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Effect of Whey Protein Supplementation and/or Fermentation on Biochemical and Sensory Characteristics of Sorghum Flour

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Abstract: Changes in pH, titrable acidity, protein, non-protein nitrogen, total soluble solids, protein fractions and *in vitro* protein digestibility were investigated during fermentation and/or supplementation of sorghum flour with whey protein. The pH of the fermenting material was decreased sharply with a concomitant increase in the titratable acidity. The total soluble solids increased with progressive fermentation time. The crude protein and. non-protein nitrogen were increased with fermentation time. The albumin plus globulin fraction was increased significantly (P = 0.05) during the first 8 h of fermentation. Other fractions contents were observed to fluctuate during fermentation time. Supplementation of the cultivar flour with whey protein greatly increased the protein content as well as albumin plus globulin fraction while other fractions were significantly decreased. The *in vitro* protein digestibility was significantly (P = 0.05) improved during fermentation time and even after supplementation. Sensory evaluation of local processing methods of sorghum products (Kisra, Asida and Nasha) before and after supplementation showed no difference between the supplemented samples and the control ones as judged by trained panelists.

Key words: Sorghum, Fermentation, Whey protein, Supplementation, Protein digestibility, Sensory evaluation

Introduction

With continuing increase in the world population and pressure on land use, man has been greatly concerned not only about the quantity of food, but also about the quality of it. Cereals are the most important crops for thousands of years. Therefore, successful production, storage and use of it will contribute in the development of modern civilization. According to recent epidemiological reports, cereals and cereal products may exert beneficial effects by reducing the risk of cancer and it was observed that high intake of whole grain associated with a reducing risk of cancer, particularly those of the alimentary tract, such as colorectal cancer and gastric cancer (Andlauer and Fürst, 1999). Sorghum (*Sorghum bicolor* (L.) Moench.) is considered as one of the 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 one of the oldest cultivated crops and has been used for centuries in the regions of its origin (nile valley and central India). 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 proteins are classified according to solubility as albumins, globulins, prolamins and glutelins. The prolamin fraction of sorghum (kaffirin) is further divided into α-, β- and γ- kaffirins (Skoch, Deyoe, Shoup, Bathrust and Liang, 1970). The nutritional quality of sorghum was poor due to deficiency in lysine and low quantities of threonine and tryptophan (Au and Fields, 1981) It was observed that sorghum grains had low starch and protein digestibilities due to the presence of certain antinutritional factors which contributing also to poor sensory characteristics of processed sorghum grains. Recently large efforts have been directed to improve the nutritional quality of cereal grains particularly to improve the level of essential amino acids as well as protein digestibility. Various methods have been proposed to improve lysine content in dishes prepared from germinated and fermented sorghum (Eggum, Monowar, Bochknusden, Munck and Axtel, 1983). Fortification with synthetic lysine is very effective, but rather impracticable considering the condition under which sorghum is processed. Sudanese people consume sorghum as fermented Kisra, Asida or Nasha and providing about 97% of the protein and 75% of the calories in the diet of the people residing in Central and Western Sudan. Whey protein was found to have better functional properties, such as solubility (Patel and Kilara, 1990; Jayprakasha, 1992 and Dewit and Kessel, 1996) over a wide range of pH. β-lactalbumin and α-lactalbumin are the major proteins of whey constituting 50 to 55% as β-lactalbumin and 20 to 25% α-lactalbumin. Whey protein is a key ingredient in many infant formulas because á-lactalbumin is the major protein in human breast milk. The albumin plus globulin fraction was reportedly characterized by higher level of lysine (Wu and Wall, 1980). Whey proteins have a potential

to improve the quality of food products (Kester and Richardson,1984; Kim, Morr and Surak, 1989; Morr and Ha, 1993; Jayaprakasha, Tirumalesha and Ramachandra, 1997). Also it was reported that whey protein concentrate finds numerous applications in food and dairy industries due to their excellent nutritional and functional properties (Kinsella, 1987; Huffman, 1996 and Mann, 1998). Whey protein is a high quality protein and a rich source of essential amino acids. Therefore, in this study we would like to investigate the effect of supplementation with whey protein and/or fermentation on protein fractions and digestibility of sorghum flour.

Materials and Methods

Materials: Unsalted cow milk whey was obtained from the Dairy-Land Factory Khartoum North, Sudan. Sorghum cultivar (Dabar) of low tannin content was obtained from local market at Khartoum North. The seeds were cleaned and freed from foreign materials and broken seeds. The clean seeds were milled into flour to pass a 0.4 mm screen. The flour was stored in polyethylene bags at 4°C for further use. Unless otherwise stated all reagents used in this study are reagent grade.

Whey Protein Preparation: Whey protein was obtained by precipitation with sodium hexa-meta phosphate (SHMP), 175 mg of SHMP were added to every 100 ml unsalted cow milk whey protein at pH 3.0. The precipitate was then filtered using Whatman No.1 filter paper and the residue was washed using $CaCO_3$ solution followed by distilled water and then dried at room temperature.

Natural Fermentation: Natural fermentation was carried out by mixing sorghum flour with distilled water (1:2 w/v). About 250 g of sorghum flour were mixed with 500 ml distilled water in 600 ml beaker and then incubated in an incubator (Gallenkamp, England) at 37 °C for periods of 0, 4, 8, 12, 16, 20, 24, 28, 32, 36 h. Thereafter, the samples were mixed with a glass rod and transferred to three aluminum dishes (30 cm diameter each) and dried in a hot oven (Heraus UT 5042, Germany) at 70 °C for 3-4 h. Dried samples were then ground to pass a 0.4 mm screen and stored in polyethylene bags at 4°C for further analysis.

Whey Protein Supplementation: About 2.60 and 3.25 % of whey protein on a dry matter base were added to sorghum flour to increase its protein content by 5 and 10 %, respectively. The mixtures were fermented as described above.

Processing of Sorghum Flour Before and After Supplementation (Kisra, Asida and Nasha): Kisra bread was prepared from sorghum flour before and after supplementation. The fermented dough known as "Ajean" was prepared traditionally by mixing samples with water in a round earth ware container called "Khumara". A small amount of the previously fermented dough was then added to the mixture, which acts as a starter. After thorough mixing it was baked on a hot steel plate in a process known as "Aowasa" which is a unique Sudanese art in which a small amount of the fermented dough is spread over a hot plate forming a very thin sheet within 1-2 seconds and then taken out and considered ready for eating. Asida and Nasha preparation are differ from that of kisra, they are prepared as thick and thin paste, respectively.

Determination of pH and Titratable Acidity: The pH of the fermenting dough was monitered initially and every 4 h for 36 h by using a glass electrode pH meter (PUSL, MUNCHENZ, KARL KOLB, Germany). Titratable acidity, expressed as lactic acid was determined by titration with 0.1N NaOH to pH 8.1 (Zamora and Fields, 1979).

Determination of Non-Protein Nitrogen: Non-protein nitrogen was determined according to the method of Gheyasuddin (1970). About 5 gm of the sample were suspended in 150 ml distilled water in a 200 ml volumetric flask and 2.0 ml H_2SO_4 were added to the flask followed by 2.0 ml of 12% sodium tungstate solution. The volume was made up to 200 ml with distilled water and the mixture was shaken well and allowed to stand for 2-3 h. The extract was filtered through Whatman filter paper No.1 and aliquot was taken for nitrogen determination by the standard Kjeldhl method (AOAC, 1975).

Determination of Total Soluble Solids: Total soluble solids were determined at 20°C according to Joslyn (1970) method using an Abbe refractometer (Bellingham and Stanely LTD, London).

Protein Fractionation: Nitrogen of defatted sample was extracted stepwise by series of solvents according to Landry and Moureaux technique (1970). To obtain salt soluble globulin, 0.5 M NaCl was added to the sample powder and the mixture was stirred three times, 60, 30 and 30 min at 4°C. The residue was extracted with the same volume of distilled water twice for 15 min at 4°C to obtain water-soluble albumin. Thereafter, the residue was stirred with 60% ethanol twice for 30 min at 20°C and then at 60°C for 30 min, followed by extraction with 55% isopropanol

(Pr-OH) at 20°C to obtain alcohol soluble prolamin. Then the residue was extracted with 60% ethanol plus 0.6% 2-mercaptoethanol (2-ME) and stirred twice for 30 min (20°C) then extracted with 55% Pr-OH containing 2.ME (0.6%) at 20°C twice for 30 min to obtain G_1-glutelin while G_2- and G_3-glutelin were obtained after treatment with 0.0125 M borate buffer (pH 10), 0.6% 2.ME and 0.5 M NaCl and with 0.0125 M borate buffer (pH 10), 0.6% 2.ME and 0.5M sodium dodecyl sulphate (SDS), respectively.The solid material remaining at the end of the extraction was isolated from extractants by centrifugation (BTL Bench centrifuge, England) at 30,000xg for 15 min. Nitrogen content of each fraction was determined by the micro-Kjeldahl method (AOAC, 1975).

Determination of in Vitro Protein Digestibility (IVPD): IVPD of the protein was curried out according to Saunders, Connor, Booth, Bickoff and Kohler (1973) method. About 200 mg of sorghum samples were placed into a 50 ml centrifuge tube, 15 ml of 0.1M HCl containing 1.5 mg pepsin were added, and the tube was incubated at 37 °C for 3 h. The suspension was then neutralized with 0.5 M NaOH (calculated 3.3 ml), then treated with 4 mg of pancreatin in 7.5 ml of 0.2 M phosphate buffer (pH 8.0) containing 0.005 M sodium azide, the mixture was then gently shaken and incubated at 37°C for 24 h. After incubation the sample was treated with 10 ml, 10% trichloroacetic acid, and centrifuged at 50,000xg for 20 min at room temperature. Nitrogen in the supernatant was estimated using micro-Kjeldhl method (AOAC, 1975). IVPD was calculated using the formula

$$\text{IVPD \%} = \frac{\text{N in supernatant- enzyme N}}{\text{N in sample}} \times 100$$

Sensory Evaluations: The sensory tests were conducted using conventional profiling of a trained Panel . Ten judges were selected who had successfully passed standardized tests for olfactory, taste sensibility as well as verbal abilities and creativity. The panelists were given hedonic questionnaire to test odor, taste and general acceptability of coded samples of Kisra, Asida and Nasha made from fermented Dabar as a control, and after supplementation with 5 % and 10 % whey protein. They were scored on a scale of 1–5 (1 = poor, 2 = fair, 3 = good, 4 = very good and 5 = excellent). The acceptability was expressed as percentage as follows:

$$\text{Acceptability} = \frac{\text{Number of panelists in a score}}{\text{Total number of panelists}} \times 100$$

Statistical Analyses: Each sample was analyzed in triplicate and the figures were then averaged. Data were compared using the analysis of variance (ANOVA) (Snedecor and Cochran, 1987) and by the Duncan's multiple range tests with a probability $p \leq 0.05$ (Duncan, 1955).

Results and Discussion

Changes in pH, Titratable Acidity (TA), Total Soluble Solids (TSS), Protein Content and Non-Protein Nitrogen During Natural Fermentation of Sorghum Cultivar Flour: Table 1 shows changes in pH, titratable acidity (TA), total soluble solids (TSS), protein content and non-protein nitrogen (NPN) during natural fermentation of sorghum cultivar (Dabar) flour. The pH of the fermented dough dropped from 6.2 at zero time to 4.13 at the end of the fermentation period. Concomitant with the drop in pH there was a rise in TA throughout the fermentation process. The TA increased from 13.3 to 119.6 mg/100g. This result was in agreement with the work conducted by Au and Feilds (1981); El Tinay, ElMahadi and ELSouki(1985); Chavan, Chavan and Kadam (1988); Hamad, Bocker, Vogel and Hammes (1997) and Youssif and El Tinay (2001). According to Mohammed, Steenson and Kirleis (1991), natural sorghum fermentation is mainly lactic acid by *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 of the fermented dough ranged from 3.2% at zero time to 4.5% at the end of the fermentation period. The general pattern showed an initial increase in soluble solids at the commencement of fermentation followed by a decrease towards the end of fermentation. The protein content of the fermented dough ranged from 10.9% at zero time to 12.0% at the end of the fermentation period. It fluctuated during the initial 24 h. However, after 24 h the protein content started to increase. A similar trend of protein content during fermentation was reported by Youssif and El Tinay (2001) while non-protein nitrogen as shown in Table 1 was ranged from 0.09% to 0.11%, with no significant changes during the whole fermentation period.

Changes in Protein Fractions During Natural Fermentation of Sorghum Flour: Table 2 Shows changes in protein fractions percent during natural fermentation of sorghum flour. The albumin plus globulin fraction was observed to be 15.2% at zero time and 13.5% at the end of the fermentation period. It increased significantly ($p = 0.05$) during the first 8 h and thereafter started to decrease with a slight increase at the end of fermentation period. The

albumin plus globulin fraction was characterized by high level of lysine (Wu and Wall, 1980). Thus, the nutritional value of sorghum flour would be expected to increase after fermentation. The prolamin fraction (Kaffirin) was the major fraction; it was 30.9% at zero time and 30.5% at the end of fermentation period. It decreased significantly ($p = 0.05$) during the initial 8 h but increased significantly ($p = 0.05$), reaching its maximum value (40.5%) at 24 h, thereafter it started to decrease. The G_1-glutelin (Cross linked Kaffirin) was 27.8% at zero time and 27.1% at the end of fermentation period; its content fluctuated during fermentation time. The G_2-glutelin (Glutelin-like) was 3.4% at zero time and 2.4% at the end of fermentation; also its content fluctuated during fermentation time. The G_3-glutelin (True-glutelin) content was fluctuated during fermentation and was found to be 21.6% at zero time and 24.43% at the end of the fermentation. Non-extractable or insoluble protein was fluctuated during fermentation and was found to be 1.1% at zero time and 1.6% at the end of fermentation. El Khalifa and El Tinay (1994) fractionated fermented sorghum proteins using the classical Mendel-Osborne procedure and they found that, 14 h fermented dough had lower level of prolamin fraction and a slightly increased content of albumin plus globulin fraction.

Changes in Protein Fractions During Fermentation of Sorghum Flour Supplemented with 5% Whey Protein: Table 3 shows changes in protein fractions during fermentation of sorghum flour supplemented with 5% whey protein. The protein content was increased by 42.2% upon whey protein supplementation compared to the flour and fermented dough. The protein content of supplemented dough fluctuated during the initial 24 h and thereafter started to increase. The globulin plus albumin fraction was increased by 130% after supplementation compared to the flour and fermented dough.

Table 1. Changes in pH, titratable acidity (TA), total soluble solids (TSS), crude protein and non-protein nitrogen (NPN) during natural fermentation of a sorghum cultivar (Dabar).

Fermentation period (h)	pH	TA (mg/100g)	TSS (%)	Protein (%)	NPN (%)
0	6.20 (± 0.00)^{a}	13.33 (± 0.12)^{j}	3.20 (± 0.00)^{i}	10.87 (± 0.06)^{cde}	0.09 (± 0.01)^{b}
4	6.20 (± 0.00)^{a}	16.23 (± 0.25)^{i}	3.77 (± 0.06)^{h}	11.01 (± 0.10)^{cd}	0.10 (± 0.01)^{ab}
8	6.00 (± 0.00)^{ab}	30.27 (± 0.12)^{h}	4.10 (± 0.00)^{g}	10.28 (± 0.05)^{e}	0.09 (± 0.00)^{b}
12	5.63 (± 0.20)^{abc}	34.27 (± 0.38)^{g}	4.30 (± 0.00)^{f}	10.26 (± 0.29)^{e}	0.09 (±0.01)^{b}
16	4.80 (± 2.31)^{bcd}	40.30 (± 0.17)^{f}	4.87 (± 0.06)^{d}	10.63 (± 0.08)^{de}	0.10 (± 0.01)^{ab}
20	4.3 (± 0.06)^{cd}	51.03 (± 0.06)^{e}	5.10 (± 0.00)^{c}	11.46 (± 0.03)^{bc}	0.10 (± 0.01)^{ab}
24	4.3 (± 0.06)^{cd}	80.33 (± 0.15)^{d}	5.17 (± 0.06)^{b}	10.60 (± 0.03)^{de}	0.10 (± 0.01)^{ab}
28	4.26 (± 0.00)^{cd}	88.73 (± 0.21)^{c}	6.00 (± 0.00)^{a}	11.00 (± 0.08)^{cd}	0.11 (± 0.01)^{a}
32	4.16 (± 0.00)^{d}	115.03 (± 0.06)^{b}	4.87 (± 0.06)^{d}	11.66 (± 0.03)^{ab}	0.10 (± 0.01)^{ab}
36	4.13 (± 0.06)^{d}	119.57 (± 0.23)^{a}	4.50 (± 0.00)^{e}	12.00 (± 0.06)^{a}	0.11 (± 0.01)^{a}

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

Table 2. Changes in protein fractions during fermentation of a sorghum cultivar (Dabar).

Fermentation period (h)	Protein%	(Albumin + globulin) %	Prolamin%	G_1-glutelin%	G_2-glutelin%	G_3-glutelin%	Insoluble protein%	protien Recovery %
0	10.87 (± 0.06)^{cde}	15.20 (± 1.74)^{ab}	30.87 (± 3.58)^{bc}	27.80 (±2.46)^{ab}	3.43 (± 0.49)^{cd}	21.57 (± 3.55)^{cd}	1.13 (± 0.15)^{bc}	100
4	11.01 (± 0.10)^{cd}	17.63 (± 2.31)^{a}	27.07 (± 1.01)^{c}	20.40 (± 0.52)^{d}	3.00 (± 0.50)^{de}	29.97 (± 1.70)^{a}	1.53 (± 0.06)^{a}	99.6
8	10.28 (± 0.05)^{e}	18.03 (± 1.33)^{a}	26.90 (± 1.49)^{c}	29.73 (± 0.68)^{a}	2.23 (± 0.06)^{e}	22.60 (± 0.50)^{bc}	0.90 (± 0.10)^{a}	100.39
12	10.26 (± 0.29)^{e}	15.57 (± 2.11)^{ab}	31.50 (± 0.79)^{bc}	26.93 (± 0.06)^{abc}	3.03 (± 0.90)^{de}	21.77 (± 0.58)^{cd}	1.20 (± 0.00)^{b}	100
16	10.63 (± 0.08)^{de}	11.67 (± 0.57)^{b}	35.37 (± 5.77)^{ab}	24.73 (± 0.25)^{bc}	4.37(±0.12)^{ab}	19.43 (± 0.58)^{de}	1.10 (± 0.10)^{bcd}	96.67
20	11.46 (± 0.03)^{bc}	11.67 (± 0.57)^{b}	38.57 (± 0.12)^{a}	22.83 (± 6.79)^{cd}	4.40 (± 0.17)^{ab}	18.80 (± 0.52)^{e}	0.93 (± 0.06)^{de}	97.2
24	10.60 (± 0.03)^{de}	10.33 (± 4.66)^{b}	40.47 (± 5.22)^{a}	28.13 (± 0.12)^{ab}	4.13 (± 0.75)^{bc}	15.93(±0.29)^{f}	1.00 (± 0.10)^{cde}	99.99
28	11.00 (± 0.08)^{cd}	11.37 (± 0.45)^{b}	38.50 (± 0.00)^{a}	28.13 (± 0.12)^{ab}	2.70(± 0.36)^{de}	16.17 (± 0.29)^{f}	1.03(± 0.12)^{bcde}	97.9
32	11.66 (± 0.03)^{ab}	12.07 (± 0.93)^{b}	32.53 (± 0.46)^{b}	28.07 (± 0.23)^{ab}	5.13 (± 0.12)^{a}	22.93±(0.29)^{bc}	0.93 (± 0.06)^{de}	101.66
36	12.00 (± 0.06)^{a}	13.53 (± 0.86)^{ab}	30.50 (± 0.00)^{bc}	27.10 (± 0.00)^{ab}	2.40 (± 0.52)^{e}	24.43 (± 0.58)^{b}	1.60 (± 0.10)^{a}	99.56

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

Results indicated that, supplementation with whey protein significantly increased albumin and globulin content which indicated that lysine level will significantly increased (Wu and Wall, 1980). The prolamin fraction, which was the major fraction in the flour and fermented dough, decreased by 32%. The G_1-glutelin (cross linked kaffirin) decreased by 30% upon whey protein supplementation compared the flour and fermented dough. Its content fluctuated during fermentation of supplemented dough. The G_2-glutelin (glutelin-like) decreased by 4.5% upon supplementation with whey protein compared to flour and fermented dough.

Table 3. Changes in protein fractions during fermentation of sorghum cultivar (Dabar) flour supplemented with 5%whey protein.

Fermentation period (h)	Protein%	((Albumin + globulin) %	Prolamin%	G_1-glutelin%	G_2-glutelin%	G_3-glutelin%	Insoluble protein%	Protein recovery %
0	15.52 (± 0.06)^{cd}	40.85 (± 1.14)^{ab}	20.95 (± 2.35)^{b}	18.93 (± 1.62)^{ab}	2.92 (± 0.32)^{cd}	14.84(±2.33)^{cd}	1.41(± 0.10)^{bc}	99.09
4	15.66 (± 0.10)^{cd}	41.36 (± 3.00)^{ab}	18.45 (± 0.66)^{c}	14.07 (± 0.35)^{d}	2.63 (± 0.33)^{de}	20.35(±1.12)a	1.67 (± 0.04)^{a}	98.53
8	14.93 (± 0.05)^{a}	41.06 (± 1.61)^{ab}	18.34 (± 0.98)^{c}	20.20 (± 0.45)^{a}	2.13 (± 0.04)^{e}	15.51(±0.33)bc	1.26(±0.07)^{a}	98.5
12	14.91 (± 0.29)^{a}	42.85 (± 2.24)^{a}	21.36 (± 0.52)^{b}	18.36 (± 0.04)^{abc}	2.66 (± 0.59)^{de}	14.97(±0.38)^{cd}	1.45 (± 0.00)^{b}	101.65
16	15.28 (± 0.08)^{de}	40.37 (± 4.18)^{ab}	26.09 (± 0.00)^{a}	16.91 (± 0.17)^{bc}	3.53 (± 0.08)^{ab}	13.43(±0.38)^{de}	1.39(± 0.07)^{cd}	101.72
20	16.11 (± 0.03)^{bc}	38.83 (± 0.38)^{b}	26.00 (± 0.08)^{a}	15.67 (± 4.46)^{cd}	3.55 (± 0.12)^{ab}	13.01(±0.34)^{a}	1.28 (± 0.03)^{de}	98.34
24	15.25 (± 0.03)^{de}	37.65 (± 3.06)^{b}	27.25 (± 3.43)^{a}	19.15 (± 0.08)^{ab}	3.38 (± 0.50)^{bc}	11.13(±0.19)^{f}	1.32 (± 0.07)^{de}	99.91
28	15.65 (± 0.08)^{cd}	38.34 (± 0.30)^{b}	25.96 (± 0.00)^{a}	19.10 (± 0.15)^{ab}	2.44 (± 0.23)^{de}	11.29(±0.19)^{f}	1.35 (± 0.08)^{de}	98.48
32	16.31 (± 0.03)^{ab}	38.34 (± 0.30)^{ab}	22.04 (± 0.31)^{b}	18.47 (± 0.00)^{abc}	4.04 (± 0.08)^{a}	15.73(±0.19)^{bc}	1.28(± 0.03)^{de}	99.9
36	16.65 (± 0.06)^{a}	39.76 (± 0.56)^{ab}	20.71 (± 0.00)^{a}	19.72 (± 0.57)^{ab}	2.24 (± 0.34)^{a}	16.72(±0.38)^{b}	1.72 (± 0.07)^{a}	100.87

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

Table 4. Changes in protein fractions during fermentation of sorghum cultivar (Dabar) flour supplemented with 10 % whey protein.

Fermentation period (h)	Protein %	(Albumin + globulin) %	Prolamin %	G_1-glutelin %	G_2-glutelin %	G_3-glutelin %	Insoluble Protein%	Protein Recovery%
0	20.57 (± 0.12)^{cde}	50.67 (± 0.92)^{ab}	17.28 (± 2.04)^{b}	15.58 (± 1.30)^{a}	2.77 (± 0.26)^{bcd}	12.30 (± 1.87)^{cd}	1.56 (± 0.08)^{ab}	100.16
4	20.71 (± 0.12)^{cd}	51.95 (± 1.21)^{a}	15.19 (± 0.53)^{c}	11.69 (± 0.28)^{c}	2.54 (± 0.26)^{bcd}	16.70 (± 0.92)^{a}	1.77 (± 0.03)	99.84
8	19.98 (± 0.11)^{a}	52.16 (± 0.70)^{a}	15.10 (± 0.78)^{c}	16.29 (± 0.19)^{a}	2.14 (± 0.03)^{d}	12.85 (± 0.27)^{bc}	1.44 (± 0.05)^{a}	99.98
12	19.96 (± 0.06)^{a}	50.86 (± 1.11)^{ab}	17.53 (± 0.42)^{b}	15.13 (± 0.03)^{a}	2.56 (± 0.47)^{bcd}	12.41 (± 0.30)^{c}	1.60 (± 0.00)^{b}	100.09
16	20.33 (± 0.07)^{de}	50.29 (± 3.34)^{ab}	21.25 (± 0.10)^{a}	13.97 (± 0.14)^{ab}	2.60 (± 0.64)^{bcd}	11.18 (± 0.30)^{de}	1.64(± 0.06)^{bcd}	100.83
20	21.16 (± 0.03)^{bc}	48.82 (± 0.30)^{b}	21.24 (± 0.06)^{a}	12.97 (± 3.57)^{bc}	2.94 (± 0.54)^{bc}	10.85 (± 0.28)^{a}	1.46 (± 0.03)^{de}	98.28
24	20.3 (± 0.03)^{de}	48.12 (± 2.45)^{b}	22.24 (± 2.74)^{a}	15.75 (± 0.06)^{a}	3.14 (± 0.39)^{a}	9.34 (± 0.15)^{f}	1.49 (± 0.05)^{cde}	100.08
28	20.7 (± 0.03)^{cd}	48.66 (± 0.24)^{b}	21.20 (± 0.00)^{a}	15.72 (± 0.12)^{a}	2.38 (± 0.18)^{cd}	9.47 (± 0.15)^{f}	1.51 (± 0.06)^{cde}	98.94
32	21.36 (± 0.08)^{ab}	49.02 (± 0.49)^{b}	18.07 (± 0.24)^{b}	15.21 (± 0.00)^{a}	3.66 (± 0.06)^{a}	13.02 (± 0.15)^{bc}	1.46(± 0.03)^{de}	100.44
36	21.7 (± 0.06)^{a}	49.80 (± 0.45)^{a}	17.00 (± 0.00)^{bc}	16.21 (± 0.46)^{a}	2.23 (± 0.27)^{d}	13.81 (± 0.31)^{b}	1.81 ±(± 0.06)^{a}	100.16

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

Table 5. Changes in *in vitro* protein digestibility (IVPD) during fermentation of sorghum cultivar (Dabar) supplemented with 5 % or 10 % whey protein.

Fermentation period (h)	IVPD%		
	Control	5 % Whey protein	10 % whey protein
0	52.97 (± 3.00)^{f}	58.07 (± 2.04)^{g}	63.83 (± 0.85)^{f}
4	57.50 (± 3.92)^{ef}	63.33 (± 3.49)^{ef}	64.07 (± 2.57)^{f}
8	59.60 (± 0.26)^{de}	65.67 (± 0.12)^{de}	67.00 (± 0.75)^{e}
12	63.60 (± 3.03)^{cd}	69.97 (± 3.09)^{cd}	72.57 (± 2.48)^{cd}
16	63.57 (± 1.62)^{cd}	69.67 (± 1.53)^{cd}	71.93 (± 1.00)^{cd}
20	66.63 (± 0.42)^{bc}	72.87 (± 0.60)^{bc}	74.73 (± 1.02)^{bc}
24	69.43 (± 1.29)^{b}	75.20 (± 1.54)^{b}	76.70 (± 0.44)^{b}
28	75.23 (± 5.16)^{a}	82.43 (± 5.83)^{a}	80.10 (± 2.13)^{a}
32	65.23 (± 1.89)^{bcd}	73.03 (± 1.10)^{bc}	71.40 (± 1.99)^{d}
36	55.57 (± 5.05)^{ef}	60.30 (± 2.39)^{fg}	61.47 (± 2.02)^{f}

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

The G_3-glutelin (true-glutelin) decreased by 31.4% upon supplementation with whey protein compared to other fractions. The insoluble protein increased by 40% upon supplementation. Whey protein was found to have better functional properties, such as solubility (Patel and Kilara ,1990; Dewit and Kessel, 1996) over a wide range of pH. â-lactalbumin and á-lactalbumin are the major proteins of whey constituting about 50 to 55% â-lactalbumin and 20 to 25% á-lactalbumin. Whey protein is a key ingredient in many infant formulas because á-lactalbumin is the major protein in human breast milk. The albumin plus globulin fraction was reportedly characterized by higher level of lysine (Wu and Wall 1980). Kester and Richardson (1984); Kim, Morr and Surak (1989); Morr and Ha (1993); Jayaprakasha, Tirumalesha and Ramachandra (1997), reported that whey proteins have a potential to improve the quality of food products. Kinsella (1987); Huffman (1996) and Mann (1998) reported that whey protein concentrate finds numerous applications in food and dairy industries due to their excellent nutritional and functional properties. Whey protein is a high quality protein and a rich source of essential amino acids. Thus the nutritional value of sorghum would be expected to increase as a result of supplementation with whey protein.

Table 6: Effect of whey protien supplementation on sensory quality of sorgum (dabar) flour after local processings

Whey protien%	Taste acceptability		Odor acceptability		General acceptability	
	Score	Percent	Score	Percent	Score	Percent
Kisra processed dough:						
0	3	20	3	20	3	20
	4	50	4	50	4	50
	5	30	5	30	5	30
5	3	20	3	10	3	20
	4	50	4	50	4	50
	5	30	5	40	5	30
10	3	10	3	10	3	10
	4	50	4	50	4	50
	5	40	5	40	5	40
Asida processed dough:						
0	3	20	3	10	3	30
	4	50	4	50	4	50
	5	30	5	40	5	20
5	3	10	3	50	3	10
	4	50	4	60	4	50
	5	40	5	40	5	40
10	3	10	3	10	3	20
	4	50	4	50	4	50
	5	40	5	40	5	30
Nasha processed dough:						
0	3	30	3	20	3	20
	4	50	4	60	4	50
	5	20	5	20	5	30
5	3	10	3	20	3	20
	4	60	4	50	4	60
	5	30	5	30	5	20
10	3	10	3	10	3	20
	4	50	4	50	4	50
	5	40	5	40	5	30

Changes in Protein Fractions During Fermentation of Sorghum Supplemented with 10% Whey Protein: Table 4 shows changes in protein fractions during fermentation of sorghum supplemented with 10% whey protein. The protein content was increased by 94% after supplementation compared to the fermented flour and that supplemented with 5% whey protein. The protein content of supplemented dough fluctuated during the initial 24 h of fermentation and thereafter it started to increase. The albumin plus globulin fraction was increased by 188% upon 10% whey protein supplementation. The prolamin fraction which was the major fraction in the control samples, its content was decreased by 43.9% upon whey protein supplementation. The G_1-glutelin (cross linked kaffirin) was decreased after supplementation and was observed to fluctuate during fermentation of supplemented dough. The G_2-glutelin (glutelin-like) was decreased by 4.6% after supplementation and was fluctuated during fermentation of the supplemented dough. The G_3-glutelin (true-glutelin) was decreased by 42.9% after supplementation. The insoluble protein was increased by 55.6% after supplementation and was fluctuated during fermentation of the supplemented dough. Compared to sorghum flour supplemented with 5% whey protein, supplementation with 10% whey protein greatly increased the amount of albumin plus globulin fraction and greatly reduced other fractions. Increment in albumin plus globulin fraction will increase the amount of lysine while reduction in other fractions may be attributed to the difference in weight before and after supplementation.

Changes in In-Vitro Protein Digestibility (IVPD) During Natural Fermentation of Sorghum Flour Supplemented with 5 or 10% Whey Protein: Table 5 shows Changes in *in vitro* protein digestibility (IVPD) during natural fermentation of sorghum cultivar supplemented with 5 or 10% whey protein. IVPD of naturally fermented dough increased from 52.97% at zero time to 75.2% when fermented for 28 h and thereafter was gradually decreased. This observation indicates that fermentation of sorghum improves protein digestibility. Similar results were observed by Romo-Parada, Simord and Larrea-Reynoso (1985) who reported that controlled fermentation decreases the IVPD of low tannin sorghum cultivars by 6.3% and increase IVPD of high tannin sorghum cultivar by 17.5% while natural fermentation increases the IVPD of low tannin cultivar by 8.6%, and IVPD of high tannin cultivar by 25.6%.

Chavan, Chavan and Kadam (1988) found that the IVPD of sorghum increased markedly after fermentation for 24 h Youssif and El Tinay (2001) reported that IVPD of sorghum increased with fermentation period. Supplementation of sorghum flour with 5% whey protein was found to increase by 10.2%. IVPD values were significantly ($p \leq 0.05$) increased during the first 28 h, but gradually decreased thereafter. This observation indicates that protein digestibility increased as a result of supplementation with whey protein which was reported to have a higher value of IVPD. Supplementation of sorghum flour with 10% whey protein causes greater improvement in IVPD compared to sorghum flour and that supplemented with 5%. Results indicated that both supplementations (5 and 10%) improved the quality of sorghum protein.

Effect of Whey Protein Supplementation on Sensory Quality of Processed Fermented Sorghum Flour: Table 6 shows the effect of whey protein supplementation on sensory quality of processed fermented sorghum flour (Kisra, Asida and Nasha). According to performance of panelists the samples of supplemented processed flour (Kisra, Asida and Nasha) were tasty, with a pleasant odor and very good general acceptability as that of commonly consumed products of sorghum flour. Whey protein had unique functional properties and functional ingredients such as flavour, texture, colour and aeration, enable it to be capable of fulfilling diverse functional properties to satisfy different forms of utilization (Morr, 1987). Moreover, whey protein is generally recognized as safe (GRAS) for food product applications and not specifically restricted by standard of identity beside that it is a good source of proteins(Morr and Foegeding 1990). Therefore, addition of such type of protein to sorghum flour expected to solve problems related to proteins quality and quantity.

Conclusion

Yousif and El Tinay (2001) found that processing of fermented sorghum flour had no adverse effect on protein quality. Due to this fact we recommended supplementation of sorghum with whey protein in order to improved its nutritional value and acceptability even after cooking.

References

Andlauer, W. and P. Fürst, 1999. Does cereal reduce the risk of cancer? Cereal food world. 44: 76 - 78.

AOAC, 1975. Official methods of analysis, 12th edition Washington D. C: Association of Official Analytical Chemists.

Au, P. M. and M. L. Fields, 1981. Nutritive quality of fermented sorghum. J. Food Science, 46: 662 - 654.

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 Science, 53: 1574-1575.

Dewit, J. N and J. Kessel, 1996. Effects of ionic strength on the solubility of whey protein products: A colloid chemical approach. Food Hydrocolloids, 10: 143 - 149.

Duncan, D. B., 1955. Multiple ranges and multiple F test. Biometrics, 11: 1-42.

Eggum, B. O., L. Monowar, K. E. Boch Knusden, L. Munck and J. Axtell, 1983. Nutritional quality of sorghum foods from sSudan, J.Cereal Sci. 1: 127-137.

El Khalifa, A. O. and A. H. El Tinay, 1994. Effect of fermentation on protein fractions and tannin content of low– and high– tannin cultivars of sorghum. Food Chem. 49: 265 - 269.

El Tinay, A. H., Z. M. EL Mahadi and A. El Soubki, 1985. Supplementation of fermented sorghum kisra bread with legume protein. J. Food Tech. 20: 679 - 687.

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

Hamad, S. H., G. Bocker, R. F. Vogel and W. P. Hammes, 1992. Microbiological and chemical analysis of fermented sorghum dough for Kisra production. Applied Microbiolology and Biotechnology, 37: 728 - 731.

Hamaker, B. R., A. W. Kirleis, E. T. Mertz and J. D. Axtell, 1986. Effect of cooking on the protein profile and *in vitro* protein digestibility of sorghum and maize. J. Agri. and Food Chem. 34: 647-649.

Huffman, L. M., 1996. Processing of WPC for use as food ingredients. Food Technol. 2: 49 - 53.

Jayaprakasha, H. M., A. Tirumalesha, H. G. Ramachandra Rao, 1997. Whey protein concentrate and butter milk solids in the formation of ready- to- use dried ice cream mix. In: Proceedings of Indian convention of Food Scientists and technologists. ICFOST-97, Mumbai pp 7 - 21.

Joslyn, A. M., 1970. Methods in food analysis, 2nd edn. Academic Press, New York.

Kester, J. J, T. Richardson, 1984. Modification of whey protein to improve functionality. J. Dairy Sci. 2757 - 2774.

Kim, S. H, C. V. Morr, A. Seo, J. G. Surak, 1989. Effect of whey pretreatment on composition and functional properties of whey protein concentrate. J. Food Sci. 54: 25 - 32.

Kinsella, J. E., 1987. Physical properties of food and milk components; Research needs extend uses. J. Dairy Sci., 70: 2419 - 2424.

Landry, J., T. Moureaux, 1970. Heterogeneity of the glutelins of grain of corn: Selective extraction and composition in amion acid of the three isolated fractions: Bull. Soc. Chem. Biol. 52: 1021-1037.

Mann, E. J., 1998. Various dairy products. Dairy industries Int. 63: 16-17.

Mohammed, S. I., L. R. Steenson, A. W. Kirleis, 1991. Isolation and characterization of microorganisms associated with the traditional sorghum fermentation for production of Sudanese kisra. App. Env. Micro., 57: 2529-2533.

Morr, C. V. and E. A. foegeding, 1990. Composition and functionality of commercial whey and milk protein concentrates and isolates. A status report. Food Technol. 44: 100-112.

Morr, C. V and H. A. Y. W., 1993. Whey protein concentrate and isolates: Processing and functional properties–CRC critical Reviews Food Sci. Nutri., 33: 431-476.

Morr, C. V., 1987. The effect of HTST pasteurization of milk, cheese whey and cheese whey UF retentates upon the composition, physio-chemical and functional properties of whey protein concentrate. J. Food Sci., 52: 312-319.

Patel, M. T and A. Kilara, 1990. Studies of WPC–1. Compositional and thermal properties. J. Dairy Sci. 73: 1439-1445.

Romo–Parado, M. L., R. E. Simard and S. Larrea–Reynoso, 1985. Influence of germination, nixtamalizacion and fermentation on the nutritional value of sorghum protein. Microbio.-Alements- Nutrition, 3: 125-132.

Saunders, R. M., M. A. Connor, A. N. Booth, E. N. Bickoff and C. O. Kohler, 1973. Measurement of alfalfa protein concentrate by *in vitro* and *in vivo* methods. J. Nutrition, 103: 530-535.

Skoch, L. V., C. W. Deyoe, F. K. Shoup, J. Bathrust and D. Liang, 1970. Protein fractionation of sorghum grain. Cereal Chemistry, 47: 472-481.

Snedecor, G. W. and W. G. Cochran, 1987. Statistical Methods. 17th edn. P. 221-222. Ames. 1A:The Iowa State University Press.

Wu, Y. V. and J. S. Wall, 1980. Lysine content of protein increased by germination of normal and high lysine sorghum. J. Agri. and Food Chem. 28: 455-458.

Youssif, N. E. and A. H. El Tinay, 2001. Effect of fermentation on sorghum protein fractions and *in vitro* protein digestibility. Plant Foods for Human Nutrition, 56: 175-182.

Zamora, A. F. and M. L. Fields, 1979. Nutritive quality of cowpea (*Vigna sinenis*) and chickpeas (*Cicer arietinum*). J. Food Sci. 44: 234-236.