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Effect of fermentation on biochemical and sensory characteristics of sorghum flour supplemented with whey protein

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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 after supplementation of sorghum flour with whey protein. The pH of the fermenting material decreased sharply with a concomitant increase in the titrable acidity. The total soluble solids increased with progressive fermentation time. The crude protein and non-protein nitrogen both increased with fermentation time. The protein content and fractions were significantly ($p \leqslant 0.05$) increased after supplementation with whey protein. The albumin plus globulin fraction increased significantly ($p \leqslant 0.05$) during the first 8 h of fermentation after supplementation with 5% whey protein. Other fraction contents were observed to fluctuate during the fermentation time. Supplementation of the cultivar flour with 10% whey protein greatly increased the protein content as well as the albumin plus globulin fraction while other fractions were significantly decreased. The in vitro protein digestibility was significantly ($p \leqslant 0.05$) improved during fermentation and even after supplementation. Sensory evaluation of locally processed 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 panellists.
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1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench.) is considered to be one of the most important food crops in the world, following wheat, rice, maize and barley (FAO, 1997). Grain sorghum provides the staple food for large populations 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 of many countries and it forms a major source of proteins and calories in the diet of large segments of the populations of India and Africa. Besides being a staple food, it is also used as a 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 are important sources of calories and proteins for 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, & Liang, 1970). The nutritional quality of sorghum is poor, due to deficiency in lysine and low quantities of threonine and tryptophan (Au & Fields, 1981). Sorghum grains have low starch and protein digestibilities, due to the presence of

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certain antinutritional factors which also contribute to poor sensory characteristics of processed sorghum grains. Recently, great efforts have been directed toward improve next of 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, Boch Knusden, Munck, & Axtell, 1983). Fortification with synthetic lysine is very effective, but rather impracticable considering the conditions under which sorghum is processed. Sudanese people consume sorghum as fermented Kisra, Asida or Nasha which provide about 97% of the protein and 75% of the calories in the diet of the people residing in central and western Sudan. Whey protein has better functional properties, such as solubility (Dewit & Kessel, 1996; Jayaprakasha, Tirumalesha, & Ramachandra Rao, 1997; Patel & Kilara, 1990) over a wide range of pH. β-Lactalbumin and α-lactalbumin are the major proteins of whey, constituting 50–55% as β-lactalbumin and 20–25% α-lactalbumin. Whey protein is a key ingredient in many infant formulas because α-lactalbumin is the major protein in human breast milk. The albumin plus globulin fraction is reportedly characterized by a high level of lysine (Wu & Wall, 1980). Whey proteins have the potential to improve quality of food products (Jayaprakasha et al., 1997; Kester & Richardson, 1984; Kim et al., 1989; Morr & Ha, 1993). Also, whey protein concentrate finds numerous applications in food and dairy industries due to its excellent nutritional and functional properties (Huffman, 1996; Kinsella, 1987; Mann, 1998). Whey protein is a high quality protein and a rich source of essential amino acids. Therefore, in this study we investigate the effects of supplementation with whey protein and/or fermentation on protein fractions and digestibility of a sorghum flour.

2. Materials and methods

2.1. Materials

Unsalted cow's milk whey was obtained from the Dairy-Land Factory, Khartoum North, Sudan. Sorghum cultivar (Wad Ahmed) of low tannin content was obtained from the 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 of reagent grade.

2.2. 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's 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.

2.3. 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 a 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 aluminium 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.

2.4. 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.

2.5. 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 earthen ware container called "Khumara". A small amount of the previously fermented dough was then added to the mixture, which acted 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 s and then taken out and considered ready for eating. Asida and Nasha preparations differ from that of Kisra; they are prepared as thick and thin pastes, respectively.

2.6. Determination of pH and titrable acidity

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

2.7. Determination of crude and non-protein nitrogen

Crude protein (N × 6.25) of the control and processed samples was determined by the standard Kjeldahl method (AOAC, 1975). Non-protein nitrogen was determined according to the method of Gheyasuddin (1970). About 5 g 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 an aliquot was taken for nitrogen determination by the standard Kjeldahl method (AOAC, 1975).

2.8. Determination of total soluble solids

Total soluble solids were determined at 20 °C by the Joslyn (1970) method, using an Abbe refractometer (Bellingham and Stanely LTD, London).

2.9. Protein fractionation

Nitrogen of a defatted sample was extracted, stepwise, by series of solvents according to the 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, for 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-glutelins 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 0.5 M 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 30,000*g* for 15 min. Nitrogen content of each fraction was determined by the micro-Kjeldahl method (AOAC, 1975).

2.10. Determination of in vitro protein digestibility

In vitro protein digestibility (IVPD) of the protein was curried out according to the Saunders, Connor, Booth, Bickoff, and Kohler (1973) method. About 200 mg of sorghum samples were placed in 50 ml centrifuge tubes, 15 ml of 0.1 M 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 of 10% trichloroacetic acid, and centrifuged at 50,000*g* for 20 min at room temperature. Nitrogen in the supernatant was estimated by the micro-Kjeldhl method (AOAC, 1975). IVPD was calculated using the formula:

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

2.11. Sensory evaluations

The sensory tests were conducted, using conventional profiling, by a trained panel. Ten judges were selected who had successfully passed standardized tests for olfactory and taste sensitivities as well as verbal abilities and creativity. The panellists were given a hedonic questionnaire to test odour, taste and general acceptability of coded samples of Kisra, Asida and Nasha made from fermented Wad Ahmed 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 a percentage as follows:

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

2.12. Statistical analyses

Each sample was analyzed in triplicate and the figures were then averaged. Data were compared using analyses of variance (ANOVA) (Snedecor & Cochran, 1987) and by the Duncan's multiple range test with a probability $p \leqslant 0.05$ (Duncan, 1955).

3. Results and discussion

3.1. Changes in pH, titrable acidity, total soluble solids, protein content and non-protein nitrogen during natural fermentation of sorghum cultivar flour

Table 1 shows changes in pH, titrable acidity (TA), total soluble solids (TSS), protein content and non-protein nitrogen (NPN) during natural fermentation of sorghum cultivar (Wad Ahmed) flour. The pH of the fermented dough dropped from 6.0 at zero time to 4.3 at the end of the fermentation period. Concomitant with the drop in pH there was a rise in TA throughout the

Table 1
Changes in pH, titrable acidity (TA), total soluble solids (TSS), crude protein and non-protein nitrogen (NPN) during natural fermentation of sorghum cultivar (Wad Ahmed)

Fermentation period (h)	pH	TA (mg/100 g)	TSS (%)	Protein (%)	NPN (%)
0	6.00 (±0.00)^{a}	12.6 (±0.06)^{j}	3.30 (±0.00)^{i}	9.90 (±0.12)^{g}	0.01 (±0.01)^{b}
4	6.00 (±0.00)^{a}	14.3 (±0.25)^{i}	3.87 (±0.06)^{h}	10.1 (±0.12)^{f}	0.09 (±0.01)^{a}
8	5.83 (±0.06)^{b}	16.5 (±0.25)^{h}	4.07 (±0.06)^{g}	10.3 (±0.11)^{e}	0.01 (±0.01)^{b}
12	4.87 (±0.06)^{c}	31.7 (±0.06)^{g}	4.47 (±0.06)^{f}	10.9 (±0.06)^{c}	0.01 (±0.01)^{b}
16	4.60 (±0.00)^{d}	39.1 (±0.06)^{f}	5.00 (±0.00)^{d}	10.3 (±0.07)^{e}	0.11 (±0.01)^{a}
20	4.43 (±0.06)^{e}	49.4 (±0.15)^{e}	5.20 (±0.00)^{c}	11.2 (±0.03)^{b}	0.11 (±0.01)^{a}
24	4.43 (±0.06)^{e}	70.3 (±0.30)^{d}	5.27 (±0.06)^{b}	10.4 (±0.03)^{e}	0.10 (±0.01)^{a}
28	4.31 (±0.00)^{f}	81.6 (±0.15)^{c}	6.07 (±0.06)^{a}	10.7 (±0.03)^{d}	0.11 (±0.01)^{a}
32	4.33 (±0.00)^{f}	111 (±0.12)^{b}	5.17 (±0.06)^{c}	11.0 (±0.08)^{c}	0.12 (±0.01)^{a}
36	4.30 (±0.00)^{f}	122 (±0.12)^{a}	4.80 (±0.00)^{e}	11.4 (±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 $p \leqslant 0.05$.

fermentation process. The TA increased from 12.6 to 122 mg/100 g. This result was in agreement with the work conducted by Au and Fields (1981), El Tinay, ELMahadi, and El Soubki (1985), Chavan,Chavan,and Kadam (1988), Hamad, Bocker, Vogel, and Hammes and Youssif (1997) and El Tinay (2001). According to Mohammed, Steenson, and Kirleis (1991), natural sorghum fermentation is mainly lactic (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 3.3% at zero time to 6.1% after 28 h fermentation; thereafter, it started to drop significantly ($p \leqslant 0.05$). The general pattern showed an initial increase in soluble solids at the commencement of fermentation, followed by a decrease toward the end of fermentation. The protein content of the fermented dough ranged from 9.9% at zero time to 11.4% 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 (Table 1) ranged from 0.01% to 0.12%, with significant changes at the end of fermentation period. Increments in TSS, protein and NPN are likely to be due to solubilization of sorghum flour constituents as a result of fermentation (Youssif & El Tinay, 2001).

3.2. 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 14.2% at zero time and 12.5% at the end of the fermentation period. It increased significantly ($p \leqslant 0.05$) during the first 8 h and thereafter started to decrease with a slight increase at the end of the fermentation period. The albumin plus globulin fraction is characterized by high level of lysine (Wu & 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 52.0% at zero time and 54.5% at the end of the fermentation period. It decreased significantly ($p \leqslant 0.05$) dur-

Table 2
Changes in protein fractions during fermentation of sorghum cultivar (Wad Ahmed)

Fermentation peroid (h)	Protein (%)	(Albumin + globulin) (%)	Prolamin (%)	G_1-glutelin (%)	G_2-glutelin (%)	G_3-glutelin (%)	Insoluble protein (%)	Protein recovery (%)
0	9.90 (±0.12)^{g}	14.2 (±1.74)^{ab}	52.0 (±3.06)^{cd}	18.5 (±1.10)^{ab}	2.27 (±0.21)^{abc}	15.3 (±2.94)^{cde}	1.20 (±0.10)^{bc}	103
4	10.1 (±0.12)^{f}	16.6 (±2.31)^{a}	49.6 (±2.32)^{de}	13.4 (±0.64)^{af}	1.67 (±0.29)^{cde}	21.6 (±2.83)^{a}	1.42 (±0.06)^{a}	104
8	10.3 (±0.11)^{e}	17.0 (±1.30)^{a}	48.7 (±1.62)^{e}	19.1 (±0.00)^{a}	1.10 (±0.17)^{e}	19.2 (±1.85)^{ab}	1.08 (±0.00)^{d}	106
12	10.9^{c} (±0.06)	14.5^{ab} (±2.11)	54.5^{bc} (±0.56)	17.3 (±0.35)^{bcd}	2.07 (±0.06)^{bcd}	15.6 (±0.90^{cd})	1.24 (±0.06)^{b}	105
16	10.3 (±0.07)^{e}	13.5 (±0.36)^{ab}	58.4 (±5.53)^{a}	11.1 (±0.17)^{e}	2.50 (±0.00)^{ab}	15.7 (±1.33)^{cd}	1.18 (±0.12)^{bcd}	102
20	11.2 (±0.03)^{b}	10.7 (±0.07)^{b}	62.0 (±0.06)^{a}	12.1 (±1.70)^{f}	2.93 (±0.31)^{a}	12.5 (±0.98)^{e}	1.10 (±0.12)^{cd}	101
24	10.4 (±0.03)^{e}	9.33 (±4.66)^{b}	62.4 (±2.07)^{a}	17.1 (±1.00)^{cde}	2.67 (±0.29)^{ab}	12.7^{e} (±0.50)	1.12^{cd} (±0.15)	105
28	10.7 (±0.03)^{d}	10.3 (±0.45)^{b}	61.7 (±1.15)^{a}	17.1 (±0.17)^{cde}	1.47 (±0.46)^{de}	12.7 (±0.46)^{e}	1.18 (±0.06)^{abcd}	105
32	11.0 (±0.08)^{c}	11.0 (±0.93)^{b}	57.4 (±0.58)^{b}	16.4 (±0.58)^{de}	2.67 (±0.76)^{ab}	17.9 (±0.55)^{bc}	1.10 (±0.06)^{d}	107
36	11.4 (±0.06)^{a}	12.5 (±0.86)^{ab}	54.5 (±0.61)^{bc}	17.8 (±0.62)^{bce}	1.47 (±0.46)^{de}	18.6 (±0.64)^{b}	1.46 (±0.06)^{a}	106

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

ing the initial 8 h but increased significantly ($p \leqslant 0.05$), thereafter reaching its maximum value (62.4%) at 24 h; then it started to decrease. The G_1-glutelin (cross linked Kaffirin) was 18.5% at zero time and 17.8% at the end of fermentation, its content fluctuated during fermentation. The G_2-glutelin (Glutelin-like) was 2.27% at zero time and 1.47% at the end of fermentation; also its content fluctuated during fermentation. The G_3-glutelin (True-glutelin) content fluctuated during fermentation and was found to be 15.3% at zero time and 18.6% at the end of the fermentation period. Non-extractable or insoluble protein fluctuated during fermentation and was found to be 1.2% at zero time and 1.46% at the end of the fermentation period. El Khalifa and El Tinay (1994) fractionated fermented sorghum proteins using the classical Mendel-Osborne procedure and they found that a 14 h fermented dough had less prolamin fraction and a slightly increased content of albumin plus globulin fraction.

3.3. 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 increased by 49.1% 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 increased by 155% after supplementation compared to the flour and fermented dough. Results indicated that supplementation with whey protein significantly increased albumin and globulin content which shows that lysine level will significantly increase (Wu & Wall, 1980). The prolamin fraction, which was the major fraction in the flour and fermented dough, decreased by 38.6%. The G_1-glutelin (cross linked kaffirin) decreased by 36% upon whey protein supplementation compared to the flour and fermented dough. Its content fluctuated during fermentation of supplemented dough. The G_2-glutelin (glutelin-like) increased by 30.9% upon supplementation with whey protein compared to flour and fermented dough. The G_3-glutelin (true-glutelin) decreased by 36.5% upon supplementation with whey protein compared to other fractions. The insoluble protein increased by 21% upon supplementation. Whey protein was found to have better functional properties, such as solubility (Dewit & Kessel, 1996; Patel & Kilara, 1990) over a wide range of pH. β-Lactalbumin and α-lactalbumin are the major proteins of whey, constituting about 50–55% β-lactalbumin and 20–25% α-lactalbumin. Whey protein is a key ingredient in many infant formulas because α-lactalbumin is the major protein in human breast milk. The albumin plus globulin fraction is reportedly characterized by higher level of lysine (Wu & Wall, 1980). Kester and Richardson (1984), Kim, Morr, Seo, and Surak (1989), Morr and Ha (1993), and Jayaprakasha et al. (1997) reported that whey proteins have the potential to improve the quality of food products. Kinsella (1987), Huffman (1996), and Mann (1998) reported that whey protein concentrates find 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.

3.4. 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

Table 3
Changes in protein fractions during fermentation of sorghum cultivar (Wad Ahmed) 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	14.6 (±0.12)^{g}	42.7 (±1.64)^{a}	31.8 (±1.83^{d})	11.8 (±0.65)^{ab}	2.14 (±0.12)^{abc}	9.91 (±1.76)^{cde}	1.50 (±0.06)^{bc}	99.9
4	14.7 (±0.12)^{f}	42.6 (±0.76)^{a}	30.4 (±1.38)^{de}	8.76 (±0.38)^{f}	1.78 (±0.17)^{cde}	13.7 (±1.69)^{a}	1.72 (±0.03)^{a}	98.9
8	14.9 (±0.11)^{e}	43.4 (±0.40)^{a}	29.9 (±0.97)^{e}	12.2 (±0.00)^{a}	1.44 (±0.10)^{e}	12.2 (±1.10)^{ab}	1.38 (±0.00)^{d}	101
12	15.6 (±0.06)^{c}	40.5 (±0.94)^{ab}	33.7 (±0.43)^{bc}	11.1 (±0.21)^{bcd}	2.02 (±0.03)^{bcd}	11.3 (±0.54)^{bc}	1.54 (±0.03)^{b}	100
16	15.0 (±0.07)^{e}	40.7 (±0.03)^{ab}	37.6 (±0.31)^{a}	9.80 (±0.10)^{e}	2.28 (±0.00)^{ab}	10.1 (±0.79)^{cd}	1.48 (±0.07)^{bcd}	102
20	15.9 (±0.03)^{b}	38.8 (±0.06)^{ab}	37.8 (±0.03)^{a}	8.03 (±1.02)^{f}	2.53 (±0.18)^{a}	10.1 (±0.58)^{cde}	1.40 (±0.07)^{cd}	98.6
24	15.1 (±0.03)^{e}	36.7 (±0.06)^{ab}	38.4 (±0.65)^{a}	10.7 (±0.79)^{cde}	2.38 (±0.17)^{ab}	8.34 (±0.30)^{e}	1.42 (±0.09)^{cd}	98.0
28	15.3 (±0.03)^{d}	39.0 (±0.75)^{ab}	38.0 (±0.00)^{a}	11.6 (±0.10)^{abc}	1.66 (±0.28)^{de}	8.88 (±0.28)^{de}	1.48 (±0.03)^{bcd}	101
32	15.2 (±0.08)^{c}	39.9 (±0.35)^{ab}	35.0 (±0.35)^{b}	10.3 (±0.17)^{de}	2.38 (±0.45)^{ab}	11.5 (±0.33)^{bc}	1.40 (±0.03)^{cd}	101
36	15.0 (±0.06)^{a}	38.7 (±0.09)^{ab}	33.3 (±0.36)^{c}	11.1 (±0.88)^{bcd}	1.66 (±0.28)^{de}	11.9 (±0.38)^{b}	1.76 (±0.03)^{a}	98.4

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

Table 4
Changes in protein fractions during fermentation of sorghum cultivar (Wad Ahmed) 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	19.6 (±0.12)^{g}	52.2 (±1.32)^{h}	25.9 (±1.46)^{cd}	9.88 (±0.53)^{ab}	2.14 (±0.10)^{abc}	8.37 (±1.41)^{def}	1.63 (±0.05)^{bc}	100
4	19.8 (±0.12)^{f}	52.1 (±0.60)^{a}	24.8 (±1.11)^{de}	7.44 (±0.30)^{e}	1.86 (±0.14)^{cde}	11.4 (±1.35)^{a}	1.81(±0.03)^{a}	99.3
8	20.0 (±0.11)^{e}	52.7 (±0.32)^{a}	24.3 (±0.77)^{e}	10.2 (±0.00)^{a}	1.59 (±0.08)^{e}	10.2 (±0.88)^{ab}	1.54 (±0.00)^{d}	101
12	20.6 (±0.06)^{c}	50.4 (±0.75)^{b}	27.0 (±0.27)^{bc}	9.32 (±0.17)^{bc}	2.05 (±0.02)^{bcd}	9.48 (±0.42)^{bcd}	1.66 (±0.03)^{b}	100
16	20.0 (±0.07)^{e}	50.1 (±1.05)^{bc}	30.6 (±0.11)^{a}	8.27 (±0.08)^{d}	2.26 (±0.00)^{ab}	8.54 (±0.63)^{cde}	1.62 (±0.05)^{bc}	101
20	20.9 (±0.03)^{b}	49.0 (±0.05)^{c}	30.7 (±0.03)^{a}	6.85 (±0.81)^{e}	2.46 (±0.15)^{a}	8.48 (±0.47)^{def}	1.56 (±0.06)^{cd}	99
24	20.1 (±0.03)^{e}	49.0 (±0.51)^{c}	30.9 (±0.99)^{a}	9.23 (±0.48)^{bc}	2.34 (±0.13)^{ab}	7.11 (±0.24)^{f}	1.57 (±0.07)^{cd}	100
28	20.4 (±0.03)^{d}	49.2 (±0.60)^{bc}	30.9 (±0.00)^{a}	9.70 (±0.08)^{ab}	1.76 (±0.23)^{de}	7.54 (±0.22)^{ef}	1.62 (±0.02)^{bcd}	101
32	20.2 (±0.08)^{c}	49.8 (±0.35)^{bc}	28.3 (±0.00)^{b}	8.91 (±0.28)^{cd}	2.34 (±0.36)^{ab}	9.62 (±0.26)^{bcd}	1.56 (±0.03)^{cd}	101
36	20.4 (±0.06)^{a}	48.9 (±0.35)^{c}	27.2 (±0.31)^{b}	9.56 (±0.30)^{abc}	1.76 (±0.23)^{de}	9.93 (±0.31)^{bc}	1.84 (±0.02)^{a}	99.2

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

increase. The albumin plus globulin fraction increased by 210% upon 10% whey protein supplementation. The prolamin fraction, which was the major fraction in the control samples, was decreased by 50% upon whey protein supplementation. The G_1-glutelin (cross linked kaffirin) was decreased by 46.5% after supplementation and was observed to fluctuate during fermentation of supplemented dough. The G_2-glutelin (glutelin-like) was decreased by 45.5% after supplementation and fluctuated during fermentation of the supplemented dough. The G_3-glutelin (true-glutelin) was decreased by 46.9% after supplementation. The insoluble protein was increased by 36.4% after supplementation and 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. Increase of the 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.

3.5. Changes in in vitro protein digestibility during natural fermentation of sorghum flour supplemented with 5 or 10% whey protein

Table 5 shows changes in IVPD during natural fermentation of sorghum cultivar supplemented with 5% or 10% whey protein. IVPD of naturally fermented dough remained constant after 24 h fermentation and was found to be 47.9% at zero time and 47.6% when fermented for 36 h with a maximum value (69.6%) obtained after 28 h of fermentation. This observation indicates that fermentation of sorghum improves protein digestibility. Similar results were observed by Romo-Parado, Simard, and Larrea-Reynoso (1985) who reported that controlled fermentation decreases the IVPD of low tannin sorghum cultivars by 6.3% and increases 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 et al. (1988) found that the IVPD of sorghum increased markedly after fermentation for 24 h. Youssif and El Tinay (2001) reported that

Table 5
Changes in in vitro protein digestibility (IVPD) during fermentation of sorghum cultivar (Wad Ahmed) supplemented with 5% and 10% whey protein

Fermentation period (h)	IVPD (%)		
	Control	5% Whey protein	10% whey protein
0	47.9 (±2.43)^{d}	59.2 (±0.95)^{e}	63.1 (±0.95)^{e}
4	50.7 (±2.44)^{d}	64.3 (±2.57)^{d}	66.6 (±2.06)^{d}
8	49.3 (±6.50)^{d}	62.0^{de} (±6.25)	62.1^{e} (±4.94)
12	58.2 (±2.90)^{c}	72.6 (±0.56)^{c}	74.4 (±0.78)^{c}
16	59.6 (±1.04)^{bc}	73.7 (±0.61)^{bc}	76.0 (±0.85)^{c}
20	61.6 (±1.68)^{bc}	75.5 (±1.30)^{bc}	77.4 (±0.62)^{bc}
24	64.3 (±0.96)^{b}	77.8 (±1.42)^{b}	79.8 (±0.81)^{b}
28	69.6 (±0.76)^{a}	82.4^{a} (±0.84)^{a}	83.8 (±1.27)^{a}
32	62.2 (±1.46)^{bc}	74.9 (±0.42)^{bc}	76.0 (±1.51)^{c}
36	47.6 (±3.13)^{d}	61.6 (±1.39)^{de}	61.8 (±0.89)^{e}

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

Table 6
Effect of whey protein supplementation on sensory quality of sorghum (Wad Ahmed) flour after local processings

Whey protein (%)	Taste acceptability		Odour acceptability		General acceptability	
	Score	Percent	Score	Percent	Score	Percent
1. Kisra processed dough:						
0	3	30	3	30	3	30
	4	40	4	40	4	50
	5	30	5	30	5	20
5	3	30	3	20	3	20
	4	40	4	50	4	50
	5	30	5	30	5	30
10	3	20	3	10	3	20
	4	50	4	50	4	50
	5	30	5	40	5	30
2. Asida processed dough:						
0	3	30	3	30	3	30
	4	50	4	40	4	50
	5	20	5	30	5	20
5	3	20	3	10	3	20
	4	40	4	40	4	40
	5	40	5	50	5	40
10	3	20	3	20	3	20
	4	50	4	60	4	40
	5	30	5	20	5	40
3. Nasha processed dough:						
0	3	30	3	20	3	30
	4	60	4	50	4	50
	5	10	5	30	5	20
5	3	30	3	30	3	30
	4	50	4	40	4	50
	5	20	5	30	5	20
10	3	20	3	30	3	20
	4	40	4	40	4	50
	5	40	5	30	5	30

IVPD of sorghum increased with fermentation period. Supplementation of sorghum flour with 5% whey protein was found to increase IVPD to 82.4%. IVPD values were significantly ($p \leqslant 0.05$) increased during the first 28 h, but thereafter gradually decreased. This observation indicates that protein digestibility increases as a result of supplementation with whey protein which is reported to have a higher value of IVPD. Supplementation of sorghum flour with 10% whey protein caused better IVPD than sorghum flour or that supplemented with 5%, with a maximum value (83%) obtained after fermentation of the mixture for 28 h. Results indicated that both supplementations (5% and 10%) improved the quality of sorghum protein and also 28 h of fermentation was the suitable time to ferment the flour.

3.6. 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 scores of panellists, the samples of supplemented processed flour (Kisra, Asida and Nasha) were tasty, with a pleasant odour and very good general acceptability, similar to that of commonly consumed products of sorghum flour. Whey protein has unique functional properties, such as flavour, texture, colour and aeration, rendering it capable of fulfilling diverse functional roles (Morr, 1987). Moreover, whey protein is generally recognized as safe (GRAS) for food product applications and not specifically restricted by standard of identity; besides that, it is a good source of proteins (Morr & Foegeding, 1989). Therefore, addition of such type of protein to sorghum flour expected to solve problems related to protein quality and quantity.

4. Conclusion

It was observed that fermentation greatly improved protein digestibility without any impact on sensory quality of the samples. Moreover, Youssif and El Tinay (2001) found that processing of fermented sorghum flour had no adverse effect on protein quality. Due to these facts, we recommend supplementation of sorghum with whey protein in order to improve its nutritional value and acceptability, even after cooking.

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