibility of raw millet flour was found to be 45.75%. Supplementation of millet

flour with 5, 10 and 15% MSF slightly increased the protein digestibility to 52.62, 65.89 and 66.50%, respectively. A similar trend was obtained by Elhag (2009) who reported that the protein digestibility of sorghum was 49.3% and after supplementation with 15, 20 and 25% ground nut flour significantly ($P \leq 0.05$) increased to 56.8, 60.1 and 62.5%, respectively. Cooking of millet flour and composite flour supplemented with 5% MSF slightly decreased the protein digestibility to 45.60 and 45.89%, respectively, whereas a significant ($P \leq 0.05$) decrease was observed in protein digestibility when the flours was supplemented with 10 and 15% MSF.

As shown in Table 4, the protein digestibility of millet flour was found to be 45.75%. Supplementation with 5% FSF slightly increased the protein digestibility of millet flour to 46.29%, while the flours supplemented with 10 and 15% FSF had significantly ($P \leq 0.05$) increased the protein digestibility. A similar trend was obtained by Elhag (2009) who reported that the protein digestibility of sorghum was 49.3% and after supplementation with 15, 20 and 25% ground nut flour significantly ($P \leq 0.05$) increased to 56.8, 60.1 and 62.5%, respectively.

Table (3). Effect of cooking on protein content (%) and *in vitro* protein digestibility (%) of millet flour supplemented with different ratios of defatted MSF.

Supplementation level (%)	Treatment	Protein content (%)	IVPD (%)
0	Raw	14.29^{d} (±0.10)	45.75^{cd} (±0.004)
	Cooked	13.78^{de} (±0.39)	45.60^{cd} (±0.002)
5%	Raw	16.91^{c} (±0.19)	52.62^{bc} (±0.02)
	Cooked	16.85^{c} (±0.63)	45.89^{c} (±0.002)
10%	Raw	19.79^{a} (±0.86)	65.89^{ab} (±0.001)
	Cooked	19.36^{b} (±0.69)	45.84^{c} (±0.004)
15%	Raw	22.02^{a} (±0.75)	66.50^{a} (±0.55)
	Cooked	21.75^{a} (±0.25)	52.91^{b} (±0.01)
$Lsd_{0.05}$		**0.4671****	**0.3946****
SE		**0.1652**	**0.1536**

Mean ±SD values having same superscript within a column are insignificantly different (P≥0.05) according to DMRT.

Table (4). Effect of cooking on protein content (%) and *in vitro* protein digestibility (%) of millet flour supplemented with different ratios of fenugreek seeds flour.

Supplementation level(%)	Treatment	Protein content(%)	IVPD(%)
0	Raw	14.29^{d} (±0.10)	45.75^{bc} (±0.004)
	Cooked	13.78^{e} (±0.39)	45.60^{c} (±0.002)
5%	Raw	15.35^{cd} (±0.04)	46.29^{b} (±0.004)
	Cooked	14.26^{d} (±0.20)	44.02^{d} (±0.01)
10%	Raw	15.76^{c} (±0.08)	63.49^{ab} (±0.58)
	Cooked	15.66^{c} (±0.10)	45.61^{c} (±0.002)
15%	Raw	17.49^{a} (±0.28)	64.12^{a} (±0.21)
	Cooked	16.63^{b} (±0.21)	53.35^{b} (±0.06)
$Lsd_{0.05}$		**0.3097***	**0.7811****
SE		**0.0442**	**0.3546**

Mean ±SD values having same superscript within a column are insignificantly different (P≥0.05) according

Cooking of millet flour slightly decreased the protein digestibility from 45.75 to 45.60%, but a significant ($P \leq 0.05$) decrease was observed in protein digestibility in flours supplemented with 5, 10 and 15% FSF (Table 4). A similar findings for the effect of cooking on protein digestibility was reported by Elkhalifa *et al*.(1999) and Rom *et al*.,(1992), they reported that sorghum protein digestibility significantly ($P \leq 0.05$) decreased after cooking, Mustafa,(2008) reported that cooking significantly ($P \leq 0.05$) decreased the protein digestibility of pearl millet flour from 73.6 to 57.6%. They attributed the reduction in protein digestibility to the formation of disulphide bonds during cooking which resulted in toughening at the surface and interior of protein bodies. The negative effect of cooking on protein digestibility also was reported by Yousif (2000) for corn that attributed the reduction to the formation of disulphide bonds resulting in folding of protein molecular, and hence decreasing its susceptibility to digestive enzymes.

5. Conclusion

Supplementation with Moringa or fenugreek seeds flours improves the protein digestibility by reducing the anti-nutrients content. However, cooking decreased anti-nutrients contents and also the protein digestibility of the flour alleviate the effect of cooking on protein digestibility we recommend that both raw and supplemented flour should be soaked or fermented before processing.

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