Composition and Functional Properties of Cowpea (*Vigna unguiculata* L. Walp) Flour and Protein Isolates

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ABSTRACT

Protein isolates from dehulled defatted cowpea (*Vigna unguiculata* L.) seeds were prepared using isoelectric (CPIA) precipitation and micellization (CPIB) procedures. Proximate analysis gave 75% crude protein, 2.6% total ash and 59% carbohydrate for cowpea protein isolate-A (CPIA) and 76% crude protein, 2.3% total ash and 13.1% carbohydrate for Cowpea Protein Isolate-B (CPIB). The protein percentage of the seed was found to be 22.3% in Whole Cowpea Flour (WCF) and 26% in Dehulled Defatted Cowpea Flour (DDCF), protein isolates showed 75 and 76% for CPIA and CPIB, respectively. The minimum protein solubility for CPIA was at pH 5.0 and for CPIB at pH 4.0. Total protein isolate studied showed good solubility in both acid and alkaline pH regions. For water and oil absorption capacity, DDCF gave 1.3 mL water g⁻¹ sample and 1.04 mL oil g⁻¹ sample, respectively; while CPIA gave 2.10 mL water g⁻¹ sample and 1.93 mL oil g⁻¹ sample, CPIB gave 2.33 mL water g⁻¹ sample and 2.37 mL oil g⁻¹ sample. Thus CPIA and CPIB showed better performance than DDCF with respect to these properties. The highest Emulsifying Capacity (EC) was observed at pH 12.0 for DDCF (173 oil g⁻¹ protein) and CPIA (160 oil g⁻¹ protein) while CPIB have highest EC (137 oil g⁻¹ protein) at pH 2.0 The emulsion capacity for both cowpea protein isolates (CPIA and CPIB) was higher at pH 7.0 compare to value obtained from DDCF. Least gelation concentration for Dehulled Defatted Cowpea Flour (DDCF) and both Cowpea Protein Isolates (CPIA) and (CPIB) was noted at 12.0% (w/v) at both pH 4.0 and 7.0.

**Key words:** Cowpea, protein isolate, functional properties

INTRODUCTION

The continuous increase in population in developing countries makes likely that many people will not be able to afford buying animal products regularly. The wide prevalence of protein-calories malnutrition in developing countries is of great importance not only to food scientists, nutritionists or agricultural scientists but also for concerned governments as well (Olsen, 1975). The problem of wide spread prevalence of Protein Energy Malnutrition (PEM) has resulted in high morbidity and mortality rates, especially among infants and children in low income groupings in the third world, including Sudan. Michaelsen and Friis (1998) have shown that malnutrition among children in developing countries is mainly due to the consumption of cereal based porridge which is bulky, high in energy and anti-nutrients. Legumes will therefore, continue to play important part in diets in the foreseeable future. Legumes provide a good source of protein (18-35%). Cowpea protein
concentrates and isolates could be utilized in the production of several conventional food formulations to increase their protein content as well as in the production of texture foods.

Cowpea (*Vigna unguiculata* L.) is an important food legume indigenous to Africa. Cowpea provides more than half the plant protein in human diets (Rachie, 1985). It is a good source of calories, vitamins and minerals and provides a significant amount of dietary protein (18-35%) and lysine. In regions of chronic protein shortage, it provides food of fairly high nutritive value to both humans and domestic animals (Sosulski *et al.*, 1987; Elhardallou *et al.*, 1980). In Sudan, cowpea-based food products are utilized as weaning foods for infants. Legume seeds as protein sources, are used as flours in products such as baby formula or supplemental diet for preschool children (Jansen and Harper, 1980; Akinyele *et al.*, 1988), baking products (Mustafa *et al.*, 1986), pastas (Bahnassey *et al.*, 1986; Molina *et al.*, 1982) or extruded products (Aguilera and Kosikowski, 1976; Ringe and Love, 1988; Likeimani *et al.*, 1991). The purpose of this study was to prepare two types of protein isolates from dehulled cowpea seed flour using the isoelectric precipitation, (CPIA) and micellization precipitation (CPIB) and to study and compare the functional properties of the two isolates (CPIA and CPIB) with Dehulled Defatted Cowpea Flour (DDCF) and the influence of pH on functional properties.

Nitrogen solubility was being used as a guide for protein functionality, since this relates directly to many important properties. Protein solubility was pH dependant. At pH values higher or lower than the isoelectric point, the protein molecules carries a negative or positive charges and water molecules may interact with this charge, thus contributing to protein solubilization.

**MATERIALS AND METHODS**

**Samples:** Dehulled cowpea (*Vigna unguiculata* L. Walp) of white coloured seed (Fig. 1) was brought from the local market at Wad Medani city, Sudan. The seeds were stored in polyethylene bags at room temperature (29-30°C) until used.

![Whole cowpea seeds and Dehulled cowpea seeds](image)

**Fig. 1(a-d):** Cowpea seeds and protein isolates, CPIA: Cowpea protein isolate prepared by isoelectric precipitation, CPIB: Cowpea protein isolate prepared by micellization precipitation
Fig. 2: Preparation of cowpea protein isolates by isoelectric (CPIA) and micellization precipitate (CPIB)

**Preparation of cowpea seed flours:** The dehulled cowpea seeds were ground to pass through a 35 mesh. The flour was defatted by soaking in petroleum ether (BP. 40-60°C) at room temperature for 48 h with several changes of the solvent. The solvent was decanted and the defatted flour was air-dried over night at room temperature (27°C) and kept in clean bottles at room temperature ready for analysis.

**Protein isolates preparation**

**Preparation of Protein Isolate (PI) by isoelectric precipitation (CPIA):** Protein isolate-A, (CPIA) was prepared from cowpea seed flour as shown in Fig. 2 following the method described by Thompson (1977) slightly modified by Mccurdy and Knipfel (1990) and Fernandez-quintela et al.
(1997). The defatted flours were dispersed in distilled water in a 1:5 (w/v) ratio and the pH of the suspension was adjusted to pH 9.0 with 1 N NaOH. The mixture was stirred at room temperature for 20 min. The insoluble matrixes were separated by refrigerated centrifuge at 4×10⁶ g for 20 min and discarded. The extraction and centrifugation procedures were repeated on the residue. The supernatant was adjusted to pH 4.0 with 1.0 N HCl and stirred at room temperature for 20 min. The mixture was centrifuged in a refrigerated centrifuge (4000 g⁻¹ 20 min). The precipitate was washed by distilled water several times until it was free from the salt and then neutralized by 1.0 N NaOH to pH 7.0. The neutralized precipitate was left over night in refrigerator (4°C). The isolate was dried using freeze-drying and then ground into powder using a ceramic mortar and pestle and finally stored in a desiccators at room temperature until analysis.

Preparation of Protein Isolate (PI) using micellization precipitation (CPIB): Preparation of protein isolate-B using micella method is presented in Fig. 2 as described by Lampart-Szczepa (1996). The defatted seed flour was suspended in NaCl 1.0 N solution in a 1:10 (w/v) ratio, then stirring for 2 h at room temperature. The suspension was centrifuged at 3000xg for 30 min and then the residue was extracted again as described above. The combined supernatant was diluted ten folds by distilled water and left to stand at refrigeration temperature (4°C) for 18 h.

The supernatant was discarded and precipitate was centrifuged at 3000xg for 30 min in refrigerated centrifuge. The precipitated isolate was dried by freeze-drying and proceeded as mentioned for isoelectric isolate (CPIA).

Chemical analysis: Cowpea seed flour and protein isolate composition were determined following methodology for total nitrogen (Kjeldahl), fat (Soxhlet), carbohydrates, moisture and ash (gravimetrically) and crude fibre by a chemical-gravimetric method AOAC (1998) and the means reported on dry weight basis.

Functional properties: Protein solubility The solubility of dehulled defatted cowpea flour and protein isolates as a function of pH was determined using the method described by Sathe et al. (1982). The pH was checked and adjusted then centrifuged at 4000 rpm for 20 min at room temperature and the nitrogen in the supernatant or in aliquot (2.0 mL) of the clear supernatant was estimated by the micro Kjeldahl method (AACC, 1983).

Water and oil absorption capacity: Water and oil absorption capacity of isolates was determined according to the method described by Beuchat (1977).

Emulsifying capacity: Effect of pH on the emulsifying capacity Emulsifying capacity was determined according to the procedure of Beuchat et al. (1975). Emulsifying capacity was calculated as follows:

\[ \text{Emulsifying capacity} = \frac{\text{Weight of oil emulsified}}{\text{Weight of sample taken}} \]

where, Weight of oil emulsified = Total volume of oil emulsified×Specific gravity of oil used.
Gelation capacity: Least gelation concentration was determined using the method of Coffmann and Garcia (1977).

RESULT AND DISCUSSION

Results of the proximate composition of the seed flour and the protein isolates are presented in Table 1. The whole (WCF) and dehulled defatted (DDCF) cowpea seed flour contained 22.30-26.73% protein, 2.10-2.30% fat, 4.10-1.02% fibre, 3.77-3.87% ash and 60-69% carbohydrates, respectively (on dry weight basis) as major components. The data obtained is comparable to that reported by Abdalla et al. (2001), Ragab et al. (2004) and Sosulski et al. (1987). Protein isolates (CPIA and CPIB) showed 75 and 76% protein content and a decrease in carbohydrate content from 59.78 to 13%.

Protein solubility: Solubility of a protein is one of the critical functional attributes required for its use as a food ingredient, because solubility directly influences other functional properties such as emulsion, gelation and foaming (Wang and Kinsella, 1976).

The protein solubility profile of Dehulled Defatted Cowpea Flour (DDCF) and Cowpea Protein Isolates (CPIA) and (CPIB) at pH values ranging from 2-9 is shown in Fig. 3. Generally proteins have a U-shaped pH-solubility curve and are least soluble at pH 4-5 (their isoelectric point). The solubility behavior of cowpea seed flours gave a U-shaped curve in the pH range of 2-9 which is similar to many oil seed and most vegetable legume proteins (Lawhon et al., 1972). The minimum

Table 1: Proximate composition of Whole Cowpea Flour (WCF), Dehulled Defatted Cowpea Flour (DDCF) and protein isolates (CPIA) and (CPIB)% dry basis

<table>
<thead>
<tr>
<th>Chemical constituents</th>
<th>WCF</th>
<th>DDCF</th>
<th>CPIA</th>
<th>CPIB</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein (N x 6.25)</td>
<td>22.30±0.20</td>
<td>26.73±0.06</td>
<td>75.0±0.06</td>
<td>76.0±0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>Crude fat</td>
<td>2.10±0.10</td>
<td>2.30±0.10</td>
<td>Traces</td>
<td>Traces</td>
<td>0.43</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>4.10±0.20</td>
<td>1.02±0.08</td>
<td>Traces</td>
<td>Traces</td>
<td>0.35</td>
</tr>
<tr>
<td>Total ash</td>
<td>3.77±0.06</td>
<td>3.87±0.06</td>
<td>2.63±0.15</td>
<td>2.3±0.20</td>
<td>0.55</td>
</tr>
<tr>
<td>Carbohydrate (by difference)</td>
<td>60.07±0.06</td>
<td>56.78±0.28</td>
<td>13.0±0.17</td>
<td>13.1±0.0</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Means in the same raw with different letters are significantly different (p<0.05). Mean±Standard deviation of triplicate analysis. LSD: Least significant differences. CPIA: Cowpea protein isolate by isoelectric point precipitation. CPIB: Cowpea protein isolate by micelization precipitation.

Fig. 3: Effect of pH on the protein solubility profile of Dehulled Defatted Cowpea Flour (DDCF) and protein isolates (CPIA and CPIB)
protein solubility of DDCF and CPIA were 25 and 5% respectively at pH 5.0 and 28% for CPIB at pH 4.0. Similar isoelectric points were observed in some legume flours, such as winged bean flour (Narayana and Narasimha Rao, 1982), lablab flour and protein concentrate (Melaku, 1998), lablab and cowpea flours (Elkhalifa, 1997) and chick pea flour (Carcea-Bencini, 1986). Ragab et al. (2004) reported minimum solubility at pH 4, 5 and 6 and maximum solubility at pH 10 for cowpea protein. More cowpea protein isolate showed lower protein solubility at low pH and high NaCl concentration but increase with increase in pH (Aluko and Yada, 1997). A protein in an aqueous system has a zero net charge at its isoelectric point and no migration occurs, above and below this pH a protein usually has the highest solubility. Protein-protein interaction increases because the electrostatic forces of the molecules are at a minimum and less water interacts with the protein molecules. At pH values above and below the isoelectric point the protein solubility progressively increased. Proteins has a positive or negative charge at pH values above and bellow the isoelectric point, where more water interacts with the protein charges. Total protein isolate studied showed good solubility in both acid and alkaline pH regions which is most important characteristics for food formulation (Idouraine et al., 1991).

**Water and oil absorption capacity:** DDCF had a water absorption capacity of 1.3 mL water g⁻¹ sample and 1.04 mL oil g⁻¹ sample respectively (Table 2) which was comparable to that reported for cowpea flour (Abu et al., 2005). While CPIA gave 2.10 mL water g⁻¹ sample and 1.90 mL oil g⁻¹ sample, CPIB gave 2.33 mL water g⁻¹ sample and 2.37 mL oil g⁻¹ sample similar to that reported by Ragab et al. (2004). The data obtained within the commercial values reported for protein concentrate (1.9-2.210 mL water g⁻¹ protein) as reported by Lin and Zayas (1987). The water absorption capacity by the defatted defatted cowpea seed flour in the present investigation was lower than that for soy bean (1.80 g g⁻¹) (Chau and Cheung, 1998) but was comparable to that reported for cowpea flour (1.13 g g⁻¹) (Abu et al., 2005). The higher water absorption of soy bean flour could be due to its higher protein level (35.82%) which was higher than that of cowpea flour (26.7%). The water absorption capacity was reported to increase with increasing level of protein content (Lin et al., 1974; Kinsella, 1979; Rhee et al., 1981). Thus CPIA and CPIB showed better performance than DDCF with respect to these properties. Thus would be more useful in food system such as baking products which required hydration to improve handling characteristics.

**Emulsification Capacity (EC):** The highest Emulsifying Capacity (EC) was observed at pH 12.0 for DDCF (173 oil g⁻¹ protein) and CPIA (160 oil g⁻¹ protein) while CPIB have highest EC (137 oil g⁻¹ protein) at pH 2.0 (Table 3).

The highest Emulsifying Capacity (EC) was observed at pH 12.0 for DDCF (173 oil g⁻¹ protein) and CPIA (160 oil g⁻¹ protein) while CPIB have highest EC (137 oil g⁻¹ protein) at pH 2.0.

<table>
<thead>
<tr>
<th>Type of product (mL g⁻¹)</th>
<th>Water absorption capacity (mL g⁻¹)</th>
<th>Fat absorption capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehulled Defatted Flour (DDCF)</td>
<td>1.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cowpea Protein Isolate-A (CPIA)</td>
<td>2.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.90&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cowpea Protein Isolates-B (CPIB)</td>
<td>2.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEMs</td>
<td>0.11</td>
<td>0.11</td>
</tr>
</tbody>
</table>

SEM<sub>s</sub> is standard error mean of triplicate determinations. Means within the same column with different letters are different at p<0.05. CPIA: Cowpea protein isolate by isoelectric point precipitation, CPIB: Cowpea protein isolate by micellization precipitation.
Table 3: Effect of pH on emulsifying capacity of Dehulled Defatted Cowpea Flour (DDCF) and protein isolates (CPIA) and (CPIB)

<table>
<thead>
<tr>
<th>Type of product</th>
<th>pH</th>
<th>Emulsifying capacity (g oil g$^{-1}$ sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehulled Defatted Cowpea Flour</td>
<td>2</td>
<td>105.89</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>133.79</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>73.89</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>72.79</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>173.69</td>
</tr>
<tr>
<td>Cowpea Protein Isolate A (CPIA)</td>
<td>2</td>
<td>133.99</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>107.49</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>133.99</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>146.99</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>160.69</td>
</tr>
<tr>
<td>Cowpea Protein Isolate B (CPIB)</td>
<td>2</td>
<td>137.79</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>111.19</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>106.80</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>115.56</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>115.96</td>
</tr>
</tbody>
</table>

SEMs: 0.3973  
Coefficient of Variation (C.V%): 0.67

Means with in the same column with different letters are different at p<0.05. CPIA: Cowpea protein isolate by isoelectric point precipitation, CPIB: Cowpea protein isolate by micellization precipitation.

Table 4: Least gelaition concentrations of Dehulled Defatted Cowpea Flour (DDCF) and Cowpea Protein Isolate (CPIA and CPIB) at pH 4 and 7

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehulled defatted cowpea flour (DDCF)</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cowpea protein isolate-A (CPIA)</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cowpea protein isolate-B (CPIB)</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cowpea protein isolate-B (CPIB)</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

- : No gelation, +: Gelation

However, highest EC was obtained by DDCF (173 oil g$^{-1}$ protein) at pH 12.0. The emulsion capacity for both Cowpea Protein Isolates (CPIA and CPIB) was higher at pH 7.0 compared to value obtained from DDCF.

The emulsion capacity for both Cowpea Protein Isolates (CPIA and CPIB) was higher at pH 7.0 compared to value obtained from DDCF. The emulsion capacity for both Cowpea Protein Isolates (CPIA and CPIB) in the present investigation was higher, comparable to values reported for lupin seed protein concentrate (88.9 g g$^{-1}$). Sathe et al. (1982), the values of several oil seed flours and protein concentrates/isolates (Crenwelge et al., 1974; Sosulski and Youngs, 1979). The high emulsifying capacity of cowpea seed protein isolate may be useful for food applications.

Gelation: Gelation is an aggregation of denatured molecules. Least Gelation Concentrations (LGCs) of cowpea flour and both protein isolates (CPIA) and (CPIB) both at pH 4.0 and 7.0 are shown in Table 4. Least gelation concentration for Dehulled Defatted Cowpea Flour (DDCF) and both Cowpea Protein Isolates (CPIA) and (CPIB) was noted at 12.0% (w/v). These values are
identical to those reported for cowpea protein isolate (Horax et al., 2004), lupin seed flour (Sathe et al., 1982), (Phaseolus calcarus) (Chau and Cheung, 1998) black gram flour (Sathe et al., 1983) but lower than those reported for raw cowpea (16% W/V). Chau and Cheung (1998) and Sathe et al. (1982) reported that the least gelation concentrations of the lupin seed flour and the protein concentrate were 14 and 8% (w/v), respectively.

CONCLUSION

The variation in gelling properties of different legume flours may be due to variation in the relative ratios of different constituents such as proteins, lipids and carbohydrates make up of the legumes. Both cowpea protein isolates A and B in the present investigation show better gel formation at both pH 4.0 and 7.0 when compared to raw cowpea flour. This may be relative to protein content. Gelling depends on protein concentrate.

REFERENCES


