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

A.M. Awadalkareem1, A.I. Mustafa2 and A.H. El Tinay2
1Department of Food Science and Technology, Faculty of Agriculture, University of Al-Zaieem Al-Azhari, Khartoum North, Sudan
2Department of Food Science and Technology, Faculty of Agriculture, University of Khartoum, Khartoum North 13314, Shambat, Sudan

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 ≤ 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 ≤ 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., 1988 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 (Tseng 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-Saldívar 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 electrophoretic 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
Awadalkareem et al.: Soybean Protein Concentrate Supplementation

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sorghum flour</th>
<th>SPC</th>
<th>Meal 1</th>
<th>Meal 2</th>
<th>Meal 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>14.00 ± 0.00</td>
<td>68.25 ± 0.00</td>
<td>18.00 ± 0.00</td>
<td>22.00 ± 0.00</td>
<td>26.00 ± 0.00</td>
</tr>
<tr>
<td>Ash</td>
<td>2.29 ± 0.01</td>
<td>6.51 ± 0.03</td>
<td>2.82 ± 0.05</td>
<td>3.01 ± 0.45</td>
<td>3.67 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ±SD. Values not sharing a common superscript in a column are significantly (p ≤ 0.05) different.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sorghum flour</th>
<th>SPC</th>
<th>Meal 1</th>
<th>Meal 2</th>
<th>Meal 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>0.41 ± 0.01</td>
<td>0.85 ± 0.05</td>
<td>0.76 ± 0.01</td>
<td>0.62 ± 0.00</td>
<td>0.68 ± 0.00</td>
</tr>
<tr>
<td>Ca</td>
<td>2.43 ± 0.02</td>
<td>27.79 ± 0.15</td>
<td>6.17 ± 0.01</td>
<td>9.23 ± 0.01</td>
<td>11.38 ± 0.01</td>
</tr>
<tr>
<td>Fe</td>
<td>15.30 ± 0.01</td>
<td>7.16 ± 0.01</td>
<td>18.10 ± 0.06</td>
<td>16.84 ± 0.07</td>
<td>19.41 ± 0.30</td>
</tr>
<tr>
<td>P</td>
<td>16.30 ± 0.10</td>
<td>40.93 ± 0.42</td>
<td>28.11 ± 0.15</td>
<td>33.10 ± 0.10</td>
<td>32.10 ± 0.24</td>
</tr>
<tr>
<td>Na</td>
<td>6.18 ± 0.16</td>
<td>62.60 ± 0.10</td>
<td>7.22 ± 0.06</td>
<td>17.58 ± 0.06</td>
<td>15.61 ± 0.01</td>
</tr>
<tr>
<td>K</td>
<td>225.23 ± 0.16</td>
<td>1020.30 ± 0.10</td>
<td>310.00 ± 0.06</td>
<td>314.79 ± 0.05</td>
<td>389.99 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ±SD, values not sharing a common superscript in a column are significantly (p ≤ 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. Defatted 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 Tsuburichi and Yamauchi (1987), then centrifuged at 500 rpm for 30 min at 15°C. Soluble protein was estimated using the method of Cornassie Brilliant 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, 1955) was used to separate means. Significance was accepted at P ≤ 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 meal have increased protein content (18-28%) 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.

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Sorghum flour</th>
<th>SPC</th>
<th>Meal 1</th>
<th>Meal 2</th>
<th>Meal 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic</td>
<td>517.81</td>
<td>5304.30</td>
<td>831.12</td>
<td>1231.73</td>
<td>1202.75</td>
</tr>
<tr>
<td>Threonine</td>
<td>204.72</td>
<td>1910.50</td>
<td>324.81</td>
<td>503.32</td>
<td>456.80</td>
</tr>
<tr>
<td>Serine</td>
<td>231.55</td>
<td>1778.10</td>
<td>352.40</td>
<td>516.82</td>
<td>473.16</td>
</tr>
<tr>
<td>Glutamic</td>
<td>995.86</td>
<td>6520.40</td>
<td>1457.85</td>
<td>2130.27</td>
<td>2620.16</td>
</tr>
<tr>
<td>Glycine</td>
<td>72.22</td>
<td>2017.16</td>
<td>144.61</td>
<td>286.16</td>
<td>323.03</td>
</tr>
<tr>
<td>Alanine</td>
<td>984.00</td>
<td>2392.30</td>
<td>1108.98</td>
<td>1227.42</td>
<td>1090.40</td>
</tr>
<tr>
<td>Valine</td>
<td>504.85</td>
<td>2728.20</td>
<td>597.50</td>
<td>774.85</td>
<td>697.78</td>
</tr>
<tr>
<td>Methionine</td>
<td>134.95</td>
<td>508.50</td>
<td>139.11</td>
<td>176.72</td>
<td>150.66</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>411.73</td>
<td>2402.70</td>
<td>546.41</td>
<td>738.17</td>
<td>665.75</td>
</tr>
<tr>
<td>Leucine</td>
<td>1230.76</td>
<td>3396.10</td>
<td>1473.27</td>
<td>1721.78</td>
<td>1508.85</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>147.33</td>
<td>1398.50</td>
<td>164.06</td>
<td>224.18</td>
<td>98.61</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>443.47</td>
<td>2820.90</td>
<td>601.21</td>
<td>807.66</td>
<td>688.74</td>
</tr>
<tr>
<td>Histidine</td>
<td>219.23</td>
<td>1546.66</td>
<td>313.91</td>
<td>441.15</td>
<td>448.01</td>
</tr>
<tr>
<td>Lysine</td>
<td>105.75</td>
<td>3306.50</td>
<td>252.40</td>
<td>516.73</td>
<td>506.56</td>
</tr>
<tr>
<td>Ammonia</td>
<td>677.22</td>
<td>2460.57</td>
<td>1071.11</td>
<td>1393.46</td>
<td>1440.69</td>
</tr>
</tbody>
</table>


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 meet 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 ≤ 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 ≤ 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 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 KDa.

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 the 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 13911, 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 shown in Fig. 3. The SDS-PAGE revealed that for sorghum protein (lane 1) the MW of the protein bands centred 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.

References


Dendy, D.A.V., 1995. Sorghum and millets, Chemistry and Technology. Published by the American Association of cereal chemists, Inc., St Paul, Minnesota, USA.


