PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 7 (2): 335-341, 2008
ISSN 1680-5194
© Asian Network for Scientific Information, 2008

Effect of Fermentation, Malt-Pretreatment and Cooking on Antinutritional Factors and Protein Digestibility of Sorghum Cultivars

Wedad, H. Abdelhaleem, Abdullahi H. El Tinay, Abdelmoneim I. Mustafa and Elfadil E. Babiker
Department of Food Science and Technology, Faculty of Agriculture,
University of Khartoum, Khartoum North 13314, Shambat, Sudan

Abstract: Two sorghum cultivars namely, Mugud (low tannin) and Karamaka (high tannin) were used in this study. The flour of the seeds of both cultivars was mixed with 5% malt. Then the flour with or without malt was fermented for 16 h. Samples were taken every 2 h during fermentation to study changes in pH, total acidity, crude protein and dry matter. Fermentation of the flour with or without malt resulted in an increase in crude protein content and total acidity for both cultivars. Moreover, the fermented flour with or without malt was cooked to study changes in phytate, tannins and *in vitro* protein digestibility of the cultivars. A highly significant ($P \leq 0.05$) reduction in antinutritional factors (phytate and tannins) was observed for malted and fermented flour compared to the fermented dough. Cooking significantly ($P \leq 0.05$) reduced the *in vitro* protein digestibility of the treated cultivars but the extent of the reduction is lower in malted samples. Fermentation alleviates the adverse effect of cooking on sorghum protein digestibility after addition of malt. Results obtained revealed that addition of malt followed by fermentation is a useful method to improve the nutritional value of sorghum even after cooking.

Key words: Sorghum, fermentation, malt, cooking, antinutrients and protein digestibility

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench.] is considered as a most important food crop in the world, following wheat, rice, maize and barley (FAO, 1997). Grain sorghum provides the staple food for a large population of Africa, India and the semi-arid parts of the tropics. It is commonly consumed by the poor mass of many countries and it forms a major source of proteins and calories in the diet of large segments of the population of India and Africa. Besides being a staple food, it is also used as feed for animals and it is an industrial raw material; its stalk provides fodder, fuel, shelter and syrup. Grain sorghum is the leader cereal crop in the Sudan. It is the main staple food, prevailing throughout the country and covering more than 60% of the total cultivated cereals area, with an annual production of about 4.0 million tons (FAO, 1997). Processed sorghum seeds or flour were found to be important sources of calories and proteins to the vast majority of the population as well as for poultry and livestock (FAO, 1997). Sorghum acts as a principal source of energy, protein, vitamins and minerals for millions of the poorest people living in Africa, Asia and the semi-arid tropics worldwide (Klopfesntein and Hoseney, 1995). Unfortunately, sorghum has low nutritional value and inferior organoleptic qualities due to the presence of antinutritional factors which make complexes with food ingredients. In addition, *in vivo* and *in vitro* studies indicate that the proteins of wet cooked sorghum are significantly less digestible than the proteins of other similar cooked cereals such as wheat and maize (Doudou *et al*., 2003). They divided factors responsible for poor sorghum protein digestibility into exogenous factors (grain organizational structure, polyphenols, phytic acid, and starch and non-starch polysaccharides) and endogenous factors (disulphide and non-disulphide cross-linking, kafirin hydrophobicity and changes in protein secondary structure). The antinutritional effect of tannin and phytate in sorghum has been demonstrated by many researchers (Butler *et al*., 1984; Ryden and Selvendran, 1993; Agrwal and Chitnis, 1995). The tannin-protein interaction in sorghum involves hydrogen bonding and hydrophobic interactions (Butler *et al*., 1984). Sorghum prolamins (proline-rich proteins) bind strongly to sorghum tannins and these results in reduced protein digestibility. The phytate molecule, containing six phosphate groups, is highly charged. This makes it an excellent chelator and it can form insoluble complexes with proteins leading to reduced digestibility (Ryden and Selvendran, 1993). Fermentation (Hassan and El Tinay, 1995; Lorri and Svanberg, 1993) and germination (Agrwal and Chitnis, 1995 and Bhise *et al*., 1988) has been reported as good options for increasing digestibility of sorghum proteins. In the present study we would like to establish a simple and suitable processing method to improve the nutritional quality of two common cultivars consumed in Central and Western Sudan.

Materials and Methods

Source of sorghum grains: Two sorghum cultivars were used in this study; Mugud (low tannin) obtained from the Agricultural Research and Technology Corporation and

Table 1: pH and percent titratable acidity, crude protein and dry matter of fermented flour with or without malt of Mugud cultivar.

Fermentation period (h)	Fermented samples			
	pH	Titratable acidity	Crude protein	Dry matter
0	6.12^{b}(±0.00)	0.36^{i}(±0.02)	10.58^{i}(±0.16)	92.22
2	5.98^{c}(±0.005)	0.43^{h}(±0.04)	11.05^{h}(±0.08)	92.08
4	5.23^{d}(±0.01)	0.46^{g}(±0.00)	11.5^{g}(±0.22)	92.02
6	4.88^{e}(±0.00)	0.59^{f}(±0.01)	11.81^{f}(±0.01)	91.80
8	3.95^{f}(0.00)	0.98^{e}(±0.01)	12.11^{e}(±0.01)	91.60
10	3.65^{g}(±0.00)	1.11^{d}(±0.02)	12.45^{d}(±0.11)	91.50
12	3.52^{h}(±0.005)	1.32^{c}(±0.03)	12.65^{c}(±0.01)	91.28
14	3.46^{i}(±0.00)	1.50^{b}(±0.00)	12.98^{b}(±0.01)	90.80
16	3.07^{j}(±0.01)	1.60^{a}(±0.02)	13.11^{a}(±0.02)	90.20

Table 1: (Continued).

Fermentation period (h)	Malted and fermented samples			
	pH	Titratable acidity	Crude protein	Dry matter
0	6.08^{b}(±0.01)	0.35^{h}(±0.03)	10.36^{i}(±0.01)	91.90
2	5.52^{c}(±0.01)	0.42^{g}(±0.01)	11.04^{h}(±0.10)	91.60
4	4.79^{d}(±0.00)	0.47^{g}(±0.01)	11.73^{g}(±0.25)	91.40
6	4.38^{e}(±0.01)	0.68^{f}(±0.02)	12.12^{f}(±0.01)	91.60
8	3.73^{f}(±0.01)	0.97^{e}(±0.02)	12.52^{e}(±0.02)	90.96
10	3.53^{g}(±0.01)	1.35^{d}(±0.12)	12.83^{d}(±0.21)	90.40
12	3.43^{h}(±0.06)	1.62^{c}(±0.00)	12.94^{c}(±0.01)	90.07
14	3.26^{i}(±0.00)	1.80^{b}(±0.06)	13.48^{b}(±0.00)	89.63
16	3.03^{j}(±0.05)	1.95a(±0.01)	14.82^{a}(±0.04)	89.36

Values are means of triplicate samples (± SD). Means not sharing a common letter in a column are significantly different at $P \leq 0.05$ as assessed by Duncan's multiple range test.

Karamaka (high tannin) obtained from Western Sudan as a local cultivar during the season 2004/2005. All chemicals used in this study were of analytical grade.

Sample preparation: The grains of both cultivars were cleaned manually to remove husks, damaged grains and other extraneous materials. The cleaned grains of each cultivar were divided into two groups. One group was milled into fine flour with a hammer mill (Gibbons Electric, Essex, UK) to pass through a 0.4 mm mesh size screen. The other group was malted according to the method of Bhise *et al.* (1988) with a slight modification. The grains were steeped in thrice the quantity of water for 10 h with l h air rest after 6 h of steeping. For each air rest, the steeping water was changed. After steeping, the grains were sterilized by soaking in 1% sodium hypochlorite for 20 min before it was drained prior to germination. The steeped grains were spread on wet jute bags and covered with a moist cotton cloth and left to sprout at room temperature (28 ± 3°C) for 6 days. After germination, the grains were dried in a Gallenkamp oven (BS model OV-160; Manchester, UK) at 50°C for 24 h. Rootlets and shoots of the grains were separated from the kernels by rubbing the grains in a sieve (Endecotts Ltd, London, UK) of 0.6 mm mesh size. The malted grains were milled into fine flour with a hammer mill (Gibbons Electric, Essex, UK) to pass through a 0.4 mm mesh size screen. About 5% of the malt was mixed with 95% of the flour in 200 ml distilled water. Then the flour with or without malt was fermented according to El Tinay *et al.* (1979) method with a minor modification. The dough was prepared in the ratio of 5% starter obtained from previously fermented dough and 95 % sorghum flour with or without malt. About 200 ml distilled water was added and mixed well with a glass rod. The slurry was allowed to ferment at room temperature ((28 ± 3°C)). Samples were withdrawn at zero time and then every 2 h intervals up to 16 h. The pH was measured every 2 h during fermentation using pH meter (PUSL München 2, Karl Kolb, Germany). Thereafter, the samples were dried in a Gallenkamp oven (BS model OV-160; Manchester, UK) at 50°C for 24 h. The dried samples were milled into fine flour with a hammer mill (Gibbons Electric, Essex, UK) to pass through a 0.4 mm mesh size screen for determination of crude protein and dry matter. The fermented flour with or without malt was cooked in a water bath for 20 min. The viscous mass was spread in petri dishes and dried using Gallenkamp oven (BS model OV-160; Manchester, UK) at 50°C for 24 h. The dry flakes were milled into fine flour with a hammer mill (Gibbons Electric, Essex, UK) to pass through a 0.4 mm mesh size screen for determination of tannins, phytate and *in vitro* protein digestibility.

Determination of crude protein and dry matter: The crude protein (NX6.25) and dry matter were determined according to the AOAC (1984) methods.

Determination of total acidity: Total titratable acidity was estimated according to the method described in the AOAC (1984).

Table 2: pH and percent titratable acidity, crude protein and dry matter of fermented flour with or without malt of Karamaka cultivar.

Fermentation	Fermented samples			
period (h)	pH	Titrable acidity	Crude protein	Dry matter
0	5.91^{b} (±0.01)	0.36^{i} (±0.01)	10.85^{i} (±0.01)	93.90
2	5.53^{c} (±0.00)	0.43^{h} (±0.01)	10.91^{h} (±0.00)	93.64
4	5.00^{d} (±0.00)	0.46^{g} (±0.01)	10.99^{g} (±0.03)	93.80
6	4.59^{e} (±0.01)	0.63^{f} (±0.04)	11.11^{f} (±0.02)	93.60
8	4.23^{f} (±0.005)	0.94^{e} (±0.01)	11.34^{e} (±0.01)	93.50
10	4.00^{g} (±0.00)	1.17^{d} (±0.03)	11.48^{d} (±0.01)	93.27
12	3.75^{h} (±0.01)	1.51^{c} (±0.01)	11.62^{c} (±0.00)	92.76
14	3.61^{i} (±0.01)	1.61^{b} (±0.01)	11.84^{b} (±0.04)	92.40
16	3.39^{j} (±0.005)	1.80^{a} (±0.02)	12.25^{a} (±0.01)	92.00

Table 2: (Countinued).

Fermentation	Malted and fermented samples			
period (h)	pH	Titratable acidity	Crude protein	Dry matter
0	5.61^{b} (±0.00)	0.34^{i} (±0.02)	11.02^{i} (±0.01)	93.60
2	5.49^{c} (±0.005)	0.41^{h} (±0.01)	11.32^{h} (±0.00)	93.37
4	4.97^{d} (±0.00)	0.46^{g} (±0.02)	11.41^{g} (±0.00)	93.07
6	4.47^{e} (±0.01)	0.66^{f} (±0.03)	11.60^{f} (±0.02)	92.60
8	4.12^{f} (±0.00)	0.97^{e} (±0.01)	11.83^{e} (±0.01)	92.76
10	3.86^{g} (±0.05)	1.36^{d} (±0.00)	11.85^{d} (±0.02)	92.50
12	3.55^{h} (±0.01)	1.62^{c} (±0.01)	12.06^{c} (±0.02)	92.15
14	3.25^{i} (±0.005)	1.85^{b} (±0.02)	12.34^{b} (±0.01)	91.74
16	3.00^{j} (±0.00)	1.89^{a}(±0.05)	12.74^{a} (±0.03)	91.20

Values are means of triplicate samples (± SD). Means not sharing a common letter in a column are significantly different at $P \leq 0.05$ as assessed by Duncan's multiple range test.

Determination of tannin content: Quantitative estimation of tannins was carried out using the modified vanillin-HCl method as described by Price *et al.* (1978). A standard curve was prepared expressing the results as catechin equivalent, i.e. amount of catechin (mg/ml) which gives a color intensity equivalent to that given by tannin after correcting for blank.

Determination of phytic acid: Phytic acid content was determined by the method described by Wheeler and Ferrel (1971) using 2.0 g dried sample. A standard curve was prepared expressing the results as $Fe(NO_3)_3$ equivalent.

Determination of *in vitro* protein digestibility: The *in vitro* protein digestibility was carried out using pepsin alone according to the method of Maliwal (1983) as described by Monjula and John (1991) with a minor modification.

Statistical analysis: Three samples for each parameter were prepared, each sample was analyzed in triplicate and the values were then averaged. Data were assessed by analysis of variance (ANOVA) as described by Snedecor and Cochran (1987) and by Duncan's (Duncan, 1955) multiple range test with probability $P \leq 0.05$.

Results and Discussion

Changes in pH, total titratable acidity, crude protein and dry matter of fermented flour with or without malt: Table 1 and 2 show the effect of fermentation with or without malt on pH, total titratable acidity (TTA), crude protein and the dry matter of the flour of Mugud and Karamaka cultivars, respectively. Unfermented flour of Mugud (Table 1) and Karamaka (Table 2) cultivars had a pH value of 6.12 and 6.00, respectively. Fermentation gradually reduced the pH of both cultivars flour with time. Fermentation of the flour for 16 h had significantly ($P \leq 0.05$) dropped the pH to 3.1 and 3.4 for the cultivars, respectively. Further reduction in pH values was observed during fermentation of malted flour with time with a minimum pH (3.0) obtained after 16 h fermentation for both cultivars. The drop in pH during fermentation is consistent with the gradual increase in total acidity. Chavan and Kadam (1989a) reported that during fermentation, the pH decreased with a concomitant increase in acidity as lactic acid accumulates due to microbial activity. During fermentation the total titratable acidity (TTA) of Mugud cultivar was significantly ($P \leq 0.05$) increased from 0.36% to 1.60% (Table 1) while for Karamaka it was significantly ($P \leq 0.05$) increased from 0.36% to 1.80% (Table 2). The increment in TTA was more pronounced after 16 h fermentation of malted flour at which the TTA was found to be 1.95% for Mugud (Table 1) and 1.89% for Karamaka (Table 2) cultivar. The dry matter loss after 16 h fermentation was found to be 2.20% and 2.02% for Mugud and Karamaka, respectively. The loss in dry matter is likely to be due to physiological activities of fermenting organisms that utilized part of the meal nutrients, causing a decrease in dry matter. Chavan (1988) observed a loss of 2.3% during 2 days fermentation. The loss of dry matter after 16 h

Table 3: Effect of cooking on antinutritional factors content of fermented flour with or without malt of Mugud cultivar.

	Fermented samples			
	Phytate (mg/100g)		Tannin (%)	
Fermentation period (h)	Uncooked	Cooked	Uncooked	Cooked
0	210.9^{b} (±0.04)	192.8^{b} (±0.04)	0.55^{b} (±0.06)	0.38^{b} (±0.04)
2	184.8^{c} (±0.02)	168.8^{c} (±0.15)	0.48^{c} (±0.01)	0.36^{c} (±0.01)
4	157.3^{d} (±0.06)	143.5^{d} (±0.13)	0.46^{d} (±0.00)	0.32^{d} (±0.01)
6	116.54^{e} (±0.06)	106.2^{e} (±0.06)	0.43^{e} (±0.01)	0.29^{e} (±0.02)
8	109.8^{f} (±0.02)	99.6^{f} (±0.54)	0.31^{f} (±0.01)	0.20^{f} (±0.10)
10	97.65^{g} (±0.12)	88.45^{g} (±0.43)	0.29^{g} (±0.00)	0.18^{g} (±0.01)
12	88.46^{h} (±0.02)	80.25^{h} (±0.21)	0.27^{h} (±0.01)	0.15^{h} (±0.01)
14	82.32^{i} (±0.02)	74.50^{i} (±0.10)	0.24^{i} (±0.01)	0.12^{i} (±0.01)
16	73.12^{j} (±0.13)	66.12^{j} (±0.06)	0.18^{j} (±0.00)	0.09^{j} (±0.15)

Table 3: (continued)

	Malted and fermented samples			
	Phytate (mg/100g)		Tannin (%)	
Fermentation period(h)	Uncooked	Cooked	Uncooked	Cooked
0	206.4^{b} (±0.15)	181.0^{b} (±0.12)	0.51^{b} (±0.06)	0.36^{b} (±0.02)
2	162.15^{c} (±0.04)	142.5^{c} (±0.14)	0.47^{c} (±0.01)	0.32^{c} (±0.01)
4	126.8^{d} (±0.13)	111.2^{d} (±0.15)	0.46^{d} (±0.00)	0.31^{d} (±0.06)
6	109.16^{e} (±0.21)	95.18^{e} (±0.11)	0.45^{e} (±0.06)	0.29^{e} (±0.01)
8	96.67^{f} (±0.06)	83.9^{f} (±0.13)	0.31^{f} (±0.04)	0.19^{f} (±0.06)
10	85.34^{g} (±0.16)	74.1^{g} (±0.06)	0.26^{g} (±0.01)	0.15^{g} (±0.04)
12	70.60^{h} (±0.15)	61.3^{h} (±0.10)	0.23^{h} (±0.01)	0.10^{h} (±0.12)
14	62.31^{i} (±0.01)	54.2^{i} (±0.08)	0.17^{i} (±0.12)	0.06^{i} (±0.02)
16	51.10^{j} (±0.21)	44.25^{j} (±0.13)	0.13^{j} (±0.02)	0.04^{j} (±0.10)

Values are means of triplicate samples (±SD). Means not sharing a common letter in a column are significantly different at $P \leq 0.05$ as assessed by Duncan's multiple range test.

fermentation of malted flour was 2.80% and 2.60% for Mugud (Table 1) and Karamaka (Table 2) cultivars, respectively. When the flour was fermented for 16 h, the total protein content increased significantly ($P \leq 0.05$) from 10.58 to 13.11% (Table 1) for Mugud cultivar and from 10.85% to 12.25% (Table 2) for Karamaka cultivar. Fermentation of malted flour for 16 h caused additional increment in total protein content and was found to range from 10.36% to 14.82% for Mugud cultivar and from 11.02% to 12.74% for Karamaka cultivar. Chavan and Kadam (1989a and 1989b) and Ahmed *et al.* (1991) reported that the observed increment in protein content after processing of the samples (fermented or germinated) was probably due to shift in dry matter content through depletion of carbohydrates, during both germination and fermentation by action of the fermenting microorganisms. It may thus be apparent and not real increment. However, cells of the fermenting microorganisms could have contributed to the protein, therefore, germination or fermentation of sorghum results in an observable increase in crude protein content. However, a combination of the two treatments generally may result in additional increment. In most human diet, the protein is more limiting than carbohydrates. Therefore, application of any process that appears to increase the protein content even at the expense of carbohydrates may be advantageous nutritionally (Asiedu *et al.*, 1993).

Changes in antinutritional factors of fermented and cooked flour with or without malt: Table 3 shows changes in phytic acid and tannins content during cooking of fermented flour with or without malt of Mugud cultivar. Cooking of the cultivar dough after fermentation for different period of time with or without malt reduces both phytate and tannins of the cultivar. Fermentation of the cultivar flour for 6 h significantly ($P \leq 0.05$) reduced total phytate from 210.9 to 116.54 mg/100g. The reduction rate continued and reached its maximum value (73.12 mg/100g) when the flour was fermented for 16 h. It has been suggested that the loss of phytate during fermentation could be a result of activity of native phytase and/or the fermentative microflora. Most of the reduction rate of phytate occurred during the first 4 hour of fermentation. This may be due to the prevailing pH at this period which is considered to be an optimum pH for microbial phytase activity. Faridi *et al.* (1983) also stated that the phytate is insoluble at pH 6 and the microbial phytase activity is inhibited below pH 5.0. When fermented dough was cooked, further reduction in phytic acid content (66.12 mg/100g) was observed especially when the flour was fermented for 16 h and then cooked for 20 min. Fermentation of the malted flour significantly ($P \leq 0.05$) reduced phytate content with fermentation time from 206.4 to 51.10 mg/100g. Cooking of malted and fermented product significantly ($P \leq 0.05$) reduced phytic acid content to 44.25 mg/100g. The results

Table 4: Effect of cooking on antinutritional factors content of fermented flour with or without malt of Karamaka cultivar

	Fermented samples			
	Phytate (mg/100g)		Tannin (%)	
Fermentation period (h)	Uncooked	Cooked	Uncooked	Cooked
0	249.20^{b} (±0.06)	224.3^{b} (±0.14)	2.74^{b} (±0.01)	1.98^{b} (±0.01)
2	207.50^{c} (±0.01)	185.0^{c} (±1.00)	2.31^{c} (±0.02)	1.75^{c} (±0.02)
4	173.60^{d} (±0.16)	155.6^{d} (±0.60)	2.10^{d} (±0.03)	1.40^{d} (±0.05)
6	130.57^{e} (±0.06)	116.9^{e} (±0.10)	1.86^{e} (±0.01)	1.20^{e} (±0.01)
8	117.80^{f} (±0.20)	105.6^{f} (±0.53)	1.72^{f} (±0.04)	1.10^{f} (±0.03)
10	106.97^{g} (±0.06)	95.80^{g} (±0.15)	1.60^{g} (±0.03)	1.08^{g} (±0.06)
12	100.00^{h} (±0.30)	89.72^{h} (±0.13)	1.54^{h} (±0.05)	1.05^{h} (±0.02)
14	88.700^{i} (±050)	79.30^{i} (±0.10)	1.35^{i} (±0.01)	1.02^{i} (±0.08)
16	80.300^{i} (±0.10)	71.20^{i} (±0.60)	1.07^{i} (±0.05)	0.73^{i} (±0.02)

Table 4: (continued)

	Malted and fermented samples			
	Phytate (mg/100g)		Tannin (%)	
Fermentation period (h)	Uncooked	Cooked	Uncooked	Cooked
0	236.13^{b} (0.16)	206.16^{b} (0.03)	2.72^{b} (±0.02)	1.80^{b} (0.02)
2	156.31^{c} (±0.02)	136.1^{c} (±0.10)	2.40^{c} (±0.01)	1.56^{c} (±0.02)
4	115.5^{d} (±0.15)	100.23^{d} (±0.07)	1.97^{d} (±0.01)	1.28^{d} (±0.02)
6	81.86^{e} (±0.01)	70.73^{e} (±0.06)	1.62^{e} (±0.03)	1.03^{e} (±0.01)
8	70.57^{f} (±0.07)	61.03^{f} (±0.06)	1.53f (±0.01)	0.92^{f} (±0.01)
10	60.51^{g} (±0.01)	52.17^{g} (±0.24)	1.32^{g} (±0.00)	0.79^{g} (±0.01)
12	51.15^{h} (±0.16)	44.15^{h} (±0.13)	1.12^{h} (±0.02)	0.66^{h} (0.01)
14	46.67^{i} (±0.07)	40.3^{i} (±0.12)	0.96^{i} (±0.01)	0.56^{i} (0.02)
16	41.17^{i} (±0.17)	35.5^{i} (±0.15)	0.87^{i} (±0.06)	0.40^{i} (±0.01)

Values are means of triplicate samples (±SD). Means not sharing a common letter in a column are significantly different at $P \leq 0.05$ as assessed by Duncan's multiple range test.

obtained revealed that phytic acid content for the cultivar reduced significantly ($P \leq 0.05$) when malt (5%) was added throughout the course of fermentation (Table 3). It was found that after 16 h fermentation, the percentage decrease in phytate content was 75.2. The results obtained also indicated that malt followed by fermentation enhances the loss of phytate compared to fermentation alone. The results agree with that reported by Kheterpaul and Chauhan (1991), who stated that fermentation of pearl millet sprouts by mixed pure culture, produces significant further loss in phytate compared to germination alone. In sorghum, processes such as pretreatment with malt (El Khalil *et al.*, 2001) or microbial phytase (Ravindran *et al.*, 1994) have greatly reduced phytic acid content leading to enhanced protein digestibility. However, the observed effect of phytase addition is believed to be due to structural or chemical properties of both phytic acid and protein rather than the total concentration of phytic acid (Ravindran *et al.*, 1994). Tannin content of the cultivar before fermentation was 0.55% and after fermentation for 16 h reduced to 0.18 (Table 3). The reduction in tannin content of malted and fermented samples is highly significant ($P \leq 0.05$) compared to unmalted and fermented samples. Cooking of fermented dough significantly ($P \leq 0.05$) reduced tannin content from 0.38% to 0.09% after 16 h fermentation. Tannin content of cooked malted and fermented samples was lowered from 0.36% to 0.04% after 16 h fermentation. Tables 4 shows changes in phytic acid and tannins content during cooking of fermented flour with or without malt of Karamaka cultivar. The effect of the processes applied for Karamaka on phytate and tannins gave results similar to those obtained for Mugud cultivar. For both cultivars the results revealed that combination of the three processes (malting, fermentation and cooking) greatly enhances the removal of the antinutritional factors which are believed to be responsible for unavailability of both proteins and divalent minerals.

Changes in *in vitro* protein digestibility (IVPD) of fermented and cooked flour with or without malt: In complete digestion (single-enzyme digestion) by pepsin alone was carried out to study the effect of cooking on fermented dough with or without malt of Mugud and Karamaka cultivars (Table 5). The IVPD of untreated flour for Mugud cultivar was 12.26% and that of Karamaka was 12.09%. Mugud (low in tannin) gave high values of IVPD than Karamaka (high in tannin) for most treatments. Fermentation alone improved the IVPD of the cultivars. However, cooking reduced the IVPD significantly ($P \leq 0.05$) with the fermentation time. Malting of the flour slightly reduces the effect of cooking on IVPD. It is well documented that condensed polyphenols have

Table 5: *In vitro* protein digestibility of fermented flour with or without malt of sorghum cultivars.

	Mugud cultivar			
	Fermented dough		Malted and fermented flour	
Fermentation period (h)	Uncooked	Cooked	Uncooked	Cooked
0	12.26^{ef} (±0.01)	10.65^{i} (±0.01)	12.72^{h} (±0.02)	11.34^{i} (±0.01)
2	12.32^{ef} (± 0.01)	11.06^{h} (±0.01)	13.17^{g} (±0.02)	11.70^{h} (±0.13)
4	12.45^{e} (±0.01)	11.55^{g} (±0.03)	13.18^{g} (±0.00)	11.98^{g} (±0.01)
6	12.5^{e} (±0.00)	11.87^{f} (±0.01)	13.26^{f} (±0.01)	12.56^{f} (±0.01)
8	12.98^{d} (±0.03)	12.16^{e} (±0.01)	13.48^{e} (±0.01)	12.73^{e} (±0.01)
10	13.10^{d} (± 0.00)	12.46^{d} (±0.01)	14.56^{d} (±0.03)	13.14^{d} (±0.01)
12	14.32^{c} (±0.04)	12.70^{c} (±0.03)	15.44^{c} (±0.01)	13.87^{c} (±0.01)
14	15.22^{b} (± 0.01)	12.98^{b} (± 0.01)	16.56^{b} (± 0.30)	14.27^{b} (±0.02)
16	16.10^{a} (±0.10)	13.16^{a} (± 0.01)	17.96^{a} (±0.05)	15.57^{a} (±0.01)

Table 5: (Continued).

	Karamaka cultivar			
	Fermented dough		Malted and fermented flour	
Fermentation period (h)	Uncooked	Cooked	Uncooked	Cooked
0	12.09^{i} (± 0.01)	10.56^{i} (± 0.02)	12.57^{i} (± 0.01)	11.06^{i} (± 0.01)
2	12.17^{h} (±0.02)	11.13^{h} (± 0.04)	12.97^{h} (±0.04)	11.41^{h} (±0.20)
4	12.43^{g} (±0.04)	11.50^{g} (±0.02)	13.07^{g} (± 0.02)	11.63^{g} (±0.01)
6	12.56^{f} (± 0.01)	11.95^{f} (±0.10)	13.13^{f} (± 0.01)	12.09^{f} (± 0.01)
8	12.79^{e} (±0.01)	12.11^{e} (± 0.01)	13.22^{e} (± 0.02)	12.32^{e} (±0.50)
10	13.16^{d} (± 0.01)	12.34^{d} (± 0.01)	14.31^{d} (± 0.03)	12.57^{d} (±0.14)
12	13.85^{c} (±0.01)	12.65^{c} (± 0.01)	14.98^{c} (±0.01)	12.94^{c} (±0.01)
14	14.01^{b} (± 0.03)	12.95^{b} (± 0.04)	15.26^{b} (±0.02)	13.88^{b} (±0.01)
16	15.31^{a} (± 0.02)	13.11^{a} (±0.02)	17.65^{a} (± 0.01)	14.80^{a} (±0.01)

Values are means of triplicate samples (±SD). Means not sharing a common letter in a column are significantly different at $P \leq 0.05$ as assessed by Duncan's multiple range test.

a negative impact on digestibility of high tannin sorghum (Butler *et al.*, 1984 and Hassan and El Tinay 1994). It has been proposed that oxidation of plant polyphenols leads to formation of quinines and highly reactive peroxides, which are oxidizing agents; these peroxides may then bring about oxidation of amino acid residues and subsequently, polymerization of proteins which may then lead to a reduced protein digestibility (Doudou *et al.*, 2003). The fall in *in vitro* protein digestibility on cooking has been well documented (Klopfesntein and Hoseney, 1995) but not fully explained. However, as one of the sorghum varieties used in this study has been shown to be low in tannin (Mugud), the reduction in IVPD on cooking is not due to the presence of tannin alone. Doudou *et al.* (2003) reported that the low protein digestibility of cooked sorghum must result from changes in the proteins themselves during cooking. The formation of enzyme resistant disulphide bonded oligomers may be the cause of the low digestibility. The IVPD of both cultivars increased significantly ($P \leq 0.05$) throughout the period of fermentation. Kheterpaul and Chauhan (1991) reported that fermentation appears to protect the proteins from becoming resistant to digestion by pepsin on cooking. Fermented products, e.g. Kissra (a fermented Sudanese bread made from sorghum flour), Abray (a fermented Sudanese drink) and Nasha (a fermented Sudanese baby food showed an improvement in digestibility over that of unfermented cooked sorghum flour (El Tinay *et al.*, 1979). Fermentation after addition of 5% malt caused higher increment in IVPD throughout the period of fermentation. El Khalil *et al.* (2001) reported that the IVPD increment was significantly affected by addition of sorghum malt. Therefore, fermentation with or without malt partially alleviates the adverse effect of cooking on sorghum proteins digestibility. This confirms the findings of Fageer and El Tinay (2004) that malting significantly improved the IVPD of cooked samples.

Conclusion: Processing of sorghum (boiling, germination, fermentation and cooking) greatly improved its nutritive value. However, combination of these processes further improved the quality of sorghum as a food by removing the antinutritional factors as well as by alleviating the effect of heating.

References

Agrawal, P. and U. Chitnis, 1995. Effect of treatment on phytate phosphorus, iron bioavailability, tannin and *in vitro* protein digestibility of grain sorghum. J. Food Sci. and Tech., 32: 453-458.

Ahmed, R.U., H.M. Kabirulla and S.A. Khan, 1991. Effect of traditional processing on the nutritive value of sorghum. Bangladesh J. Sci. Ind. Res., 26:195-199.

AOAC, 1984. Official methods of analysis. 14th Edn. Washington, DC: Association of Official Agricultural Chemists.

Asiedu, M., E. Lied, R.Nilsen and K. Sandes, 1993. Effect of processing (sprouting and/or fermentation) on sorghum and maize. II: Vitamins and amino acid composition, Biological utilization of maize protein. Food Chemistry, 48: 201-204.

Bhise, V.J., J.K. Chavan and S.S. Kadam, 1988. Effects of malting on proximate composition and *in vitro* protein digestibilities of grain sorghum. J. Food Sci. and Tech., 25: 327-329.

Butler, L.G., D.J. Riedl, D.G. Lebryz and H.J. Blytt, 1984. Interaction of proteins with sorghum tannin: Mechanism, specificity and significance. J. Am. Oil Chem., 61: 916-920.

Chavan, U.D., J.K. Chavan and S.S. Kadam, 1988. Effect of fermentation on soluble protein and *in vitro* protein digestibility of sorghum, green gram and sorghum-green gram blend, J. Food Sci., 53: 1574–1575.

Chavan, J.K. and S.S. Kadam, 1989a. Nutritional improvement of cereals by sprouting. Food Sci. Nutr. Crit. Rev., 28: 401-437.

Chavan, J.K. and S.S. Kadam, 1989b. Nutritional improvement of cereals by sprouting. Food Sci. Nutr. Crit. Rev., 28: 349-400.

Duncan, D.B., 1955. Multiple range and multiple F test. Biometrics, 11: 142-149.

Doudou, K.G., J.R.N. Taylor, P.S. Belton and B.B. Hamaker, 2003. Factors affecting sorghum protein digestibility. Mini Review. J. Cereal Sci., 38: 117-131.

El Khalil, E.A.I., A.H. El Tinay, B.E. Mohamed and E.A.E. Elsheikh, 2001. Effect of malt pre-treatment on phytic acid and *in vitro* protein digestibility of sorghum flour. Food Chem., 72: 29-32.

El Tinay, A.H., A.M. Abdel Gadir and M. El Hidai, 1979. Sorghum fermented kisra bread. 1. Nutritive value of kisra. J. Sci. Food and Agric., 30: 859-863.

Fageer, A.S.M. and A.H. El Tinay, 2004. Effect of genotype, malt pretreatment and cooking on *in vitro* protein digestibility and protein fractions of corn. Food Chem., 84: 613-619.

FAO, 1997. Production Yearbook. Food and Agriculture Organization of the United Nation. Rome, Italy., 51: 59-79.

Faridi, H.A., P.I. Finney and G.I. Rubenthaler, 1983. Iranean bread: Relative bioavailability of zinc. J. Food. Sci., 48: 107-110.

Hassan, I.A.G. and A.H. El Tinay, 1995. Effect of fermentation on tannin content and *in vitro* protein digestibility of two sorghum cultivars. Food Chemistry, 53: 149-151.

Kheterpaul, N. and B.M. Chauhan, 1991. Effect of natural fermentation on phytate and polyphenolic content and *in vitro* digestibility of starch and protein of pearl millet (P. typhodeum). J. Sci. Food Agric., 55: 189-195.

Klopfesntein, C.F. and R.C. Hoseney, 1995. Nutritional properties of sorghum and millets. In: Sorghum and Millets: Chemistry, Technology (edited by D.A.V. Dendy) St Paul, MN: Am. Assoc. Cereal Chemists., pp: 125-168.

Lorri, W. and U. Svanberg, 1993. Lactic fermented gruels with improved *in vitro* protein digestibility. Int. J. Food Nutr., 44: 29-36.

Maliwal, B.P., 1983. *In vitro* method to assess the nutritive value of leaf concentrate. J. Agric. and Food Chemistry, 31: 315-319.

Monjula, S. and E. John, 1991. Biochemical changes and *in vitro* protein digestibility of the endosperm of germinating Dolichos lablab. J. Sci. Food Agriculture, 55: 229-538.

Price, M.L., S. VanScoyoc and L.G. Butler, 1978. A critical evaluation of the vanillin reactions as an assay for tannin in sorghum grain. Food Chemistry, 26: 12-14.

Ravindran, V., G. Ravindran and S. Sivalogan, 1994. Total and phytate phosphorus contents of various foods and food stuffs of plant origin. Food Chemistry, 50: 133-137.

Ryden, P. and R.R. Selvendran, 1993. Phytic acid: Properties and determination. In: Macrae, R. Robinson R.K., Sadler M.J. (Eds.), Encyclopaedia of food sci. Food Tech.Nutr. Academic Press, London, pp: 3582-3587.

Snedecor, G.W. and W.G. Cochran, 1987. Statistical methods. 7th Edn. Ames, IA: The Iowa State University Press., pp: 221-224.

Wheeler, E.L. and R.E. Ferrel, 1971. A method for phytic acid determination in wheat and wheat fractions. Cereal Chem., 48: 312-320.