

Biochemical Characteristics of Sorghum Flour Fermented and/or Supplemented with Chickpea Flour

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Abstract—Sorghum flour was supplemented with 15 and 30% chickpea flour. Sorghum flour and the supplement were fermented at 35 °C for 0, 8, 16, and 24 h. Changes in pH, titrable acidity, total soluble solids, protein content, *in vitro* protein digestibility and amino acid composition were investigated during fermentation and/or after supplementation of sorghum flour with chickpea. The pH of the fermenting material decreased sharply with a concomitant increase in the titrable acidity. The total soluble solids remained unchanged with progressive fermentation time. The protein content of sorghum cultivar was found to be 9.27 and that of chickpea was 22.47%. The protein content of sorghum cultivar after supplementation with 15 and 30% chickpea was significantly ($P \leq 0.05$) increased to 11.78 and 14.55%, respectively. The protein digestibility also increased after fermentation from 13.35 to 30.59 and 40.56% for the supplements, respectively. Further increment in protein content and digestibility was observed when supplemented and unsupplemented samples were fermented for different periods of time. Cooking of fermented samples was found to increase the protein content slightly and decreased digestibility for both supplements. Amino acid content of fermented and fermented and cooked supplements was determined. Supplementation was found to increase the lysine and threonine content. Cooking following fermentation decreased lysine, isoleucine, valine and sulfur containing amino acids.

Keywords—Amino acid, Chickpea, Cooking, Fermentation, protein, Sorghum.

I. INTRODUCTION

THE present trend in population growth indicates that the protein gap may continue to increase in the future unless well-planned measures are taken to tackle the situation. Provision of adequate proteins of animal origin is difficult and expensive. An alternative for improving nutritional status of the people is to supplement the diet with plant proteins. Attention, therefore, has to be directed to the nutritional evaluation of proteins from plant species. Legumes (poor man's meat) play an important role in human nutrition since they are rich sources of protein, calories, minerals and vitamins and therefore can be good supplements [1]. Sorghum (*Sorghum bicolor* (L.) Moench) is an important

cereal crop grown in the semi-arid tropics of Africa and Asia due to its drought tolerance. It is a staple food crop cultivated on a substantial level by farmers in these areas for human consumption [2]. In many African countries, sorghum is milled into flour before fermentation and cooking. Fermentation is an ancient method of food processing aimed at prolonging shelf-life and improving palatability. It may also improve digestibility and nutritional value of food and feed. The preservation by fermentation of vegetables and cereals is mostly due to the lactic acid bacteria, often in combination with yeasts. However, other types of bacteria e.g. *Bacillus spp.* are involved [3]. Cooking reduced the protein digestibility of sorghum grain. When sorghum is cooked, enzymatically resistant protein polymers are formed through disulphide bonding of beta- and gamma-kafirins [4], [5]. This is perhaps one of the most important factors contributing to reduced protein digestibility of cooked sorghum. In Africa and Asian countries, sorghum porridges are generally prepared by cooking slurry of fermented or unfermented flour in boiling water with continuous stirring; the resulting thick porridge after cooling is known by different names such as tuwo, aseda, ugali, etc, depending on geographical region [6]. Other traditional foods prepared from sorghum include unfermented bread (chapatti, roti and tortilia), fermented bread (kirsra, injera, dosa and dosai), alcoholic (pito and dold) and nonalcoholic (mahewu, marewa and magou). Sorghum is eaten in areas where the populations are frequently undernourished, therefore it is important to consider the quality, quantity and availability of the nutrients in the grain. At the heart of the issue of sorghum nutritive effectiveness is the fact that almost 60% of the protein is in the highly cross-linked form called prolamin, which human digestive enzymes are unable to break it and often some form of fermentation and germination are employed as a way to improve the protein quality. Sorghum like other cereals has some limitations in some essential amino acids particularly lysine, while legumes and oilseeds are high in both protein and lysine, but legumes usually deficient in sulphur containing amino acids, methionine and cystine. On the other hand, cereal-grain in general has adequate amounts of sulphur amino acids [7]. It is often, therefore, emphasized that legume seed grain proteins are the natural supplement to cereal grain protein in producing and overall essential amino acid balance. Also, there is a

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growing recognition that legumes and their products are good sources of vitamins and minerals [8]. In this study in order to improve sorghum protein quality and quantity we would like to supplement it with chickpea at different levels (15 and 30%) and then to investigate the protein content and digestibility and amino acid composition of the supplements.

II. MATERIALS AND METHODS

A. Materials

Sorghum (*Sorghum bicolor* L., Moench) cultivar Tabat and chickpea (*Cicer arietinum* L.) cultivar Shendi were used in this study. Tabat was obtained from Food Research Center, Shambat, Sudan and chickpea obtained from Alhodieba Research Station, Sudan. The grains were carefully cleaned and milled into fine flour to pass a 0.32 mm screen. Three replicates of each sample were used for the analysis. Unless otherwise stated all chemicals used in this study are of reagent grade.

B. Fermentation of sorghum flour

Sorghum flour (Tabat) fermented with 10% starter [10% (starter) + 90% (sorghum flour)] in an incubator (35 °C) for different periods of time (0, 8, 16, and 24 hours). The water to flour ratio was 2:1. After fermentation the samples were dried in a hot air oven drier at 65°C. Dried samples were reground to pass a 0.32 mm screen and stored at 4°C until used for subsequent analysis.

C. Cooking

Cooking of the samples was performed according to the method described by Arbab and El Tinay [9]. Cooked samples were prepared by suspending the flour of each sample in distilled water in the ratio of 1:10 flour to water and stirring to avoid lumps while boiling in water bath for 20 min. The viscous mass was spread out thinly and then dried at 65 °C in an oven. The dry flakes were milled into fine flour to pass a 0.32 mm screen and kept in nylon bags at 4°C for further analysis.

D. Chickpea supplementation

About 15% chickpea and 30% of chickpea flour, on a dry matter base, were added to sorghum flour to increase its protein content. The mixtures were fermented and cooked as described above.

E. Determination of pH and titratable acidity

The pH of the fermenting dough was monitored for different period of time by using a glass electrode pH meter (PUSL, MUNCHENZ, KARL KOLB, Germany). Titratable acidity, expressed as lactic acid, was determined by titration with 0.1 N NaOH to pH 8.1 [10].

F. Determination of total soluble solids

Total soluble solids were determined at 20 °C by the Joslyn [11] method, using an Abbe refractometer (Bellingham and Stanley LTD, London).

G. Protein content

The protein content of the samples was determined by the micro-kjeldahl method as described by AOAC [12].

H. Protein digestibility

The *in vitro* protein digestibility of the samples was carried out according by Monjula *et al.* [13]. A known weight of the sample containing 16 mg nitrogen was taken in triplicate and digested with 1 mg pepsin in 15 ml of 0.1 M HCl at 37 °C for 2 hours. The reaction was stopped by the addition of 15 ml 10% trichloro-acetic acid (TCA). The mixture was then filtered quantitatively through Whatman No. 1 filter paper. The TCA soluble fraction was assayed for nitrogen using the micro-kjeldahl method. Digestibility was calculated using the following equation:

$$\text{Protein digestibility (\%)} = \frac{\text{N in supernatant} - \text{Blank N}}{\text{N in sample}} \times 100$$

I. Determination of amino acid content

The amino acid content was determined according to the official methods of analysis [12]. About 500 mg of pulverized sample was hydrolyzed with 5 ml of 6 N HCl in an evacuated sealed tube for 24 hours at 110°C, before and after oxidation (H₂O₂/HCOOH, 24 h, chilled), the pH was adjusted to 2.2 with NaOH and filled to 100 ml with a buffer (pH 2.2) and about 2 ml were then filtrated (membrane filter). The liberated amino acids were separated by LKB Biochrom 4150 (Alpha0 Automatic Amino Acid Analyzer based on ion-exchange chromatography. 1/Tyrosine, histidine and tryptophane (oxidized sample). 2/Cysteine, methionine and tryptophane (hydrolyzed sample without pervious oxidation). Prolin is detected from a separate detector channel at 440 nm, all the other were detected at 570 nm, and then calculated as µg of amino acid per mg of protein.

J. Statistical analysis

Samples were analyzed in triplicate and the figures were then averaged. Data was assessed by analysis of variance (ANOVA) [14] and by Duncan's Multiple-range test with a probability $P \leq 0.05$.

III. RESULTS AND DISCUSSION

A. Changes in pH, titratable acidity and total soluble solids during fermentation and/or supplementation of sorghum flour

Table 1 shows changes in pH, titratable acidity (TA) and total soluble solids (TSS) during fermentation of sorghum cultivar (Tabat) flour with and without chickpea supplement. The pH of the fermented dough of supplemented and unsupplemented sorghum flour greatly dropped and reached 3.8 for the supplemented and unsupplemented flour at the end of the fermentation period (24 h). Concomitant with the drop in pH there was a rise in TA throughout the fermentation process for both supplemented and unsupplemented flour. According to Mohammed [15], sorghum fermentation is mainly lactic (*Lactobacillus spp.*), yeast and acetic acid fermentation occur to a lesser extent during the latter stages of fermentation. This could explain the apparent increase in lactic acid towards the end of fermentation, accompanied by lack of changes in pH. The TSS remained constant for both supplemented and unsupplemented fermented dough. The general pattern showed an initial increase in soluble solids at the commencement of fermentation, followed by a decrease toward the end of fermentation.

TABLE I
EFFECT OF FERMENTATION ON pH, TOTAL SOLUBLE SOLIDS (TSS) AND TITRATABLE ACIDITY (TA) OF SORGHUM SUPPLEMENTED WITH CHICKPEA (15 AND 30%)

Fermentation period (h)	Unsupplemented			15% Supplement			30% Supplement		
	pH	TSS	TA	pH	TSS	TA	pH	TSS	TA
0	6.05	1.34	0.44	6.07	1.34	0.57	6.07	1.35	0.72 ^c
8	5.89	1.34	0.53	5.91	1.35	0.71	5.90	1.36	0.84 ^c
16	4.30	1.34	1.08	4.17	1.34	1.63	4.52	1.34	1.63 ^b
24	3.79	1.34	1.73 ^a	3.80	1.34	2.47	3.81	1.34	3.03 ^a

Values are means of triplicate samples

B. Changes in protein content and in vitro digestibility during fermentation and/or cooking of supplemented and unsupplemented sorghum flour

The protein content of both sorghum and chickpea flour was determined (data not shown). The protein content of sorghum flour was found to be 9.69% which is within the range (9–11.3%) reported by Torres *et al.* [16]. The crude protein content of chickpea flour was found to be 22.47% (data not shown) which is higher than that reported by Osman [17]. Table 2 shows the effect of fermentation of on protein content and digestibility (%) of sorghum supplemented with chickpea (15 and 30%). The protein content of the fermented dough ranged from 9.69% at zero time to 10.15% at the end of the fermentation period (24 h). A similar trend of protein content during fermentation was reported by Mohammed [15]. An increment in protein is likely to be due to solubilization of

TABLE II
EFFECT OF FERMENTATION ON PROTEIN CONTENT (%) AND DIGESTIBILITY (%) OF SORGHUM SUPPLEMENTED WITH CHICKPEA (15 AND 30%)

Fermentation period (h)	Unsupplemented		15% Supplement		30% Supplement	
	Protein	IVPD	Protein	IVPD	Protein	IVPD
0	9.69	13.35	11.78	30.59	14.55	40.56
8	10.07	15.64	11.31	35.19	13.78	41.49
16	10.24	16.74	13.48	39.28	12.14	29.29
24	10.15	15.22	12.27	29.08	14.05	33.23

Values are means of triplicate samples

sorghum flour constituents as a result of fermentation.

Supplementation increased the protein content of the flour to 11.78 and 14.55% at zero time for 15 and 30% supplements, respectively. However, after fermentation the protein content of both supplements fluctuated. The *in vitro* protein digestibility (IVPD) for sorghum flour was found to be 13.35%. The value obtained for IVPD is lower than that obtained by Arbab and El Tinay [9] for sorghum cultivars. The *in vitro* protein digestibility for chickpea flour was found to be 56.13% which is higher than that obtained by Rehman and Shah [18]. Supplementation and/or fermentation of the flour significantly ($P \leq 0.05$) increased the IVPD with a maximum value (41.49%) obtained when the flour was supplemented with 30% chickpea and fermented for 8 h. The increment in protein content before supplementation and after fermentation can be attributed to microbial synthesis from metabolic intermediates during fermentation. The increment in *in vitro* protein digestibility (IVPD) of supplemented and/or fermented sorghum flour could be attributed to antinutrients degradation by microorganisms and to partial degradation of complex storage proteins into more simple and soluble products. Moreover, addition of chickpea flour may contribute in improving the IVPD of the supplements due to its high IVPD. The results obtained also are in accordance with Taylor and Taylor [19] who found that, fermentation of sorghum-based porridge intended for young children improved protein digestibility and insoluble protein digestibility (a new index) suggested that fermentation causes structural changes in the sorghum storage proteins (prolamins and glutelins) making them more accessible to enzymatic attack. Table 3 shows the variation in protein content and digestibility of supplemented and/or fermented sorghum flour after cooking. The protein content supplemented and unsupplemented sorghum flour increased slightly after cooking. The IVPD of the cooked samples (supplemented and unsupplemented) increased initially with fermentation time and thereafter started to decline. The results obtained agree with those of Eggum *et al.* [7] who reported that sorghum protein digestibility decreased significantly after cooking. The losses in protein content could be attributed to partial removal of certain amino acids, along with other nitrogenous compounds, on heating as has already been reported by other workers [20], [21]. Rom *et al.* [5] and Hamaker *et al.* [4]

reported that when sorghum is cooked enzymatically resistant protein polymers are formed through disulphide bonding of beta- and gamma-kafirins. The negative effect of cooking on IVPD was reported by Rom *et al.*, [5] and for legumes by Rehman and Shah [18]. They attributed the reduction in IVPD to the formation of disulphide bonds resulting in folding of protein molecule and hence decreasing its susceptibility to digestive enzymes.

C. Changes in amino acid composition during fermentation and/or cooking of supplemented and unsupplemented sorghum flour

Table 4 shows the effect of fermentation of supplemented and unsupplemented sorghum flour on amino acid composition. In cereals lysine represent the second limited amino acid, the first one is threonine. For both supplemented and unsupplemented sorghum flour, amino acid content was fluctuated and either slightly increased or decreased. Fermentation of unsupplemented sorghum flour slightly decreased threonine and lysine contents from 1.2 to 0.9 and from 3.0 to 2.4g/100g protein after 8 h fermentation, respectively. Supplementation of sorghum flour greatly increased both threonine and lysine. However, fermentation of supplements slightly decreased the content of both amino acids with maximum value (4.5 g/100g protein) obtained for lysine when sorghum flour was supplemented with 30% chickpea which is higher than that of reference pattern recommended by FAO/WHO/UNU [22] and Dendy [23]. Table 5 shows the amino acid profile of supplemented and/or fermented sorghum flour after cooking. Cooking supplemented and/or fermented sorghum flour decreased the lysine, isoleucine, valine and the sulfur amino acids. However, other amino acids were increased for both supplements as well as sorghum flour. The reduction in amino acids may be attributed to the denaturation of the protein during the heat treatment. Among the non-essential amino acid alanine, arginine, aspartic acid and glycine are markedly decreased after cooking.

TABLE III
EFFECT OF FERMENTATION FOLLOWED BY COOKING ON PROTEIN CONTENT (%) AND DIGESTIBILITY (%) OF SORGHUM SUPPLEMENTED WITH CHICKPEA (15 AND 30%)

Fermentation period (h)	Unsupplemented		15% Supplement		30% Supplement	
	Protein	IVPD	Protein	IVPD	Protein	IVPD
0	9.69	13.35	11.42	22.92	13.69	32.23
8	10.07	15.64	11.84	25.70	12.66	36.76
16	10.24	16.74	13.45	35.06	13.01	24.90
24	10.15	15.22	12.22	27.43	14.18	37.80

Values are means of triplicate samples

TABLE IV
EFFECT OF FERMENTATION FOR DIFFERENT PERIOD OF TIME (H) ON AMINO ACID COMPOSITION (G/100G PROTEIN) OF SORGHUM SUPPLEMENTED WITH CHICKPEA (30%)

Amino acid	Unsupplemented				30% Supplement			
	Fermentation period (h)							
	0	8	16	24	0	8	16	24
Histidine	2.20	1.50	1.90	1.40	1.30	2.10	1.60	1.70
Isoleucine	11.2	9.90	10.8	10.1	7.20	10.6	10.3	10.8
Leucine	16.5	15.4	12.2	15.9	9.88	14.0	12.1	12.1
Lysine	2.98	2.40	2.60	2.40	4.50	4.50	2.98	2.03
Methionine	0.60	0.70	0.99	0.85	0.89	0.92	0.75	0.85
Cystine	1.80	2.85	0.85	1.80	2.00	0.86	1.60	1.79
Phenylalanine	3.80	4.40	2.60	4.10	3.90	5.10	3.13	5.50
Tyrosine	2.20	1.70	1.00	2.10	3.86	2.50	1.70	2.10
Threonine	1.20	0.90	1.10	0.91	1.00	2.50	0.90	1.40
Valine	17.1	15.7	16.2	14.8	9.07	13.4	14.7	13.9
Alanine	23.8	21.2	21.3	22.9	11.8	15.4	21.4	19.9
Arginine	NP	10.3	9.60	9.30	6.70	8.80	NP	9.0
Aspartic acid	5.80	4.90	6.20	5.10	4.60	10.4	6.70	7.70
Glutamic acid	5.40	5.70	6.10	5.80	4.10	8.10	4.70	5.60
Glycine	0.99	0.78	0.85	0.77	0.40	0.98	1.00	0.57
Serine	2.20	1.50	2.30	1.70	1.10	2.60	1.00	1.60

TABLE V
EFFECT OF FERMENTATION FOR DIFFERENT PERIOD OF TIME (H) FOLLOWED BY COOKING ON AMINO ACID COMPOSITION (G/100G PROTEIN) OF SORGHUM SUPPLEMENTED WITH CHICKPEA (30%)

Amino acid	Unsupplemented				30% Supplement			
	Fermentation period (h)							
	0	8	16	24	0	8	16	24
Histidine	2.35	0.30	0.60	2.40	3.20	2.88	1.30	3.22
Isoleucine	6.48	6.03	4.80	5.90	6.56	6.60	10.4	6.60
Leucine	16.6	14.7	6.80	14.3	11.9	12.2	16.2	11.3
Lysine	2.40	2.34	1.20	2.60	4.40	4.08	1.88	4.25
Methionine	0.29	0.32	0.55	0.49	0.50	0.50	1.20	0.60
Cystine	0.10	0.27	1.00	0.26	0.10	0.33	0.27	0.37
Phenylalanine	6.80	6.40	1.40	6.40	7.50	7.10	5.50	7.50
Tyrosine	1.04	1.40	0.75	3.50	1.99	1.09	1.40	3.25
Threonine	3.80	3.90	0.56	3.90	4.50	4.60	1.70	4.55
Valine	8.49	7.90	7.50	7.90	7.30	7.50	13.9	7.50
Alanine	14.3	13.5	11.8	13.4	10.9	11.3	11.7	11.5
Arginine	4.70	1.40	3.40	1.30	5.60	5.70	5.40	4.10
Aspartic acid	7.90	7.50	3.40	7.20	10.9	10.7	8.30	10.7
Glutamic acid	17.0	16.9	2.30	16.8	16.1	16.1	7.70	16.1
Glycine	3.00	3.10	0.59	2.90	3.60	3.70	1.20	3.70
Serine	3.60	3.60	1.10	3.70	4.60	4.40	2.45	4.40

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