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Protein, Mineral Content and Amino Acid Profile of Sorghum Flour as Influenced by Soybean Protein Concentrate Supplementation

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Abstract: This study was conducted to investigate the affect of supplementation of sorghum flour with soy protein concentrate (SPC) on ash, protein, mineral composition (Cu, Ca, Fe, Na and K) amino acids profile and electrophoretic patterns of their blends. The protein contents were found to be 14, 68, 18, 22 and 26% for sorghum flour, SPC, meal one, meal two and meal three, respectively. However, their ash contents were 2.29, 6.51, 2.82, 3.01 and 3.67%, respectively. No significant difference ($P \leq 0.05$) in terms of the ash content was observed between meal one and two, while meal three was significantly different from the remaining meals. Mineral composition of the tested samples significantly differs between the meals ($P \leq 0.05$) Supplementation of sorghum flour with SPC showed a significant increase in lysine and threonine contents, with a slight increase in methionine level in meal one and two. Electrophoretic patterns of samples indicated the appearance of new bands in all blends as well as intensification of protein bands due to the interaction between the two native proteins.

Key words: Chemical composition, electrophoretic patterns, sorghum flour, soy-protein concentrate

Introduction

Cereals are important sources of energy and protein in human diets. Although carbohydrates are their main dietary contribution, they provide protein and smaller amount of lipid, fibre and vitamins. It is commonly known that the main nutritional drawback to cereals, particularly sorghum (*Sorghum bicolor*), is their low protein content and the limited biological quality of their protein (highly deficient in lysine and tryptophan) (Mertz, 1970; Ortega *et al.*, 1986 and Waliszewski *et al.*, 2000) compared to animal protein. Nevertheless, the protein quality cereals can be improved by combining it with other rich sources of protein. Soybean (*Glycine max* L, Merril) proteins are used in human food in a variety of forms, including infant formula, flours, protein isolate and concentrates and textured fibres. Soy foods include cheese, drinks, miso, temph, tofu, salami and vegetarian meat substitutes. New soy foods are continually being developed. (Liu, 2000 and Singh *et al.*, 2000). Effort to increase the availability of protein in human diet from plant materials, particularly soybean concentrate and isolate have partially replaced wheat flour in some baked products (Tsen *et al.*, 1982). Soybean blend have been used as an excellent source of protein for corn tortilla fortification (Figueroa *et al.*, 2003) with improved essential amino acid balance, specially lysine and tryptophan which are low in cereals (Serna-Saldivar *et al.*, 1988 and Waliszewski *et al.*, 2000). Fortification of wheat flour with soy proteins improved the protein quality reflected in amino acid profile (Stark *et al.*, 1975). The present investigation was undertaken to study the nutritive value of sorghum as affected by supplementation with soybean protein concentrate and electrophretic assay of native proteins.

Materials and Methods

Commercial soy protein concentrate (SPC) was obtained from Loli Meat Manufacturing Company, Khartoum and Sudan. Sorghum (*Feterita*) was obtained from the local market. The seeds were cleaned and freed from foreign material 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 analysis. Unless otherwise stated, all reagents used in this study are of analytical - grade.

Sample preparation

Cooking: Cooked samples were prepared by suspending the flour of each sample in distilled water in the ratio of 1:10 (W/V) flour to water and stirring to avoid lumps, while boiling in a water-bath for 20min. Then the samples were freeze-dried and kept in polyethylene bags at 4°C for further analysis according to Arbab and El Tinay (1997).

Soybean protein concentrate (SPC) supplementation: The supplementation of sorghum with soybean protein concentrate was elevated by 4, 8 and 12%.

Protein and ash content: Crude protein (N x 6.25), ash

Table 1: Protein and ash content (%) of sorghum flour, soy protein concentrate (SPC) and their meals.

	Treatments				
Parameter	Sorghum flour	SPC	Meal 1	Meal 2	Meal 3
Protein	14.00^{e} (± 0.00)	68.25^{a} (±0.00)	18.00^{d} (±0.00)	22.00^{c} (±0.00)	26.00^{b} (±0.00)
Ash	2.29^{d} (±0.01)	6.51^{a} (±0.03)	2.82^{c} (±0.05)	3.01^{c} (±0.45)	3.67^{b} (±0.05)

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

Table 2: Mineral Contents (mg/100g) of Sorghum Flour, Soy Protein Concentrates (SPC) and their Meals.

	Treatments				
Parameter	Sorghum flour	SPC	Meal 1	Meal 2	Meal 3
Cu	0.41^{e} (±0.01)	0.85^{a} (±0.05)	0.76^{b} (±0.01)	0.62^{d} (±0.00)	0.68^{c} (±0.00)
Ca	2.43^{e} (±0.02)	27.79^{a} (±0.15)	6.17^{d} (±0.01)	9.23^{c} (±0.01)	11.38^{b} (±0.01)
Fe	15.54^{d} (±0.01)	7.15^{d} (±0.01)	18.10^{b} (±0.06)	16.64^{c} (±0.07)	19.41^{a} (±0.30)
P	263.30^{e} (±0.10)	469.63^{a} (±0.42)	282.11^{d} (±0.19)	331.40^{c} (±0.10)	342.08^{b} (±0.07)
Na	6.18^{e} (±0.16)	82.50^{a} (±0.10)	7.23^{d} (±0.06)	17.68^{b} (±0.05)	15.61^{c} (±0.01)
K	225.23^{e} (± 0.16)	1020.30^{a} (±0.10)	310.00^{d} (±0.06)	314.79^{c} (±0.05)	389.99^{b} (±0.01)

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

content of the raw material (sorghum and SPC) and cooked samples were determined according to AOAC (1984).

Determination of mineral content: Minerals were extracted from the samples by dry ashing method as described by Chapman and Pratt (1982) the amount of iron, Ca and Cu were determined using atomic absorption spectroscopy (Perkin-Elmer 2380). Phosphorus was determined according to Chapman and Pratt (1982). Sodium and potassium were determined by flame photometer (CORNIGEEL) according to AOAC (1984).

Determination of amino acids: The amino acid content was determined according to official method of analysis AACC (2000) using LKB Biochrom 4150 (Alpha) Automatic Amino acid Analyzer based on ion-exchange Chromatography.

SDS-polyacrylamide gel electrophoresis: Electrophoretic pattern of sorghum flour and soy protein concentrate and their blends was conducted as follow: Deffated soybean and sorghum blends were extracted with 0.03 M Tris-HCl (pH 8.0) buffer at room temperature to yield whole buffer extract as described by Iwabuchi and Yamauchi (1987), then centrifuged at 500 rpm for 30min at 15^{o}C. Soluble protein was estimated using the method of Comassie Brillent Blue G-250 according to Bradford (1976). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Laemmli (1970).

Statistical Analysis: Each determination was carried out on three separate samples and analyzed in triplicate and figures were then averaged. Data was assessed by the analysis of variance (ANOVA) (Snedecor and Cochran, 1987). Duncan Multiple Range Test (DMRT,

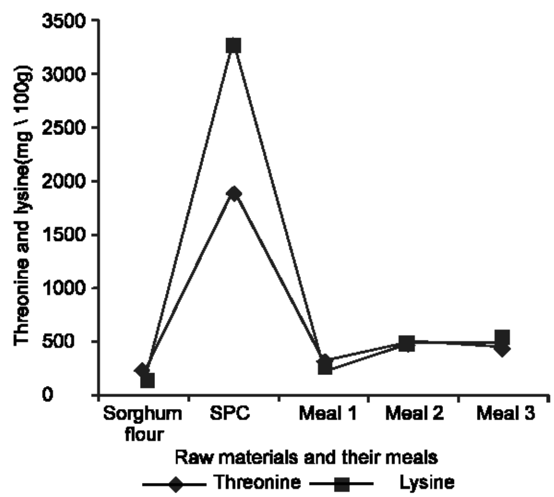

Fig. 1: Effect of supplementation of sorghum flour with SPC on Lysine and Threonine.

1955) was used to separate means. Significance was accepted at $P \leq 0.05$.

Results and Discussion

Chemical composition of sorghum flour, soy protein concentrates (SPC) and their meals: Table 1 shows the protein and ash content of sorghum flour, SPC and soy-sorghum flour meals. The soy-sorghum flour meals have increased protein content (18-26%) and ash (2.82-3.67%). In all meals there were significant improvements in the quality of soy-enriched sorghum. The protein content of sorghum increased significantly with the addition of soy protein concentrate. There were significant differences ($P < 0.05$) in ash content. The protein and ash contents were higher in meal three. This work confirms earlier report by Fashakin (1994) on the beneficial effect of vegetable protein. Supplementation of sorghum flour with the soy protein concentrate (SPC)

Table 3: Amino acids profile (mg/100g) of sorghum flour, soy protein concentrate (SPC) and their meals.

Amino acids	Sorghum flour	SPC	Meal 1	Meal 2	Meal 3
Aspartic	517.81	5304.30	831.12	1281.73	1202.75
Thrionine	204.72	1910.50	324.81	503.32	456.80
Serine	231.55	1778.10	352.40	515.82	473.16
Glutamic	995.86	6520.40	1457.65	2130.27	2020.15
Glycine	72.22	2017.16	144.61	286.16	323.03
Alanine	984.00	2392.30	1108.98	1227.42	1090.40
Valine	504.85	2728.20	597.50	774.85	697.78
Methionine	134.95	608.50	139.11	176.72	150.66
Isoleucine	411.73	2402.70	546.41	738.17	685.75
Leucine	1230.76	3896.10	1473.27	1721.78	1508.85
Tyrosine	147.33	1398.50	164.06	224.18	96.51
Phenylalanine	443.47	2620.90	601.21	807.66	688.74
Histidine	219.23	1546.66	313.91	441.15	448.01
Lysine	105.75	3306.50	252.40	510.73	506.58
Ammonia	877.22	2460.57	1071.11	1393.46	1440.69

Means are of duplicate samples, Meal 1: 18% on protein base. Meal 2: 22% on protein base. Meal 3 :24% on protein base.

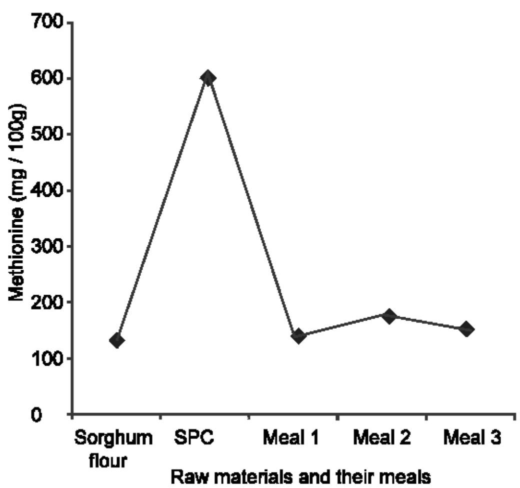

Fig. 2: Effect of supplementation of sorghum with SPC on methionine level.

reaching 18, 22 and 26% protein which meets the RDI of infant fed solely on sorghum-soy meals. About 285 - 310g to meat the daily protein requirements of 55g proteins (FAO/WHO, 1974). The increase in protein values of food that was supplemented with soy protein concentrate had been reported earlier by many investigators (Shuey and Tipples, 1982).

Mineral content of sorghum flour, soy protein concentrates (SPC) and their meals: Table 2 shows the results of mineral composition of sorghum flour, soy protein concentrate (SPC) and soy-sorghum flour blends. Sodium and potassium contents of the meal samples varied from 7.23 to 15.61 mg/100g and 310 to 389.99 mg/100g, respectively. The 12% SPC supplementation had significantly ($P \leq 0.05$) higher sodium and potassium contents. All meals were significantly different in sodium and potassium contents. Phosphorus was found to be varying from 263.30 to 469.63 mg/100g. The values of phosphorus in all the meal samples were significantly different ($P \leq 0.05$) from each other. Iron, calcium and copper contents of meal samples varied from 7.15 to 19.41 mg/100g, 2.43 to 27.79 mg/100g and .41 to .85 mg/100g, respectively. The copper and calcium contents of SPC were significantly higher than others while the iron content was significantly higher in sorghum flour compared to SPC and the three meals. The samples differ significantly among themselves with regard to all cations tested. The results obtained are in full agreement with Ijarotimi and Ashipa (2005) who studied the mineral content of weaning foods made of sweet potato supplemented with soybean flours.

Amino acid profile of sorghum flour, soy protein concentrates (SPC) and their meals: Table 3 shows the essential amino acids composition of sorghum flour, soy protein concentrate (SPC) and their meals. The amino acids content of sorghum flour was in agreement with Dendy (1995) who studied the amino acid composition of sorghums (regular and brown cultivars). The results revealed that lysine and threonine have the lowest values among all meals. Amino acids content of SPC was higher than sorghum flour. Methionine content was the lowest one. Result agreed with the those reported by Friedman *et al.* (1991). Supplementation of sorghum by soy protein concentrate (SPC) elevated protein content as well as protein quality (essential amino acid profile). The results obtained are in agreement with Stark *et al.* (1975) who reported that fortification of wheat flour with soy proteins increased protein quality by improving amino acids profile. Lysine and threonine contents of sorghum flour and soy protein concentrate (SPC) and soy-sorghum flour meals are shown in Fig. 1. Sorghum proteins have been reported to be limited in lysine and threonine (Shelton *et al.*, 1951). The presence of relatively high concentration of leucine in sorghum has been suggested as possible

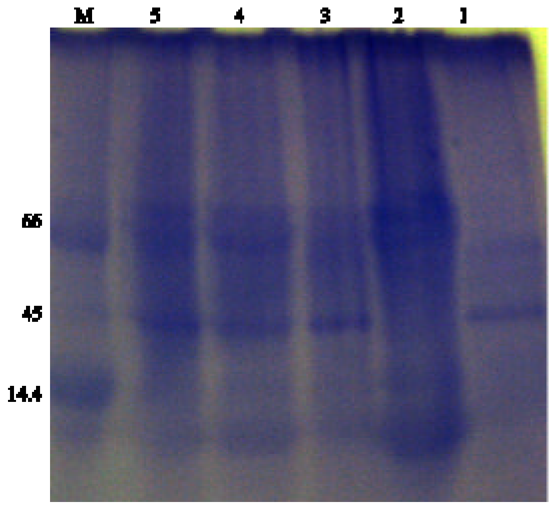

Fig. 1: Electrophoretic separation of protein subunits in Tris - glycine buffer pH 8.3 of sorghum and soybean samples in SDS - PAGE 5 and 15 % With 0.1% SDS.
1 = Sorghum, 2 = SPC, 3 = Blend 1, 4 = Blend 2, 5 = Blend 3. M = Molecular weight protein markers; Albumin bovine 66.0, Ovalbumin 45.0, Lysozyme 14.4 K Da.

factor in the development of pellagra in a population group subsisting principally on this crop (Belavady *et al.,* 1967). The correlation between protein and lysine is significantly negative while that between the protein and leucine is positive. Lysine content of soy proteins falls between that of cereal proteins such as wheat gluten and that of animal proteins such as casein (Betschart, 1978 and Friedman and Finot, 1990).Supplementation of sorghum flour with soy protein concentrate (SPC) increased the lysine content from 105.7 mg/100g in sorghum flour to 252.40 mg/100g in meal one (based on 18% proteins content) and this increment was doubled (510.73 mg/100g) in meal two (22% protein content) while no change was observed in meal three (26% protein content). Threonine contents were 204.72mg/100g in sorghum flour and 813.12, 1281.73 and 1202.75 mg/100g in supplemented meals 1, 2 and 3, respectively. Fig. 2 shows the methionine levels of sorghum flour, soy protein concentrate and soy-sorghum flour meals. The low contents of the essential acid L-methionine in soy protein limits its nutritive value. Methionine content of soy protein concentrate was 608.50 mg/100g which agrees with Brandon *et al.* (1991) who reported methionine content of soy flour as only 650mg/100g. This value is much lower than that of other cereals and meat (Friedman, 1996 and Sarwar *et al.*, 1993). Although cysteine has a sparing effect on methionine, it does not make up for the low methionine levels. The problem is further compounded for two reasons, first, during food processing and storage L-methionine and other amino acids are chemically modified, further reducing nutritional quality in case of methionine, such modification included oxidation to methionine sulfoxide and methionine sulfone, racemization to D-methionine and degradation to compounds with undesirable flavors, second, protein bound methionine in some plant foods is poorly utilized, presumably because of poor digestibility (Begbie and Pusztai, 1989 and Gumbmann *et al.,* 1983). The methionine content of soy-sorghum flours meals were 139.11, 176.72 and 150.66mg/100g, respectively. Methionine content was low in sorghum flour and SPC but higher in meal one and two (18 and 22% protein content and respectively) while no increase was observed in meal three (based on 26% protein content) this may be due to SPC being subjected to excessive heat during cooking.

Electrophoretic patterns of soy-sorghum blends: Results of electrophoretic patterns of soy protein concentrate (SPC), sorghum flour and soy-sorghum blends are shows in Fig. 3. The SDS-PAGE revealed that for sorghum protein (lane 1) the MW of the protein bands confined between 14.4 to 45KD, while those of SPC were extended in the range of 14.4 to 66 KD, considering that other bands had molecular weight lower than 14.4 KD. In composite flour samples bands of molecular weight of 45 to 66 KD were observed .Bearing in mind that the detectable bands in blends of higher intensity, suggesting the effect of addition of SPC proteins to the sorghum flour native protein. However, in composite flour samples with SPC have higher protein quantity and quality (Stark *et al.,* 1975) and the intensification of bands in soy blends indicate protein aggregation, suggesting interaction between two native proteins in the blends.

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