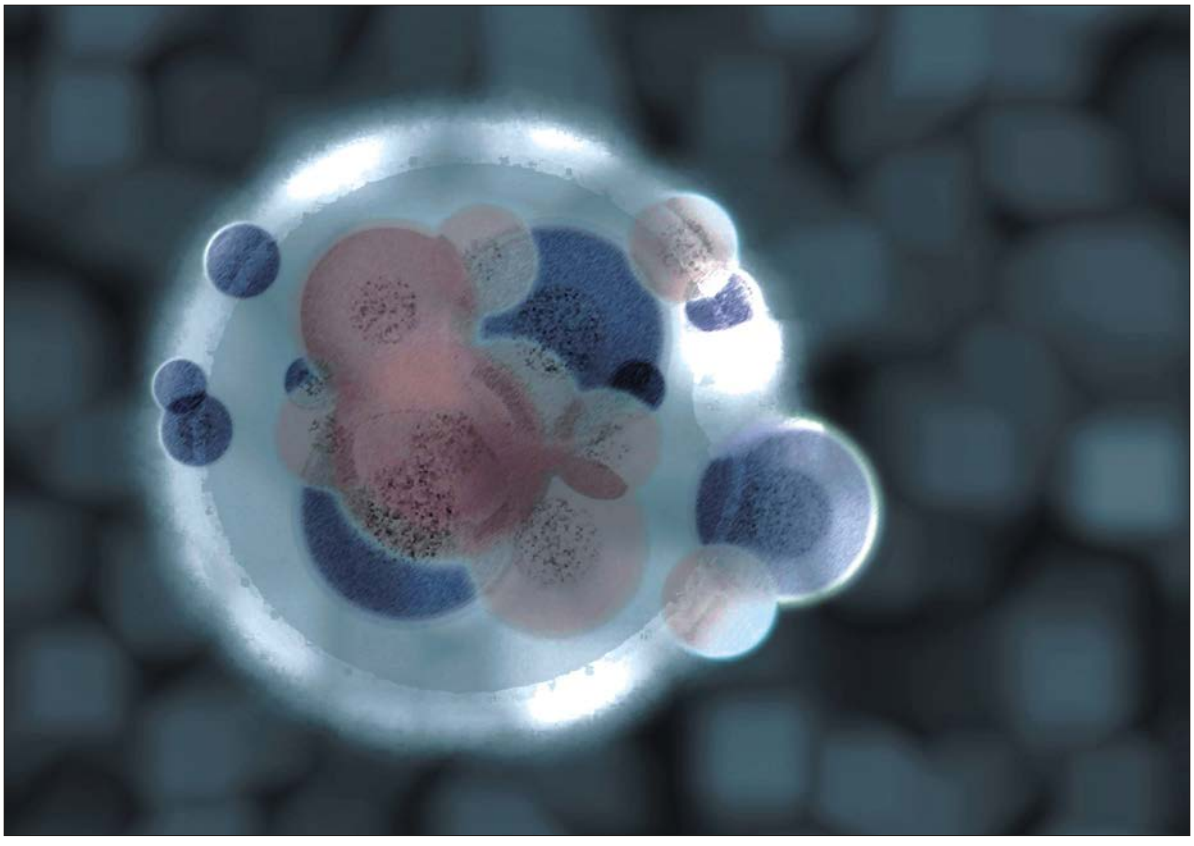

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Biochemical Characteristics of Sorghum (*Sorghum bicolor* L. Moench) Flour Supplemented with Cluster Bean (*Cyamopsis tetragonolaba* L.): Influence of Fermentation and/or Cooking

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Abstract: The aim of the present study is to investigate the effect of cluster bean supplementation followed by fermentation and cooking on biochemical characteristics of sorghum cultivars flour. Two Sudanese sorghum cultivars (Dabar and WadAhmed) were supplemented with cluster bean. The flour of the two cultivars and supplements were fermented for different periods of time and then cooked. The proximate composition of the cultivars flour and cluster bean showed that the protein was found to be 8.36, 9.76 and 44.65% for Dabar, WadAhmed and cluster bean, respectively. Fermentation of the cultivars flour for different periods of time significantly ($p \leq 0.05$) changed the titratable acidity, non protein nitrogen, crude protein and the dry matter for both cultivars. The protein digestibility of the cultivars flour and supplements was significantly ($p \leq 0.05$) increased with fermentation time even after cooking. The protein fractions contents of the flour before and after cooking and that of the supplements were fluctuating for both cultivars. Lysine content of the cultivars flour was significantly ($p \leq 0.05$) increased with fermentation time even after supplementation. However, other amino acids contents were fluctuating with fermentation time before and after supplementation for both cultivars.

Key words: Fermentation, supplementation, sorghum, cluster bean, protein fractions, amino acids

INTRODUCTION

Malnutrition and under nutrition are prevalent in several parts of the developing countries in the world. The reasons behind this situation include high population density, poor socioeconomic status for the people, inadequate sanitary and health facilities and non-availability of enough quantity and quality of foods (FAO, 1997). Although these factors are closely interrelated, major food sources, dietary habits and the processing methods used in the preparation of food significantly influence the nutritional status of the populations. Animal foods, although excellent in nutritional quality, are not available in enough quantity to these populations mainly due to their higher costs and certain religious traditions and customs. Hence, greater emphasis has been placed throughout the world on increasing the production of plant foods, improving their nutritional quality and developing simple and economical methods for their storage and processing. Cereals, legumes and oil seeds form a major bulk of dietary proteins, calories, vitamins and minerals to the developing nations (Steller, 1993). With increasing dependence upon cereal grains to provide both energy and protein requirements for man living in the developing countries, the need for raising the overall nutritional status of cereal grains has become increasingly important and much effort has been made to improve the amount and quality of cereal proteins. Many methods employed to improve the nutritional quality and organoleptic properties of cereal-based foods include genetic improvement, amino acid fortification, supplementation or complementation with protein rich sources such as grain legumes and defatted oil seed meals (Ibrahim *et al*., 2005). In recent years large and concentrated efforts have been directed to enhance the nutritional quality of almost all agriculturally significant cereal grains and in particular aimed at attaining the most favorable levels in the essential amino acids in cereal proteins such as sorghum and millet. Sorghum like other cereals is known to be deficient in lysine which creates amino acid imbalance and subsequent growth retardation. Therefore, various means have been proposed to improve the nutritional quality of dishes prepared from sorghum; these include germination and fermentation to increase the available lysine level (Ibrahim *et al*., 2005). According to FAO (1997) sorghum (*Sorghum bicolor* (L.) moench) is considered as one of the most important food crops in the world, following

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wheat, rice, maize and barely. It represents an important source of calories and protein to the vast majority of the population as well as for poultry and livestock. Sorghum was consumed by Sudanese as fermented Kisra (unleavened bread) and Asida or thick porridge (Dewit and Kessel, 1996). Sorghum can be supplemented with other ingredients, such as legumes to improve its nutritional value particularly the protein (Ibrahim *et al*., 2005). Cluster bean or cluster bean (*Cyamopsis tetrgonoloba*) belongs to family Fabaceae is bushy, drought tolerant, nitrogen fixing and protein rich summer legume. Cluster bean has acquired an economic and industrial importance after the discovery of the gummy substance (galactomannan) in the seed endosperm, which is used in food and industrial products. The present study was carried out to investigate the effect of cluster bean supplementation followed by fermentation and cooking on biochemical characteristics of sorghum cultivars flour.

MATERIALS AND METHODS

Two sorghum cultivars Wad Ahmed and Dabar were obtained from the Agricultural Research Station, Wad Medani, Sudan. Cluster bean seeds were obtained from Cluster Bean Notational Company (Khartoum). The study was conducted during the season 2005/2006.

The grains of both cultivars were cleaned manually to remove husks, damaged grains and other extraneous materials. The cleaned grains of each cultivar were milled into fine flour with a hammer mill (Gibbons Electric, Essex, UK) to pass through a 0.4 mm mesh size screen. The flour of the cultivars was supplemented with cluster bean flour in the ratio of 1:2.1, 1:2.4 for Dabar and Wad Ahmed, respectively, to raise the protein content of each cultivar to 19.86 and 19.82%, respectively. The cultivars flour with or without cluster bean was fermented according to El-Tinay *et al*. (1979) method with a minor modification. About 200 ml distilled water was added to the flour and mixed well with a glass rod. The slurry was allowed to ferment naturally at room temperature ((28±3°C). Samples were withdrawn at different periods of time (0, 8, 24 and 36 h). The pH was measured during fermentation using pH meter (PUSL Munchen 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. The fermented flour with or without cluster bean 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 and kept for further analysis.

The dry matter and crude protein (N×6.25) were determined according to AOAC (1984).

Total titratable acidity was determined according to AOAC (1984) method. About 10 g of material were weighed into 250 mL beaker and added to 150 mL of distilled water and mixed well. The mixture was filtered through Whatman No. 1 filter paper. The filtrate was then titrated against 0.1 N NaOH using 0.30 mL phenolphthalein indicator. Titratable acidity was expressed as lactic acid using the following equation:

$$\text{Acidity (\% lactic acid)} = \frac{\text{Milliliter of 0.1 N NaOH} \times 0.009 \times 100}{\text{Weight of the sample}}$$

0.009 = g lactic acid equivalent to 0.1 N sodium hydroxide.

Non-protein nitrogen was determined according to the method of Gheyasuddin (1970) using 5.0 g of the sample suspended in 2 mL H_2SO_4 diluted in 150 mL distilled water in a 200 mL volumetric flask.

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.

The proteins from the defatted flours of the samples were fractionated according to the technique of Osborne as described by Abd El-Aal *et al*. (1986) using distilled water, 1 M NaCl, 70% ethanol and 0.2% NaOH solutions for albumins, globulins, prolamins and glutelins, respectively. The nitrogen content of each fraction was determined using the micro-Kjeldahl procedure (AOAC, 1984). The residue left after extraction was also analyzed for nitrogen content. Each fraction was expressed as a percent of the total nitrogen.

The amino acid composition of the samples was determined using an amino acid analyzer Sykam System 7130 (Widner and Eggum, 1966) after hydrolyzing the samples with 6 N HCl at 110°C for 24 h. The sulphur-containing amino acids were oxidized using performic acid before the acid hydrolysis. The contents of different amino acids recovered were presented as g/100 g.

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 (1955) multiple range test with probability $p \leq 0.05$.

RESULTS AND DISCUSSION

Table 1 shows changes in pH, Titratable Acidity (TA), Crude Protein (CP), Non-Protein Nitrogen (NPN)

Table 1: Changes in pH and percent Titratable Acidity (TA), Crude Protein, (CP) Non-Protein Nitrogen (NPN) and Dry Matter (DM) during natural fermentation of sorghum cultivars

	Cultivars									
	Dabar					WadAhmed				
Fermentation time (h)	pH	TA	NPN	CP	DM	pH	TA	CP	NPN	DM
0	6.26±0.02^{a}	0.17±0.04^{i}	0.10±0.01^{g}	9.34±0.07^{h}	95.37	6.19±0.10^{e}	0.19±0.01^{d}	10.20±0.01^{h}	0.04±0.01^{g}	95.60
8	5.85±0.01^{b}	0.21±0.01^{h}	0.12±0.01^{f}	10.67±0.06^{e}	94.91	5.22±0.00^{c}	0.34±0.01^{c}	10.99±0.03^{c}	0.07±0.01^{fg}	95.07
24	4.13±0.01^{e}	0.89±0.02^{c}	0.28±0.01^{b}	11.02±0.01^{f}	92.84	4.16±0.01^{g}	0.89±0.02^{b}	10.67±0.06^{e}	0.11±0.01^{cde}	93.22
36	4.10±0.00^{e}	1.25±0.01^{a}	0.30±0.01^{a}	12.23±0.01^{a}	91.47	3.91±0.01^{j}	1.42±0.00^{a}	11.52±0.05^{a}	0.18±0.01^{a}	90.33

Values are means±SD. Means not sharing a common superscript letter(s) in a column are significantly different at $p \leq 0.05$

Table 2: Changes in protein digestibility (%) of sorghum cultivars (Dabar and WadAhmed) supplemented with cluster bean during fermentation and cooking

	IVPD							
	Dabar		Dabar+Cluster bean		WadAhmed		WadAhmed+Cluster bean	
Fermentation time (h)	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked	Uncooked	Cooked
0	10.32±0.65^{g}	9.48±0.21^{d}	18.72±0.52^{e}	14.24±0.53^{b}	09.28±0.23^{f}	8.49±0.18^{c}	17.18±0.23^{e}	13.31±0.23^{b}
8	12.84±0.68^{e}	10.91±0.22^{c}	19.63±0.54^{de}	14.98±0.31^{b}	10.73±0.27^{e}	9.64±0.31^{b}	18.56±0.13^{cd}	14.10±0.05^{ab}
24	17.10±0.72^{ab}	13.15±0.44^{a}	19.79±0.51^{cde}	16.65±0.27^{a}	13.12±0.13^{bc}	10.35±0.13^{ab}	18.59±0.66^{cd}	14.51±0.38^{a}
36	14.95±0.38^{d}	11.26±0.11^{c}	21.08±0.18^{ab}	17.07±0.26^{a}	14.68±0.64^{a}	10.76±0.42^{a}	19.34±0.42^{bc}	13.41±0.21^{b}

Values are means±SD. Means not sharing a common superscript letter(s) in a column are significantly different at $p \leq 0.05$

and Dry Matter (DM) during fermentation of sorghum cultivars flour. Fermentation of sorghum cultivars flour remarkably decreased the pH of the media with time. Fermentation up to 36 h significantly ($p \leq 0.05$) reduced the pH to 4.1 and 3.9 for Dabar and Wad Ahmed cultivars, respectively. Simultaneously with drop in pH there was a gradual increase in the TA for the two cultivars. Chavan and Kadam (1989) stated that during fermentation, pH decreases with a concomitant increase in acidity as lactic acid accumulates due to microbial activity. According to El Hidai (1978) natural fermentation of sorghum is mainly due to lactic acid by *Lactobacillus* sp., yeast and acetic acid fermentations during the later stages of fermentation. During fermentation TA significantly ($p \leq 0.05$) increased from 0.17 to 1.25% and from 0.19 to 1.42% for Dabar and Wad Ahmed, respectively. These results are in agreement with many researchers (El-Tinay *et al*., 1985; Hamad and Field, 1997; Yousif and El-Tinay, 2001). The protein content of unfermented flour of Dabar cultivar was 9.34% while that of WadAhmed cultivar was 10.20%. The protein content increased significantly ($p \leq 0.05$) from 9.34% to 12.20% and from 10.20% to 11.50% at the end of fermentation period for Dabar and Wad Ahmed cultivars, respectively. The results obtained agree with previous studies conducted by Yousif and El Tinay (2001) and Ibrahim *et al.* (2005) for sorghums. The observed increase in the protein content of treated samples was probably due to loss in dry matter content through depletion of carbohydrates (Ahmed *et al*., 1991). They elucidated that it thus may be an apparent and not real increase. However, cells of the fermented micro-organisms could have contributed to the protein content. Therefore, they suggested that fermentation of sorghum results in an observable increase in crude protein content.

For Dabar cultivar NPN increased from 0.10% at zero time to 0.30% at the end of fermentation time while for Wad Ahmed cultivar it was increased from 0.04% at zero time to 0.18% at the end of fermentation period. An increase in NPN was reported by El-Tinay *et al.* (1979). The gradual decrease in dry matter towards the end of fermentation for both cultivars is mainly due to the utilization of part of the meal nutrients by the fermenting organisms; hence the two cultivars showed a gradual loss in dry matter of about 4.4 and 5.7% for Dabar and Wad Ahmed cultivars, respectively after 36 h of fermentation.

Changes in *in vitro* Protein Digestibility (IVPD) as affected by supplementation, fermentation and cooking are shown in Table 2. After supplementation of the cultivars flour with cluster bean, the protein content significantly increased to 20.02% for both cultivars. Further increment was observed when the supplemented flour was fermented for different periods of time. Fermentation after supplementation of the two cultivars flour caused additional increase in total protein content to 22.93 and 20.79% for Dabar and Wad Ahmed cultivars, respectively. Au and Field (1981) indicated that the protein is the more limiting nutrient in most human diets than carbohydrates. Therefore, any process that appears to increase its content even at the expense of carbohydrates may be advantageous nutritionally.

Values of pepsin *in vitro* protein digestibility of naturally fermented dough of Dabar cultivar increased significantly ($p \leq 0.05$) from 10.32% at zero time to 17.4% for 24 h dough. Thereafter it decreased and reached a value of 14.95% at the end of fermentation period (36 h). For Wad Ahmed cultivar the IVPD increased significantly ($p \leq 0.05$) from 9.28% at zero time to 14.68% at the end of fermentation period (36 h). The current findings agreed

Table 3: Effect of fermentation, supplementation and cooking on protein fractions (%) of Dabar cultivar

Fermentation time (h)	Cultivar flour						
	Globulin+Albumin	Prolamin	G_1-Glutelin (%)	G_2-Glutelin	G_3-Glutelin	Insoluble protein	Protein recovered
0	13.33±0.02^{d}	28.56±0.03^{d}	25.38±0.34^{e}	3.26±0.06^{d}	24.49±0.64^{a}	1.28±0.03^{c}	97.30
8	18.30±0.01^{a}	30.62±0.03^{b}	26.66±0.23^{b}	3.82±0.08^{c}	20.02±0.36^{b}	1.69±0.02^{a}	101.11
24	14.63±0.02^{c}	29.25±0.26^{c}	27.33±0.30^{c}	5.67±0.36^{a}	20.01±0.35^{b}	1.54±0.12^{b}	98.43
36	12.99±0.15^{e}	28.93±0.64^{c}	36.60± 0.22^{a}	5.67± 0.07^{a}	20.99±1.19^{b}	1.10±0.03^{d}	106.28
Cooked flour							
0	11.04±0.11^{d}	13.01±0.15^{e}	34.37±0.15^{b}	3.02±0.09^{e}	32.36±0.11^{b}	6.16±0.02^{a}	99.96
8	13.89±0.37^{a}	14.47±0.09^{b}	34.67±0.36^{b}	2.63±0.16^{b}	32.49±0.31^{b}	4.23±0.07^{d}	102.38
24	12.72±0.31^{b}	16.53±0.29^{a}	35.33±0.08^{a}	2.12±0.04^{d}	31.15±0.01^{c}	3.40±0.08^{c}	101.25
36	11.56±0.16^{c}	16.42±0.08^{a}	34.43±0.06^{b}	2.01±0.13^{d}	34.42±0.23^{a}	4.58±0.38^{c}	103.42
Supplemented flour							
0	30.97±0.45^{bc}	14.01±0.21^{c}	6.43±0.54^{c}	24.53±0.52^{c}	22.53±0.12^{a}	1.57±0.03^{b}	100.04
8	31.61±0.85^{ab}	13.56±0.57^{c}	6.91±0.44^{c}	25.75±0.14^{b}	20.68±0.21^{b}	1.51±0.06^{b}	100.02
24	30.05±0.29^{cd}	15.28±0.38^{b}	7.62±0.43^{ab}	25.66±0.06^{b}	19.05±0.58^{c}	1.81±0.01^{a}	99.47
36	29.66±0.66^{d}	16.15±0.32^{a}	7.92±0.26^{a}	24.08±0.03^{d}	19.24±0.38^{c}	1.14±0.06^{c}	98.19
Supplemented and cooked flour							
0	20.45±0.18^{b}	11.17±0.06^{b}	18.04±0.06^{b}	18.43±0.19^{d}	30.44±0.21^{b}	4.22±0.03^{b}	102.75
8	24.44±0.20^{a}	11.24±0.11^{b}	16.16±0.02^{c}	18.46±0.12^{d}	28.23±0.07^{c}	4.55±0.07^{a}	103.08
24	19.21±0.02^{d}	10.04±0.08^{c}	18.17±0.11^{b}	19.70±0.17^{b}	32.67±0.20^{a}	3.14±0.09^{c}	102.93
36	19.74±0.07^{c}	12.50±0.12^{a}	20.65±0.18^{a}	20.47±0.13^{a}	24.45±0.07^{d}	2.36±0.10^{d}	100.17

Values are means±SD. Means not sharing a common superscript letter(s) in a column are significantly different at $p \leq 0.05$

with the previous study carried by Chavan (1988) who stated that the IVPD of sorghum increased markedly after fermentation for 24 h and also that carried by Yousif and El-Tinay (2001) who indicated that the IVPD of sorghum increased from zero time up to 28 h of fermentation. Fermented sorghum products such as Kisra i.e., fermented Sudanese unleavened bread, Abrey i.e., a fermented Sudanese flakes (Axtell *et al*., 1981) and Nasha i.e., a fermented Sudanese baby food (Graham *et al*., 1986) showed an improvement in protein digestibility over that of unfermented cooked flours. Changes in IVPD of the two cultivars as a result of cluster bean supplementation followed by fermentation are shown in Table 2. The results obtained showed a marked increase ($p \leq 0.05$) in IVPD with maximum values obtained when the supplements were fermented for 36 h for both cultivars. Ibrahim *et al*. (2005) reported that the IVPD of Dabar and Wad Ahmed cultivars improved significantly ($p \leq 0.05$) during fermentation and even after supplementation with whey protein.

Cooking of unfermented sorghum flour with or without cluster bean reduced the IVPD of both cultivars. However, during fermentation the IVPD increased with time for both supplemented flour for both cultivars even after cooking. It seems likely that supplementation followed by fermentation alleviated the effect of cooking on protein digestibility. The IVPD of Dabar supplemented with cluster bean after cooking was found to be 14.24% at zero time and it was significantly ($p \leq 0.05$) increased at the end of fermentation period to 17.07% and that of Wad Ahmed supplemented with cluster bean after cooking scored 13.31% at zero time and it slightly increased after 36 h of fermentation to 13.41%. Cooking significantly ($p \leq 0.05$) reduced the IVPD of the supplemented samples from 18.7 to 14.2% and from 17.1 to 13.3% for Dabar and Wad Ahmed, respectively. However, values obtained after supplementation still higher than those obtained before supplementation.

It was concluded that the IVPD of the cultivars and their supplements were reduced for all levels of treatments after cooking. This opposing correlation between cooking and IVPD was reported by many researchers (Rom and Shull, 1992; Oria *et al*., 1995; Hamaker *et al*., 1994). They suggested that on cooking more disulphide cross linked protein oligomers and polymers are formed. During cooking enzymatically resistant protein polymers are formed through bonding of the β and γ-kafirins and possibly other proteins which are located on the outside of the protein bodies. They concluded that the disulphide cross linked proteins thus formed would then prevent access to and restrict digestion of the more digestible and centrally located γ-kafirin within the protein body. To alleviate the effect of cooking, Arbab and El-Tinay (1997) suggested that sorghum should be cooked with reducing agents to improve the protein digestibility.

Table 3 shows variation in protein solubility fractions of cooked and uncooked flour of Dabar cultivar after fermentation and/or supplementation. The albumin+globulin fraction was 13.33% at zero time and 12.99% at the end of fermentation period. It increased significantly ($p \leq 0.05$) during the first 8 h of fermentation (18.30%) but started to decrease gradually up to the end of fermentation process. Cooking of the fermented dough significantly ($p \leq 0.05$) reduced globulin+albumin fraction. Supplementation of the flour with cluster bean greatly increased the level of globulin+albumin fraction. However, cooking of the supplemented dough reduced the level of globulin+albumin fraction but still significantly ($p \leq 0.05$) greater than the values before

supplementation during all fermentation periods. This study showed an improvement in globulin and albumin fractions for both cultivars. As reported by Wu and Wall (1980) the globulin+albumin fraction is characterized by higher levels of lysine, therefore, the nutritional value of sorghum could be modified as a result of fermentation. El-Khalifa and El-Tinay (1994) fractionated fermented sorghum proteins using the classical Mendle-Osborne procedure and they reported that fermentation of sorghum flour for 14 h acquired slight increase in albumin+globulins fraction. Similar observation was noticed by Yousif and El-Tinay (2001).

The prolamin (Kafirin) was found to be a major fraction with value of 28.56% before fermentation and 28.93% at the end of fermentation period. The amount of prolamin was fluctuated with fermentation time. Cooking of the fermented dough significantly ($p \leq 0.05$) reduced the amount of prolamin. Supplementation before and after cooking significantly ($p \leq 0.05$) reduced the amount of prolamin (Table 3). The G_1-glutelin (Cross liked-kafirin) was 25.38% at zero time and increased significantly ($p \leq 0.05$) at the end of fermentation period (36.60%). Fermentation of cooked flour significantly ($p \leq 0.05$) increased the amount of the fraction with a maximum value of 35.33% obtained after 24 h fermentation. Supplementation of the flour with cluster bean significantly ($p \leq 0.05$) decreased G_1-glutelin fraction. However, fermentation of the supplemented flour increased the fraction content but to a level less than that of untreated flour. The G_2-glutelin (glutelin-like) was 3.26% at zero time thereafter it increased gradually with the fermentation time and had a significant increment ($p \leq 0.05$) after 24 h of fermentation. Cooking of the fermented flour slightly decreased the content of G_2-glutelin. Supplementation and fermentation of the flour significantly ($p \leq 0.05$) increased the amount of the fraction with higher increment obtained after the mixture was fermented for 24 h (25.66%). Cooking of the supplemented and fermented flour significantly ($p \leq 0.05$) decreased G_2-glutelin fraction but still above the level of untreated flour. The G_3-glutelin (true-glutelin) was 24.49% at zero time and reached 20.99% at the end of fermentation period.

Supplementation had no great effect on the level of the fraction. However, cooking of the supplements significantly ($p \leq 0.05$) increased G_3-glutelin fraction. Cooking of the fermented dough before and after supplementation significantly ($p \leq 0.05$) increased the insoluble protein content. The current result agreed with the findings carried out by Fageer *et al.* (2004) and El-Khalifa *et al.* (1999). Hamaker *et al.* (1986) stated that on cooking, the kafirin proteins tend to become less soluble as a result of disulphide cross linking. Cooking significantly ($p \leq 0.05$) increased the G_1-glutelin (cross linked-kafirin) while G_2-glutelin (glutelin-like) slightly decreased. G_3-glutelin (true-glutelin) significantly ($p \leq 0.05$) increased. Hamaker *et al.* (1986) reported that on cooking, the alcohol soluble proteins are converted to higher molecular weight fractions, namely G_3 and non extractable fraction. A range of 97.30 to 103.08% was the protein recovered for all treatments. The changes in protein fractions as a result of supplementation and fermentation agrees with the previous results reported by Ibrahim *et al.* (2005) when they supplemented sorghum cultivars with whey protein.

Table 4 shows variation in protein solubility fractions of the flour of WadAhmed cultivar after fermentation and/or supplementation. The results obtained for WadAhmed regarding the classification of proteins into different fractions are similar to those obtained for Dabar cultivar.

Table 5 shows the amino acid content of fermented Dabar cultivar flour before and after supplementation. The essential amino acids; threonine, methionine, tyrosine, histidine, arginine and lysine were increased after 24 h of fermentation, while valine, cystine and isoleucine were slightly increased at the initial 8 h of fermentation and thereafter decreased towards the end of fermentation. Leucine and phenylalanine fluctuating throughout the fermentation period reaching a maximum value after 24 h of fermentation.

The amino acid content of Wad Ahmed cultivar (Table 6) indicated a progressive increase in threonine, leucine, phenylalanine, histidine and lysine at the end of fermentation while sulphur-containing amino acids (cystine and methionine) and arginine increased after 24 h of fermentation. Valine and isoleucine were fluctuating throughout the fermentation process. Tyrosine was highly increased at the initial 8 h and then decreased towards the end of fermentation. The amino acid content of Dabar (Table 5) and Wad Ahmed cultivars (Table 6) supplemented with cluster bean were greatly improved particularly, threonine, histidine, arginine, the aromatic amino acids (phenylalanine and tyrosine). Lysine which is the limiting amino acid in cereals was highly increased from 0.19 and 0.2 g/100 g protein to 3.94 g/100 g and 3.97 g/100 g for supplemented Dabar and Wad Ahmed, respectively after 36 h of fermentation. Although there was a remarkable increase in lysine content but is still slightly below the required value specified by FAO/WHO (1990).

Table 4: Effect of fermentation, supplementation and cooking on protein fractions (%) of WadAhmed cultivar

Fermentation time (h)	Cultivar flour: Globulin+Albumin	Prolamin	G_1-Glutelin (%)	G_2-Glutelin	G_3-Glutelin	Insoluble protein	Protein recovered
0	14.53±0.22^{c}	28.42±0.52^{b}	24.33±0.28^{d}	5.40±0.13^{d}	21.23±0.13^{b}	2.66±0.13^{a}	96.57
8	13.52±0.10^{d}	30.32±0.32^{a}	26.56±0.40^{c}	6.34±0.10^{b}	21.73±0.48^{b}	2.32±0.06^{b}	100.79
24	15.42±0.47^{b}	31.00±0.50^{a}	28.59±0.36^{b}	7.14±0.23^{a}	19.52±0.47^{c}	1.74±0.09^{c}	103.41
36	16.00±0.17^{a}	30.33±0.11^{a}	29.61±0.57^{a}	6.07±0.06^{c}	19.47±0.19^{c}	1.52±0.10^{d}	103.00
Cooked flour							
0	11.26±0.09^{a}	13.49±0.09^{b}	32.42±0.06^{d}	4.62±0.13^{d}	28.70±0.28^{a}	8.19±0.06^{b}	98.68
8	10.56±0.11^{c}	13.52±0.11^{b}	32.54±0.19^{d}	5.57±0.10^{a}	28.13±0.21^{b}	9.49±0.13^{a}	99.81
24	10.39±0.05^{d}	12.56±0.18^{d}	36.44±0.07^{b}	4.58±0.13^{d}	27.43±0.03^{c}	9.48±0.05^{a}	100.88
36	11.08±0.11^{b}	14.51±0.10^{a}	37.05±0.11^{a}	4.83±0.09^{c}	28.42±0.06^{b}	7.36±0.10^{c}	103.26
Supplemented flour							
0	34.73±0.93^{b}	15.16±0.05^{a}	9.32±0.22^{ab}	22.70±0.28^{a}	17.46±0.21^{b}	1.81±0.02^{a}	101.18
8	36.87±0.30^{a}	13.35±0.32^{b}	8.61±0.01^{c}	22.63±0.03^{b}	18.41±0.20^{a}	1.74±0.09^{a}	101.61
24	33.66±0.88^{b}	14.82±0.41^{a}	8.97c±0.30^{bc}	23.23±0.12^{a}	16.64±0.39^{c}	1.12±0.11^{c}	98.44
36	34.87±0.73^{b}	13.61±0.42^{b}	9.02±0.10^{b}	22.48±0.32^{b}	16.24±0.11^{c}	1.84±0.09^{a}	98.06
Supplemented and cooked flour							
0	21.26±0.09^{c}	11.45±0.09^{b}	16.17±0.09^{b}	16.31±0.13^{b}	28.44±0.21^{b}	6.34±0.03^{b}	99.97
8	25.16±0.09^{a}	10.18±0.04^{e}	15.22±0.05^{e}	15.22±0.05^{e}	26.15±0.03^{e}	7.26±0.02^{a}	99.62
24	22.04±0.09^{b}	12.11±0.04^{a}	17.27±0.05^{c}	15.19±0.04^{d}	30.81±0.12^{a}	5.08±0.04^{c}	102.50
36	20.12±0.04^{d}	10.58±0.12^{c}	19.30±0.14^{b}	17.17±0.09^{a}	29.35±0.10^{c}	5.39±0.02^{d}	101.91

Values are means±SD. Means not sharing a common superscript letter(s) in a column are significantly different at $p \leq 0.05$

Table 5: Amino acid content (g/100 g protein) of Dabar cultivar flour with or without supplementation during fermentation

Fermentation time (h)	Cultivar flour: Asp	Thr	Ser	Glu	Gly	Ala	Cys	Val	Met	Iso	Leu	Tyr	Phe	His	Lys	Arg
0	5.56	1.07	1.48	2.82	0.39	21.73	2.19	12.21	1.50	9.30	18.67	0.79	3.73	0.51	0.19	4.43
8	4.40	0.69	1.19	2.31	0.47	21.73	2.51	12.77	1.42	9.41	16.76	1.20	3.46	0.33	0.21	4.75
24	5.23	1.14	1.45	8.03	0.26	19.00	1.85	10.78	1.86	8.37	19.22	1.51	4.78	0.71	0.42	4.63
36	6.49	1.54	1.93	6.43	0.45	18.75	1.78	10.29	1.99	7.68	17.51	2.17	4.66	2.20	0.54	4.97
Supplemented flour																
0	10.20	3.30	3.96	19.34	4.94	7.23	0.67	5.58	1.07	4.68	9.64	3.15	5.09	3.18	3.77	10.66
8	10.12	3.33	4.01	19.57	4.85	7.57	0.67	5.80	1.18	4.71	10.09	3.69	4.91	3.36	3.28	9.89
24	10.85	3.14	4.33	19.63	4.14	11.98	1.29	7.83	1.75	6.04	12.49	3.51	4.64	3.44	3.53	13.52
36	10.09	3.15	3.84	19.23	4.88	7.58	1.95	5.48	1.12	4.51	10.48	3.90	4.81	3.80	3.94	13.71

Values are means of duplicate determinations

Table 6: Amino acid content (m/100 g protein) of WadAhmed cultivar flour with or without supplementation during fermentation

Fermentation time (h)	Cultivar flour: Asp	Thr	Ser	Glu	Gly	Ala	Cys	Val	Met	Iso	Leu	Tyr	Phe	His	Lys	Arg
0	4.62	0.72	1.35	2.57	0.54	20.74	2.66	12.72	1.46	9.09	15.64	1.61	3.14	1.31	0.20	5.19
8	5.22	0.92	1.42	4.07	0.45	20.34	2.22	10.70	1.47	7.91	16.42	2.12	4.66	2.52	0.26	4.12
24	4.32	0.60	1.07	3.28	0.48	20.46	2.79	12.52	1.49	8.75	16.17	1.46	3.65	2.44	0.23	4.84
36	6.18	1.28	1.58	4.33	0.37	20.00	2.23	11.40	1.17	8.67	17.97	1.35	4.98	2.86	0.46	4.77
Supplemented flour																
0	9.54	2.90	3.10	10.48	2.89	11.28	0.76	7.92	1.76	5.96	11.66	2.88	5.24	3.87	2.91	13.03
8	9.72	2.90	3.21	11.22	2.93	10.91	0.53	7.28	1.27	5.38	11.40	2.47	4.88	3.58	2.66	11.99
24	9.79	3.24	3.74	18.95	4.69	9.90	0.61	6.74	1.04	4.66	10.27	2.93	5.07	3.59	3.44	10.94
36	9.89	3.44	3.70	18.96	4.70	9.91	0.55	6.64	1.18	4.63	10.17	2.25	5.00	3.93	3.97	10.81

Values are means of duplicate determinations

In conclusion, sulphur-containing amino acids (cystine and methionine) decreased upon supplementation with cluster bean this mainly because the amino acid profile of the legume protein isolates is characterized by high lysine content and low sulphur-containing amino acids (Pusztia *et al*., 1979). Moreover, the content of valine, isoleucine, leucine were noticeably diminished.

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