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

I.I. Khalid, S.B. Elhardallou and E.A. Elkhalifa
Department of Food Science and Technology, Faculty of Engineering and Technology, University of Gezira, Sudan

Corresponding Author: I.I. Khalid, Department of Food Science and Technology, Faculty of Engineering and Technology, University of Gezira, Sudan

ABSTRACT

Protein isolates from dehulled defatted cowpea (*Vigna ungiculata* 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.

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

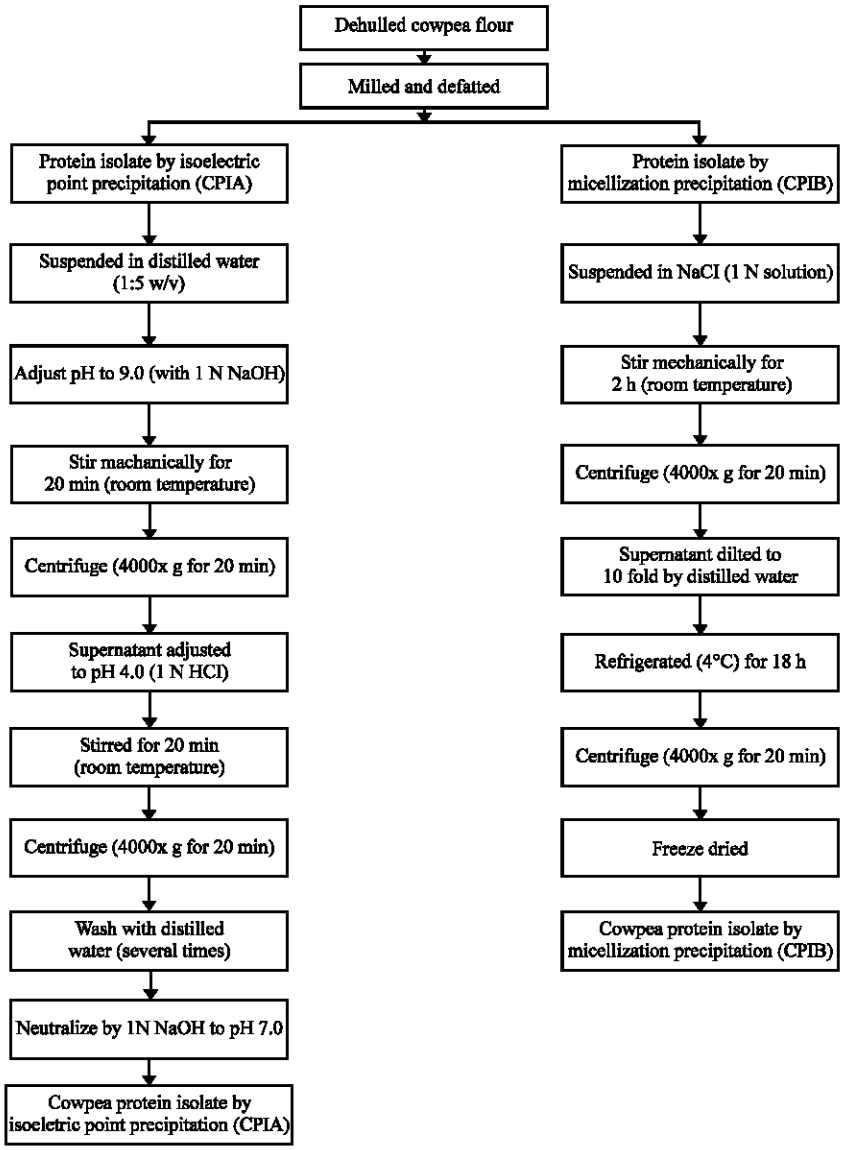

Fig. 2: Preparation of cowpea protein isolates by isoelectric (CPIA) and micellization precipitate (CPIB)

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 matrices were separated by refrigerated centrifuge at 4×10^3 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 (4.0°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-Szczapa (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 3000x g 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 3000x g 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 60-59% 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

Chemical constituents	WCF	DDCF	CPIA	CPIB	LSD
Crude protein (N x 6.25)	$22.30^{d}\pm0.20$	$26.73^{c}\pm0.06$	$75.0^{b}\pm0.06$	$76.0^{a}\pm0.12$	0.26
Crude fat	$2.10^{a}\pm0.10$	$2.30^{a}\pm0.10$	Traces	Traces	0.43
Crude fibre	$4.10^{a}\pm0.20$	$1.02^{b}\pm0.08$	Traces	Traces	0.35
Total ash	$3.77^{a}\pm0.06$	$3.87^{a}\pm0.06$	$2.63^{b}\pm0.15$	$2.3^{b}\pm0.20$	0.55
Carbohydrate (by difference)	$60.07^{a}\pm0.06$	$59.78^{a}\pm0.28$	$13.0^{b}\pm0.17$	$13.1^{b}\pm0.0$	0.43

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

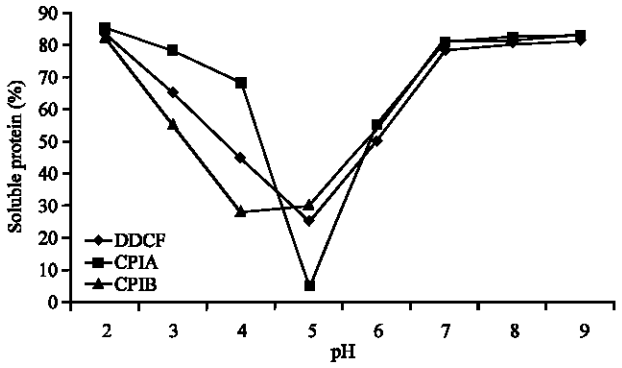

Fig. 3: Effect of pH on the protein solubility profile of Dehulled Defatted Cowpea Flour (DDCF) and protein isolates (CPIA and CPIB)

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 Narasinga 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 over 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 dehulled 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 2: Water and fat absorption capacities of dehulled defatted flour (DDCF) and Cowpea Protein Isolates (CPIA) and (CPIB)

Type of product (mL g^{-1})	Water absorption capacity (mL g^{-1})	Fat absorption capacity
Dehulled Defatted Flour (DDCF)	1.30^{b}	1.04^{c}
Cowpea Protein Isolate-A (CPIA)	2.10^{a}	1.90^{b}
Cowpea Protein Isolates-B (CPIB)	2.33^{a}	2.37^{a}
SEM±	0.11	0.11

SEM± 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)

Type of product	pH	Emulsifying capacity (g oil g^{-1} sample)
Dehulled Defatted Cowpea Flour (DDCF)	2	105.80^{i}
	4	133.70^{e}
	7	73.86^{j}
	9	72.73^{j}
	12	173.60^{a}
Cowpea Protein Isolate A (CPIA)	2	133.60^{e}
	4	107.40^{h}
	7	133.90^{e}
	9	146.90^{c}
	12	160.60^{b}
Cowpea Protein Isolate B (CPIB)	2	137.70^{d}
	4	111.10^{g}
	7	106.80^{hi}
	9	115.50^{f}
	12	115.90^{f}
SEM±		0.3978
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 gelation concentrations of Dehulled Defatted Cowpea Flour (DDCF) and Cowpea Protein Isolate (CPIA and CPIB) at pH 4 and 7

		Percent protein concentration				
Sample	pH	4	8	12	16	20
Dehulled defatted cowpea	4	-	-	+	+	+
flour (DDCF)	7	-	-	+	+	+
Cowpea protein isolate-A	4	-	-	+	+	+
(CPIA)	7	-	-	+	+	+
Cowpea protein isolate-B	4	-	-	+	+	+
(CPIB)	7	-	-	+	+	+

-: 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 compare to value obtained from DDCF.

The emulsion capacity for both Cowpea Protein Isolates (CPIA and CPIB) was higher at pH 7.0 compare 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

AACC, 1983. Approved Methods of the American Association of Cereal Chemists. 8th Edn., American Association of Cereal Chemists Inc., USA.

AOAC, 1998. Official Method of Analysis. 16th Edn., Association of Official Analytical Chemists, Washington DC, USA.

Abdalla, M.H., A.O. Elkhalifa and A.H. Eltinay, 2001. Factors affecting protein extraction from cowpea. J.Food Sci. Technol., 38: 532-533.

Abu, J.O., K. Muller, K.G. Duodu and A. Minnaar, 2005. Functional properties of cowpea (*Vigna unguiculata* L. Walp) flours and pastes as affected by γ irradiation. Food Chem., 93: 103-111.

Aguilera, J.M. and F.V. Kosikowski, 1976. Soybean extruded product: A response surface analysis. J. Food Sci., 41: 647-651.

Akinyele, I.O., M.H. Love and M. Ringe, 1988. Nutrient composition of extruded cowpea products. Int. J. Food Sci. Technol., 23: 297-301.

Aluko, R.E. and R.Y. Yada, 1997. Some physicochemical and functional properties of cowpea (*Vigna unguiculata*) isoelectric protein isolate as a function of pH and salt concentration. Int. J. Food Sci. Nutr., 48: 31-39.

Bahnassey, Y., K. Khan and R. Harrold, 1986. Fortification of spaghetti with edible legumes. I. Physicochemical, antinutritional, amino acid and mineral composition. Cereal Chem., 63: 210-215.

Beuchat, L.R., J.B. Cherry and M.R. Quinn, 1975. Physicochemical properties of peanut flour as affected by proteolysis. J. Agric. Food Chem., 23: 616-620.

Beuchat, L.R., 1977. Functional and electrophoretic characteristics of succinylated peanut flour proteins. J. Agric. Food Chem., 25: 258-261.

Carcea-Bencini, M., 1986. Functional properties of drum dried chickpea (*Cicer arietinum* L.) flour. J. Food Sci., 51: 1518-1526.

Chau, C.F. and P.C.K. Cheung, 1998. Functional properties of flours prepared from three Chinese indigenous legume seeds. Food Chem., 61: 429-433.

Coffmann, C.W. and V.V. Garciaj, 1977. Functional properties and amino acid content of a protein isolate from mung bean flour. Int. J. Food Sci. Technol., 12: 473-484.

Crenwelge, D.D., C.W. Dill, P.T. Tybor and W.A. Landmann, 1974. A comparison of the emulsification capacities of some protein concentrates. J. Food Sci., 39: 175-177.

Elhardallou, S.B., A.H. Eltinay and A.A.M. Nour, 1980. Chemical characteristics of some legumes grown in the Sudan. Sudan J. Food Sci. Technol., 12: 35-42.

Elkhalifa, E.A., 1997. Effect of heat treatment on physico-chemical and functional properties of lablab bean and cowpea flours and their utilization in Kisra and bread products. A Report of the Work Carried out under U.N.U. Fellowship. Central Food Technology Research Institute, Mysore, India.

Fernandez-Quintela, A., M.T. Macarulla, A.S. del Barrio and J.A. Martinez, 1997. Composition and functional properties of protein isolates obtained from commercial legumes grown in Northern Spain. Plant Foods Hum. Nutr., 51: 331-341.

Horax, R., N.S. Hettiarachchy, P. Chen and M. Jalaluddin, 2004. Preparation and characterization of protein isolate from cowpea (*Vigna unguiculata* L. Walp.). J. Food Sci., 69: fct114-fct118.

Idouraine, A., S.B. Yensen and C.W. Weber, 1991. Tepary bean flour, albumin and globulin fractions functional properties compared with soy protein isolate. J. Food Sci., 56: 1316-1318.

Jansen, R. and J.M. Harper, 1980. Application of low-cost extrusion cooking to weaning foods in feeding programmes. Part 1. Food Nutr. (Roma), 6: 2-9.

Kinsella, J.E., 1979. Functional properties of soy proteins. J. Am. Oil Chem. Soc., 56: 242-258.

Lampart-Szczapa, E., 1996. Preparation of protein from lupin seeds. Food/Nahrung, 40: 71-74.

Lawhon, J.T., C.M. Cater and K.F. Mattil, 1972. A comparative study of whipping potential of an extract from several oil seed flours. Cereal Sci. Today, 17: 240-243.

Likeimani, T.A., J.N. Sofos, J.A. Maga and J.M. Harper, 1991. Extraction cooking of corn/soy bean mixture in the presence of thermo stable α amylase. J. Food Sci., 56: 99-108.

Lin, C.S. and J.F. Zayas, 1987. Functionality of defatted corn germ proteins in a model system: Fat binding and water retention. J. Food Sci., 52: 1308-1311.

Lin, M.J.Y., E.S. Humbert and F.W. Sosulski, 1974. Certain functional properties of sunflower meal products. J. Food Sci., 39: 368-370.

Mccurdy, S.M. and J.E. Knipfel, 1990. Investigation of faba bean protein recovery and application of pilot scale processing. J. Agric. Food Chem., 55: 1093-1094.

Melaku, T.W., 1998. Functional properties of lablab bean protein concentrate. M.Sc. Thesis, University of Gezira Sudan.

Michaelsen, K.F. and H. Friis, 1998. Complementary feeding: A global perspective. Nutrition, 14: 763-766.

Molina, M.R., H. Gudiel, M.A. Baten and R. Bressani, 1982. Production of high-protein quality pasta products using a semolina/corn/soy flour mixture. III. Effect of cooking on the protein nutritive value of pasta. Cereal Chem., 59: 34-37.

Mustafa, A.I., M.S. Al-Wessali, O.M. Basha and R.H. Al-Amir, 1986. Utilization of cowpea flour and protein isolate in bakery products. Cereal Foods World, 31: 756-759.

Narayana, K. and M.S. Narsing Rao, 1982. Functional properties of raw and processed winged bean (*Psophocarpus tetragonolobus*) flours. J. Food Sci., 47: 1534-1538.

Olsen, R.E., 1975. Protein Calorie Nutrition. Academic Press, New York, USA.

Rachie, K.O., 1985. Nutritive Value of Cowpea. In: Cowpeas Research, Production and Utilization, Singh, S.R. and K.O. Rachie (Eds.). John Wiley and Sons, New York, USA., pp: 21-23.

Ragab, D.D.M., E.E. Babiker and A.H. Eltinay, 2004. Fractionation, solubility and functional properties of cowpea (*Vigna unguiculata*) proteins as affected by pH and/or salt concentration. J. Food Chem., 84: 207-212.

Rhee, K.C., C.K. Kuo and E.W. Lusas, 1981. Texturization. In: Protein Functionality in Foods, Cherry, J.P. (Ed.). American Chemical Society, Washington, DC.

Ringe, M.L. and M.H. Love, 1988. Kinetics of protein quality change in an extruded cowpea-corn flour and under varied steady state storage conditions. J. Food Sci., 53: 584-588.

Sathe, S.K., S.S. Deshpande and D.K. Salunkhe, 1982. Functional properties of lupin seed (*Lupirinus nutabilis*) proteins and protein concentrates. J. Food Sci., 47: 491-497.

Sathe, S.K., S.S. Deshpande and D.K. Salunkhe, 1983. Functional properties of black gram (*Phaseolous mungo* L.) proteins. Lebensm. Wiss. Technol., 16: 69-72.

Sosulski, F.W., E.N. Kasirye-Alemu and A.K. Sumner, 1987. Microscopic, nutritional and functional properties of cowpea flours and protein concentrates during storage. J. Food Sci., 52: 700-706.

Sosulski, F.W. and C.G. Youngs, 1979. Yield and functional properties of air classified protein and starch fractions from eight flours. J. Am. Oil Chem. Soc., 56: 292-295.

Thompson, L.U., 1977. Preparation and evaluation of mungbean protein isolates. J. Food Sci., 42: 202-206.

Wang, J.C. and J.E. Kinsella, 1976. Functional properties of novel proteins: Alfalfa leaf proteins. J. Food Sci., 41: 286-289.