

Global Advanced Research Journal of Food Science and Technology (ISSN: 2315-5098) Vol. 2(3) pp. 035-043, July, 2013
Available online <http://garj.org/garjfst/index.htm>
Copyright © 2013 Global Advanced Research Journals

Full Length Research Paper

Chemical composition and nutritional evaluation of a cowpea protein concentrate

Martin Alain Mune Mune^{b*}, Samuel René Minka^a, Israël Lape Mbome^b

^a Department of Biochemistry, University of Yaounde I, PO Box 812 Yaounde, Cameroon

^b The Higher Institute of Sahel, The University of Maroua, PO Box 46 Maroua, Cameroon

^c Food and Nutrition Research Centre, PO Box 6163 Yaounde, Cameroon

Accepted 10 October 2012

Cowpea protein concentrate was prepared by isoelectric precipitation, under optimum conditions determined in a previous study. The concentrate contained 86.90 % of the flour total protein, and had 85.82 % protein. Sugars and ash were in lower contents in the concentrate compared to the flour, while Fe and Zn contents were not significantly ($p > 0.05$) different. Phytates and polyphenols were eliminated in abundance during protein extraction and presence of trypsin and α -amylase inhibitors in the concentrate suggested application of an adequate heat treatment before its use in food products. The concentrate in vitro protein digestibility was high (86.81-88.74 %), and had a balanced essential amino acid composition compared to the FAO/WHO reference pattern. Tryptophan and total sulphur amino acids were first and second limiting amino acids. The predicted protein efficiency ratio, essential amino acids index and biological value indicated the good quality of cowpea protein concentrate, although elimination of tryptophan and sulphur-rich proteins during protein extraction affected the chemical score and protein digestibility corrected amino acid score.

Keywords: cowpea, protein concentrate, antinutritional factors, protein quality

INTRODUCTION

There is an increasing world demand of less expensive proteins with good nutritional and functional properties, particularly in developing and under-developed countries where the supply of food of animal origin is limited due to non-availability and high cost (Chefftel *et al.*, 1985). This situation result from constant increase of the human population and growing interest for protein to industry for application in food and non food markets.

Legumes are considered as poor man's meat. They are generally rich in protein (18-25 %), and good sources of minerals and vitamins (Tharanathan and Mahadevamma, 2003). In addition, consumption of legumes has been related to many beneficial physiological effects in controlling and preventing various metabolic diseases such as diabetes mellitus, coronary heart disease and colon cancer (Simpson *et al.*, 1981). Therefore, use of grain legumes for food is restricted by their beany flavor and the presence of antinutritional and toxic factors. Traditional processing techniques such as soaking, cooking, sprouting or roasting have limited effects on elimination of antinutritional factors, and sometimes could decreased protein quality and affected certain functional

Corresponding author Email: alainmune@yahoo.fr

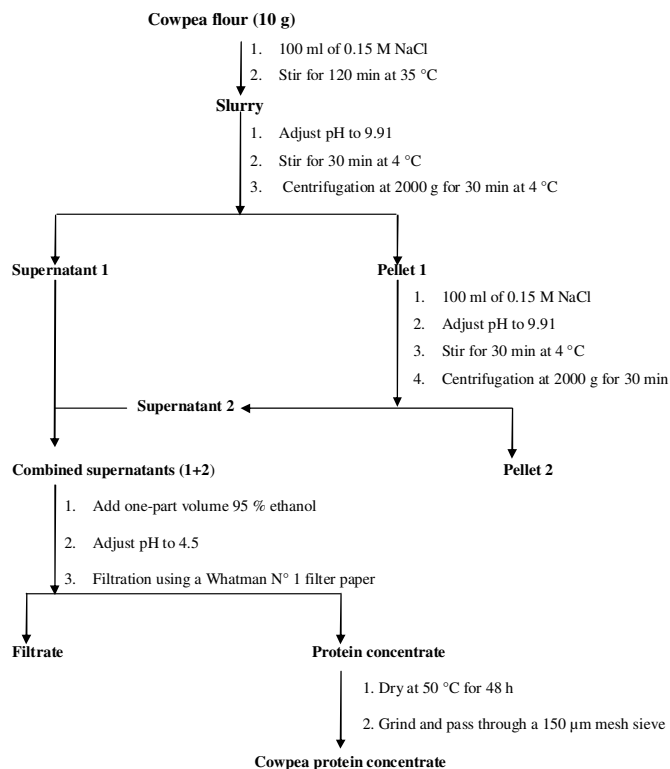


Figure 1. Schematic of cowpea protein concentrate preparation

properties (Friedman, 1992; Yusuf, Ayedun and Sanni, 2008). On the other hand, processing techniques employed during protein extraction are known to be effective in detoxification of seed material. Furthermore, protein extracts have superior functional properties than legumes flours, and are extensively used in industry as nutritional and functional ingredients (Neto *et al.*, 2001).

Cowpea (*Vigna unguiculata*) is a drought tolerant food crop, well adapted in varieties of climates and soils. This crop is widely cultivated throughout the tropics and subtropics, particularly in west and central Africa, with an annual production of 3 millions tones (Onyenekwe *et al.*, 2000). Its grains are used to prepare many traditional foods such as moin-moin or akara. cowpea grains have been used to fortify cereal-based weaning foods, in which they formed complementary amino acid profiles and improved protein quality (Bresani, 1985). Also, McWatters *et al.* (2003) prepared biscuits from cowpea composite flour, with good sensory quality.

Isoelectric precipitation is the most common technique for extracting protein from grain legumes in food industry. This technique involves the variation of physico-chemical parameters which affect product and protein yields (Berot and Davin, 1996). Our previous study reported the optimum combination of these parameters for the preparation of protein concentrate from cowpea seed flour (Mune *et al.*, 2008). In the present study, the chemical composition and the nutritional potential of the concentrate were investigated, in view of its possible use as a nutritional ingredient in the food industry.

MATERIALS AND METHODS

Materials

Cowpea seeds were purchased from Mokolo market (Yaoundé, Cameroon). The seeds were hand-picked and stored in polyethylene bags in the refrigerator (~4 °C) until used.

Methods

Preparation of cowpea flour

Cowpea seeds were washed and rinsed in deionised water at room temperature (25±2 °C). They were soaked for 1 h in water at room temperature (25 ± 2 °C), dehulled and dried in an air convection oven at 50 °C for 72 h. The dried seeds were ground into flour, passed through a 150 µm mesh sieve, stored hermetically in polyethylene bag in a refrigerator at about 4 °C.

Preparation of cowpea protein concentrate

Cowpea protein concentrate was prepared by the isoelectric precipitation method (Figure 1) as described by Mune *et al.* (2008). An aliquot (10 g) of cowpea flour was mixed with 100 ml of NaCl solution (0.15 M) and stirred at 35 °C for 120 minutes. The pH was adjusted to

Table 1. Chemical composition of cowpea flour and protein concentrate (dry weight basis)^a

	Flour	Protein concentrate
Nutritional factor (g/ 100 g)		
Moisture	5.77 ± 0.21 b	8.88 ± 0.46 a
Crude protein	21.95 ± 0.00 b	85.82 ± 1.08 a
Non-protein nitrogen	1.62 ± 0.04 a	1.63 ± 0.05 a
Protein nitrogen	20.33 ± 0.00 b	84.19 ± 1.08 a
Crude lipid	1.21 ± 0.09 b	2.39 ± 0.02 b
Fibre (NDF)	5.75 ± 0.15 a	1.73 ± 0.37 b
Starch (by difference)	57.01 ± 1.47 a	8.89 ± 1.48 b
Total sugars	9.41 ± 0.39 a	1.11 ± 0.00 b
Reducing sugars	0.30 ± 0.01 b	0.75 ± 0.06 a
Ash	4.67 ± 0.14 a	1.79 ± 0.38 b
Minerals (mg /100 g)		
Iron	19.39 ± 1.33 a	19.50 ± 1.03 a
Zinc	9.93 ± 2.15 a	8.28 ± 0.31 a

^a Means followed by different letters (a-b) in the same line are significantly ($p < 0.05$) different

9.91 using a Hanna Model HI 8521 pH-meter (Hanna Instruments, Portugal), and the mixture was further stirred at 4 °C for 30 min. The resultant slurry was then centrifuged at 2000 g for 30 min at 4 °C using a Jouan Model GR 4.11 centrifuge (Jouan, Saint Nazaire, 44600, France). The pellet obtained after recovering the supernatant, was dissolved in the initial NaCl solution at the above liquid to solid ratio under stirring. The pH was adjusted to the initial value and the slurry stirred for 30 min at 4 °C and then centrifuged as previously explained. The resultant supernatants of the two alkaline extractions were combined and one-part volume of 95 % (v/v) ethanol added. The pH was adjusted to 4.5 under stirring and the precipitated proteins were recovered by filtration under vacuum using a whatman N° 1 filter paper. The protein concentrate was dried at 50 °C for 48 h in an air convection oven, ground and passed through a 150 µm mesh sieve.

Proximate composition

Moisture, ash, total lipids and crude protein (Nx6.25) were determined according to AOAC (1990) methods. Dietary fibre was analysed using neutral acid detergent (Goering, and Van Soest, 1970). Non-protein nitrogen (NPN) was determined by the method of Bhatta and Finlayson (1973) as modified by Naczka *et al.* (1985) by which proteins were precipitated with 10 % trichloroacetic acid (TCA) solution, and the resultant non-protein nitrogen was determined according to the Kjeldahl procedure. Iron and zinc contents were determined by atomic absorption spectrophotometry using a Unicam

Model 969 atomic absorption spectrophotometer (Unicam Limited, York Street, Cambridge, CB1 2PX, United Kingdom), after digestion of 0.25 g sample with 6 ml of concentrated nitric acid at 150 ± 5 °C for 6 h according to Laurent (1981). Total simple sugars were determined by the anthron method (Montreuil *et al.*, 1981) and reducing sugars by the 3,5-dinitrosalicylic acid (DNSA), following the sugars extraction in hot 80 % (v/v) ethanol (Cerning, and Guilhot, 1973). Starch (+maltodextrins) was determined by difference.

Antinutritional factors

Polyphenols

Polyphenol content was determined according to Singleton and Rossi (1965) as gallic acid equivalents, after their extraction in 70 % (v/v) aqueous acetone (Shahidi, and Naczka, 1989).

Phytate

Phytate content was determined based on complex formation of phytic acid and Fe(III)-ion at pH 1-2 (Stone *et al.*, 1984), after extraction in 1.2 % HCl solution containing 10 % Na₂SO₄ as described by Thompson and Erdman (1980). An excess of Fe(III)-ion present in the solution would react with thiocyanate ion to form a characteristic pink complex, Fe(SCN)₃. The optical density at 465 nm was measured (Itabashi, 1985), and an inverse linear relation was found for phytate

Table 2. Amino acid composition of cowpea flour and protein concentrate (g/16 g N)

Amino acid	Flour	Protein concentrate	FAO/WHO (1991)
Histidine	3.60	3.45	1.9
Isoleucine	4.30	4.49	2.8
Leucine	8.61	9.45	6.6
Lysine	6.81	6.50	5.8
Thréonine	4.77	4.25	3.4
Tryptophan	0.97	0.27	1.1
Valine	5.54	5.46	3.5
Méthionine	0.49	0.22	
Cysteine	1.61	1.24	
Total sulfur amino acids	2.10	1.46	2.5
Tyrosine	1.44	2.13	
Phénylalanine	4.76	5.58	
Total aromatic amino acids	6.20	7.71	6.3
<u>Total essential amino acids</u>	<u>42.90</u>	<u>43.04</u>	<u>33.9</u>
Aspartic acid	12.63	13.03	
Serine	6.48	6.55	
Glutamic acid	15.04	15.56	
Proline	4.68	4.91	
Glycine	7.51	6.63	
Alanine	7.61	6.63	
Arginine	3.15	3.65	
Total non-essential amino acids	<u>60.70</u>	<u>60.41</u>	
<i>Leucine / isoleucine ratio</i>	2.00	2.10	2.36

concentration from 40 to 200 nmol/L.

Trypsin inhibitor activity

Trypsin inhibitor activity was determined based on the method described by Lqari *et al.* (2002) using soluble casein as substrate, after the extraction of protease inhibitor in a 0.02 M pH 8.0 Tris (trihydroxymethane) buffer solution containing 0.02 M CaCl₂ (Griffiths, 1984). The assay mixture contained 2 ml of inhibitory solution, 2 ml of trypsin solution [2.5 mg of bovin trypsin (SIGMA, 15,900 u/mg) in 25 ml of 0.01 N HCl], and 5 ml of soluble casein solution (0.2 % in Tris-HCl buffer, pH 8.2, 0.05 M). Before the addition of the substrate, the mixture was incubated for 30 min at 37 °C, to allow the binding of the inhibitors to the protease. Casein solution was then added and the reaction incubated for 20 min at 37 °C. The reaction was stopped by addition of 2.25 ml of 25 % TCA solution. The samples were centrifuged at 2000 g for 30 min at 4 °C. A blank set was prepared by adding TCA before the addition of the protease solution. A control set was prepared in which inhibitory solution was deleted from the assay mixture. The released tryptophan was determined in an aliquot of the supernatant by the ninhydrin reaction as described by Panasuik *et al.* (1998). The trypsin inhibitory unit (TIU) was expressed as that

producing the inhibition of 1 mg pure trypsin.

α-Amylase inhibitor activity

α-Amylase inhibitory activity was determined based on the method described by Bandary *et al.* (2008), after extraction of α-amylase inhibitors as described by Lonstaff and Mc Nab (1991). The assay mixture contained 2 ml of inhibitory solution, 50 µL of porcine pancreatic α-amylase [5 mg/ml in Tris-HCl 0.05; pH 6.9, containing 0.01 M CaCl₂], 2 ml of the Tris-HCl buffer pH 6.9, 2 ml of soluble starch (5 mg/ml in Tris-HCl buffer pH 6.9) soaked in boiling water for 5 min. Before the addition of the substrate, the mixture was incubated for 60 min at 37 °C, to allow binding of the inhibitors to the enzyme. Starch was added and the reaction incubated at 37 °C for 20 minutes. The reaction was stopped by addition of 6 ml ethanol 90 % (v/v), and the test tubes plunged in and ice bath for 10 minutes. The samples were centrifuged at 2000 g for 15 min. The release of reducing groups (calculated as maltose equivalents) was determined in an aliquot of the supernatant by the 3,5-DNSA method. A blank set was prepared by adding ethanol 90 % (v/v) before the addition of the α-amylase solution. A control set was prepared in which inhibitory solution was deleted from the assay mixture. The α-amylase inhibitory unit

Table 3. Antinutritional factors and *in vitro* protein digestibility of cowpea flour and protein concentrate ^a

Materials	Antinutritional factors				<i>In vitro</i> protein Digestibility (%)	
	Polyphenol (mg/100 g)	Phytate (g/100 g)	α -AI ^b (AIU/g)	TI ^c (TIU/g)	Pepsin-Trypsin	Pepsin-Pancreatin
Cwpc	162.98 ± 7.61 a	1.01 ± 0.03 a	1.35 ± 0.13 a	5.32 ± 0.20 a	51,53 ± 0,00 b	59,58 ± 1,62 b
Cwpc	122.26 ± 7.46 b	0.71 ± 0.01 b	1.40 ± 0.13 a	2.93 ± 0.31 b	86,81 ± 2,41 a	88,74 ± 0,63 a

^a Cwpc, cowpea flour; Cwpc, cowpea protein concentrate; α -AI, α -amylase inhibitor; TI, trypsin inhibitor; Mean in the same column followed by different letters (a-b) are significantly ($p < 0.05$) different

^b Expressed as mg pure trypsin inhibited (TIU) per g sample (in dwb)

^c Expressed as 1 μ mol maltose equivalents/min inhibited (AIU) per g sample (in dwb)

(AIU) was defined as one unit of α -amylase activity (1 μ mol maltose/min) inhibited.

measurements were performed using a Spectronic Model 601 spectrophotometer (Milton Roy company, Rochester, NY, 14625, USA).

Amino acids

Amino acids were determined using a BECKMAN 6300 amino acid analyser according to the method of Spackman *et al.* (1958). Hydrolysis of samples was performed in the presence of 6 M HCl, trifluoroacetic acid (TFA, 2:1, v/v) and 5 % thioglycolic acid, for 24 h at 100 °C. Cystein was determined by the method of Beveridge *et al.* (1974) using 5,5'-dithio-2-nitrobenzoate (DTNB), and the absorbance read at 412 nm. Tryptophan was determined by ultraviolet molar absorption coefficient of each sample in a Tris-Gly buffer (0.086 M Tris, 0.09 M glycine, 0.04 M EDTA, pH 8) containing 8 M urea as described by Pace *et al.* (1995).

In vitro protein digestibility and available lysine

In vitro digestibility was determined using trypsin-pepsin (Chavan *et al.*, 2001) and pepsin-pancreatin (Akeson, and Stahman, 1964) enzymatic systems using bovin trypsin (SIGMA, 15900 U/mg), pepsin (SIGMA, 1mAnson-E/mg) and pancreatin (SIGMA, 4 usp). The nitrogen content of the TCA-soluble matter was determined by the Kjeldahl method (AOAC, 1990). Protein digestibility was expressed as the percentage of the soluble TCA 10 % nitrogen, with respect to the total nitrogen content of the undigested sample.

Available lysine (g/16 g N) was determined by dye binding procedure using 1-phenylazo-2-naphthol-6-sulfonic acid (Orange 12), as described by Hurrell *et al.* (1979). A sample aliquot containing 15 mg of 'Arg + His + Lys' was mixed with 4 ml of half saturated sodium acetate, and 40 ml of Orange 12 reagent were added directly for 'Arg + His + Lys' determination; or after propionylation of lysine with propionic anhydride for 'Arg + His' determination. Difference in absorbance between the two at 475 nm after 2h reaction in the dark at ambient temperature was used for calculating reactive lysine. Absorbance

Determination of nutritional parameters

Nutritional parameters were determined on the basis of the amino acid profiles. A chemical scoring of amino acids was calculated using the FAO/WHO (1991) reference pattern. Essential amino acid index (EAAI) was calculated according to Oser (1959) using as standard the amino acid composition of the whole egg protein published by Cheftel *et al.* (1985). Protein efficiency ratio (PER) was estimated according to the regression equations developed by Alsmeyer *et al.* (1974), as given below:

$$PER_1 = -0.684 + 0.456(\text{LEU}) - 0.047(\text{PRO}) \quad (1)$$

$$PER_2 = -0.468 + 0.454(\text{LEU}) - 0.105(\text{TYR}) \quad (2)$$

Biological value was calculated according to Oser (1959) using the following equation:

$$BV = 1.09 \times \text{EAAI} - 11.7 \quad (3)$$

Statistical analysis

Results are expressed as mean value \pm standard deviation of three different determinations, except for amino acid contents. The data were statistically analysed by the Student-Newman-Keuls test. The computer software used in this study was SPSS (version 10.1, 2000, SPSS Inc., USA).

RESULTS AND DISCUSSION

PREPARATION OF COWPEA PROTEIN CONCENTRATE

The chemical composition of cowpea flour is presented in

Table 4. Nutritional parameters and available lysine of cowpea flour and protein concentrate ^a

Materials	Chemical score (%)	Limiting amino acids		PDCAAS (%)	EAAI (%)	PER		BV (%)	Available lysine (g/16 g N)
		First (CS, %)	Second (CS, %)			PER ₁	PER ₂		
Cwpf	84.00	Cys + Met (84 %)	Trp (88.18 %)	50.05	74.27	3.02	3.29	69.26	1.00 ± 0.04 b
Cwpc	24.55	Trp (24.55 %)	Cys+Met (58.40 %)	21.78	61.85	3.39	3.60	55.71	1.29 ± 0.07 a

^a Cwpc, cowpea protein concentrate; Cwpf, cowpea flour; CS, Chemical score; PDCAAS, protein digestibility corrected amino acid score; EAAI, essential amino acid index; PER, protein efficiency ratio calculated according to Alsmeyer *et al.* (1974) equations; BV, biological value; Means in the same column with different letters (a-b) are significantly ($p < 0.05$) different

Table 1. The high protein content (20.33 %) shows that this flour is a good raw material for the preparation of protein concentrate. Since starch is the major macromolecule (57.01 %), protein concentrate preparation in an industrial scale should take in account starch isolation to improve economical rentability.

Cowpea protein concentrate was obtained with a protein yield (86.90 %) higher than those found by Mwasaru *et al.* (1999) (36.4-53.5 %), Chavan *et al.* (2001) for beach pea (67.9-77.3 %), and Sanchez-Vioque *et al.* (1999) for chickpea (65.9 %). The loss of acid-soluble proteins may in part explain incomplete recovery of protein from the flour. Sanchez-Vioque *et al.* (1999) observed that 18 % of protein extracted from chickpea flour (80.9 %) in alkaline conditions remained soluble at the isoelectric pH. In addition, protein could be retained in the residue due to complexation with other seed material, particularly fibre. Moure *et al.* (2002) showed that enzymatic hydrolysis of fibre from chilean hazelnut seeds favoured protein extractability, and Ma (1983) observed that the fibre-rich fraction obtained after protein extraction from oat seed contained 10-20 % protein.

Chemical composition

The chemical composition of cowpea protein concentrate is presented in Table 1. The protein content was in the same range as those reported by Mwasaru *et al.* (1999), and higher than those of Bambara bean (80.2-81.5 %), benniseed (78.5-80.5 %) and mucuna bean (78.3 %) (Yusuf *et al.*, 2009; Adebawale and Lawal, 2003) protein concentrates. The concentrate (2.39 %) contained two times more lipid than the flour (1.21 %). This concentration of lipids with the protein fraction was also observed by Chavan *et al.* (2001) and Sanchez-Vioque *et al.* (1999). It was suggested that polar lipids were extracted in weak-alkaline conditions, and could interact with proteins. The fibre content of cowpea concentrate (1.73 %) was significantly ($p < 0.05$) lower than that of the flour (5.75 %), and similar to those of beach pea (1.51-1.83 %) and pigeon pea (1.54-1.63 %) protein extracts (Chavan *et al.*, 2001; Mwasaru *et al.*, 1999). Generally, water-soluble sugars and minerals are eliminated in

abundance during protein concentrate preparation. The ash and total sugars contents of the concentrate were significantly ($p < 0.05$) lower than those of the flour. Therefore, the non-significant ($p > 0.05$) Fe and Zn contents of cowpea concentrate and flour suggested that divalent cations could be associated to proteins. There is a 93.48 % reduction in the non-reducing sugars content (difference between total sugars and reducing sugars content) of the cowpea protein concentrate (0.36 %), compared to the flour (9.11 %). This suggested abundant elimination of flatus-causing oligosaccharides such as raffinose, stachyose and verbascose, which are non-reducing and found in cowpea seeds by Onwuliri and Obu (2000) and Onyenekwe *et al.* (2000).

Amino acid composition

The amino acid compositions of cowpea flour and protein concentrate are presented in Table 2. In terms of essential amino acids, cowpea protein concentrate was rich in leucine (9.45 %) and lysine (6.50 %), and poor in tryptophane (0.27 %) and total sulphur amino acids (1.46 %). The major non-essential amino acids were glutamic (15.56 %) and aspartic (13.03 %) acids. Protein concentrate preparation concentrated aromatic amino acids, leucine and isoleucine. The remaining essential amino acids were in lower contents in the concentrate compared to the flour. Sanchez-Vioque *et al.* (1999) related the lower contents of these amino acids in chickpea protein extracts compared to the flour to the high reduction of albumins, which are rich in lysine, cysteine and methionine. Albumins were probably loosen during isoelectric precipitation, since it has been reported that the acid-soluble fraction of legume protein contain a higher proportion of nutritionally essential sulphur amino acid-rich proteins, than does the acid precipitable fraction (Cerletti *et al.*, 1985; Oomah and Bushuk, 1983).

The quality of proteins as source of amino acids can usually be assessed by comparison with the FAO/WHO (1991) recommended pattern of essential amino acids. Cowpea protein concentrate had higher total essential amino acids than the FAO/WHO (1991) reference pattern. In addition, histidine, isoleucine, leucine, lysine,

threonine and valine contents met the FAO/WHO (1991) requirements for infants, while tryptophan and total sulphur amino acids were in non-adequate levels. Generally, higher lysine and leucine, lower tryptophan and sulphur amino acids contents are common to most legume proteins. These results showed that cowpea protein concentrate could be used to complement cereal proteins, which contain high amount of total sulphur amino acids and are low in lysine. The leucine/isoleucine ratio of cowpea concentrate (2.1) was in ideal range suggested by FAO/WHO (1991). Deosthale *et al.* (1970) showed that excess leucine in foods interfered with the utilization of isoleucine and lysine.

Antinutritional factors and *in vitro* protein digestibility

Antinutritional factors and *in vitro* protein digestibility of cowpea flour and protein concentrate are presented in Table 3. A 25 % reduction in polyphenolic content was observed in cowpea protein concentrate compared to the flour. The polyphenolic content of the concentrate was 122.26 mg/100g. Polyphenolic compounds can interact with protein and reduce their digestibility, as well as alter amino acid availability and functional properties (Lin *et al.*, 1974). In this regards, low levels of polyphenol are desirable. Shahidi *et al.* (1999) and Chavan *et al.* (2001) observed a 94 % reduction in polyphenolic content of beach pea protein extracts compared to the flour, and the concentrates contained 50.5-76.4 mg/100 g polyphenol. Cowpea protein concentrate was significantly ($p < 0.05$) lower in phytate content (0.71 %) than the corresponding flour (1.01 %). Phytates influence the nutritional and functional properties of grain legumes and their derivatives by forming complexes with proteins, amino acids (Reddy and Salunkhe, 1981) and trace minerals (Erdman, 1981). For nutritional application of cowpea protein concentrate, inactivation of enzymes inhibitors by an adequate heat treatment will be required, since 55 % of the trypsin inhibitor activity of the flour was found in the concentrate, and α -amylase inhibitory activity was not significantly ($p > 0.05$) different in cowpea flour and concentrate.

In vitro protein digestibility of cowpea protein concentrate (86.81-88.74 %) was in the same range than that obtained *in vivo* by Rangel *et al.* (2004) (86.90 %), and those of sweet and bitter lupin (86.90-90.80 %) protein extracts, obtained with a trypsin-pancreatin enzymatic system (El-Adawy *et al.*, 2001). Protein digestibility of cowpea concentrate was significantly ($p < 0.05$) higher than that of the flour (51.53-59.58 %). Improvement in protein digestibility was attributed to the denaturation of proteins and the reduction of antinutritional factors. Since protease inhibitors are

albumins (Richardson, 1991), they were removed in the protein isolation procedures. Sanchez-Vioque *et al.* (1999) showed that the dissociation of globulin due to action of pH, facilitated their accessibility to digestive proteases, and improved the hydrolysis.

Nutritional parameters and available lysine

The nature and quantity of amino acids contained in a dietary protein, determined the efficiency with which an organism could use the protein. Nutritional parameters and available lysine of cowpea flour and protein concentrate are presented in Table 4. The chemical score and the protein digestibility corrected amino acid score (PDCAAS) of the concentrate (24.55-21.78 %, respectively) were lower than those of the flour (84.00 and 50.05 %, respectively). Based on chemical score, the first and second limiting amino acids of the concentrate were tryptophane and total sulphur amino acids, respectively. Protein efficiency ratio of cowpea protein concentrate (3.39-3.60) was higher than those reported for beach pea (2.75-2.81) and lupin protein extracts (2.22-2.32) (El-Adawy *et al.*, 2001; Chavan *et al.*, 2001). Essential amino acids index (EAAI) and biological value were lower in the cowpea concentrate (66.85 and 55.71 %, respectively) compared to the flour (74.27 and 69.26 %, respectively). This could be attributed to the high reduction of certain essential amino acid content, particularly tryptophan, cysteine and methionine.

Available lysine was significantly ($p < 0.05$) higher in cowpea protein concentrate (1.29 g/16 g N) compared to the corresponding flour (1.00 g/16 g N). These values remained lower than those obtained by the amino acid analysis (6.81 g/16 g N for the flour and 6.50 g/16 g N for the concentrate). Nevertheless, Walker and Feather (1983) showed that a fraction of non-available lysine could be recovered *in vivo* after acid hydrolysis.

CONCLUSIONS

Cowpea protein concentrate prepared under optimum conditions (Mune *et al.*, 2008) does appear to have good potential as a source of low-cost protein, with nutritional quality comparable to those of other grain legume protein concentrates. Presence of enzyme inhibitors indicated that appropriate heat treatment should be necessary before incorporation of cowpea concentrate in food products. The ultimate success of utilizing any seed protein as a food ingredient depends largely on its functional properties. Further work will be conducted to evaluate and improve functional properties of cowpea concentrate, and then promote the use of this seed to alleviate protein malnutrition in developing and under

developing countries.

ACKNOWLEDGEMENTS

This work was done with the resources of the Food and Nutrition Research Centre (IMPM), Ministry of Scientific Research and Innovation, Yaoundé Cameroon, the authors are grateful to M. Bruneteau for Chromatography analyses.

REFERENCES

- Adebowale KO, Lawal OS (2003). Foaming, gelation and electrophoretic characteristics of mucuna bean (*Mucuna pruriens*) protein concentrates. *Food Chemistry* 83: 237-246.
- Akeson WF, Stahman MA (1964). A pepsin-pancreatin digest index of protein quality evaluation. *Journal of Nutrition* 83: 257.
- Alsmeyer RH, Cunningham AE, Happich ML (1974). Equations predict PER from amino acid analysis. *Food Technology* 28: 34-40.
- AOAC (1990). *Official methods of analysis* (15th ed.). Arlington : Association of Official Analytical Chemists.
- Berot S, and Davin A (1996). Technologie d'extraction et de purification des matières protéiques végétales. In *Protéines Végétales*, (B. GODON, ed.) pp. 280-356, Technique et Documentation Lavoisier, Paris.
- Beveridge T, Toma SJ, Nakai S (1974). Determination of SH and S-S groups in some food proteins using Ellman's reagent. *Journal of Food Science* 39: 49-51.
- Bhandari MR, Jong-Anurakkun R, Hong G, Kawabata J (2008). α -Glucosidase and α -amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata*, Haw.). *Food Chemistry* 106: 247-252.
- Bhatty RS, Finlayson AJ (1973). Extraction of non - protein nitrogen from oilseed meals with different solvents. *Cereal Chemistry* 50: 329 - 330.
- Bressani R (1985). Nutritive value of cowpea. In: *Cowpea Research, Production and Utilization* (edited by S. R. Singh and K. O. Rachie). Pp. 353-359. Chichester: JohnWiley and Sons.
- Cerletti P, Duranti M, Restani P (1983). Properties of lupine proteins relevant to their nutritional performance. *Qualitas Plantarum Plant Foods in Human Nutrition* 32(2): 145-154.
- Cerning J, Guilbot J (1973). Change in carbohydrate composition during maturation of wheat and barley kernel. *Cereal Chemistry* 50: 220-225.
- Chavan UD, McKenzie DB, Shahidi F (2001). Functional properties of protein isolates from beach pea (*Lathyrus maritimus L.*). *Food Chemistry* 74: 177-187.
- Cheftel J-C, Cuq JL, Lorient D (1985). *Protéines alimentaires. Biochimie-propriétés fonctionnelles - valeur nutritionnelle-modifications chimiques*. Paris: Technique et documentation - Lavoisier, pp. 1 - 295.
- Deosthale YG, Mohan VS, Rao KV (1970). Varietal deficiencies in protein lysine and leucine content of gram sorghum. *Journal of Agricultural and Food Chemistry* 18: 644-646.
- El-Adawy TA, Rahma EH, El-Bedawey AA, Gafar AF (2001). Nutritional potential and functional properties of sweet and bitter lupin seed protein isolates. *Food Chemistry* 74: 455-462.
- Erdman LW (1981). Bioavailability of trace minerals from cereals and legumes. *Cereal Chemistry* 58: 21- 26.
- FAO/OMS (1991). *Protein quality evaluation*. In Food and Agricultural Organization of the United Nations, Rome, Italy.
- Friedman M (1992). Dietary impact of food processing. *Annual Review of Nutrition* 12: 119-137.
- Goering HK, Van Soest PJ (1970). *Forage fiber analysis (apparatus, procedures and some applications)*. Agriculture hand-book, agricultural research service, USA.
- Griffiths DW (1984). The trypsin and chymotrypsin inhibitor activities of various pea (*Pisum spp.*) and field bean (*Vicia faba*) cultivars. *Journal of Science of Food and Agriculture* 35: 481-486.
- Hurrell RF, Lerman P, Carpenter KJ (1979). Reactive lysine in foodstuffs as measured by a rapid dye - binding procedure. *Journal of Food Science* 44: 1221 -1227, 1231.
- Itabashi E (1985). Spectroelectrochemical characterization of iron (III)-thiocyanate complexes in acidic thiocyanate solutions at an optically transparent thin-layer-electrode cell. *Inorganic Chemistry* 24 : 4024-4027.
- Laurent L (1981). Eléments minéraux. In: Deymié, B., Multon, J.-L., Simon, D.(Eds.) *Techniques d'analyse et de contrôle dans les industries agro - alimentaires* (pp. 61 - 82). Vol. 4. Paris : Technique et Documentation.
- Lin, MJY, Humbert ES, Sosulski, FW (1974). Certain functional properties of sunflower meal products. *Journal of Food Science* 39: 368-370.
- Lonstaff MA, Mc Nab, JM (1991). The effect of concentration of tannin-rich bean hulls (*Vicia faba L.*) on activities of lipase (EC 3.1.1.3) and α -amylase (EC 3.2.1.1) in digesta and pancreas on the digestion of lipid and starch by young chicks. *British Journal of Nutrition* 66: 139-147.
- Lqari H, Vioque J, Pedroche J, Millan F (2002). *Lupinus angustifolius* protein isolates: chemical composition, functional properties and protein characterization. *Food Chemistry* 76: 349-356.
- Ma C-Y (1983). Chemical composition and functionality assessment of protein concentrates from oats. *Cereal Chemistry* 60 (1): 36-42.
- Mc Watters KH, Ouedraogo JB, Resurreccion AVA, Hung Y-C, Phillips R D (2003). Physical and sensory characteristics of sugar cookies containing mixtures of wheat, fonio (*Digitaria exilis*) and cowpea (*Vigna unguiculata*) flours. *International Journal of Food Science and Technology* 38: 403-410.
- Montreuil J, Spik G, Tollier MT(1981). Dosages colorimétriques des glucides. In Deymié, B., Multon, J.- L., and Simon, D. (Eds.), *Techniques d'analyse et de contrôle dans les industries agro-alimentaires*, Vol.4 (pp. 85-143). Paris : Technique et Documentation.
- Moure A, Dominguez H, Zuniga ME, Soto C, Chamy R (2002). Characterisation of protein concentrates from pressed cakes of Guevina avellana (*Chilean hazelnut*). *Food Chemistry* 78: 179-186.
- Mune Mune MA, Minka SR, Mbome LI (2008). Response surface methodology for optimisation of protein concentrate preparation from cowpea [*Vigna unguiculata (L.) Walp*]. *Food Chemistry* 110: 735-741.
- Mwasaru MA, Muhammad K, Bakar J, Che Man YB (1999). Effects of isolation technique and conditions on the extractability, physicochemical and functional properties of pigeon pea (*Cajanus cajan*) and cowpea (*Vigna unguiculata*) protein isolates. I. Physicochemical properties. *Food Chemistry* 67(4): 435-443.
- Naczki M, Diosady LL, Rubin LJ (1985). Functional properties of canola meals produced by two phase solvent extraction system. *Journal of Food Science* 50: 1685 - 1688, 1692.
- Neto VQ, Narain N, Silva JB, Bora PS (2001). Functional properties of raw and heat processed cashew nut (*Anacardium occidentale, L.*) kernel protein isolate. *Nahrung / Food* 45: 258 - 262.
- Onwuliri AV, Obu AJ (2002). Lipids and other constituents of *Vigna unguiculata* and *Phaseolus vulgaris* grown in northern Nigeria. *Food Chemistry*, 78, 1-7.
- Onyenekwe PC, Njoku GC, Ameh DA (2000). Effect of cowpea (*Vigna unguiculata*) processing methods on flatus causing oligosaccharides. *Nutrition Research* 20(3): 349 - 358.
- Oomah BD, Bushuk W (1983). Characterization of lupin proteins. *Journal of Food Science* 48(1): 38-41.
- Oser BL (1959). An integrated essential amino acid index for predicting the biological value of proteins. In A. A. Albanese (Ed.), *Protein and amino acid nutrition* (pp. 295-311). New York: Academic Press.
- Pace CN, Vajdos F, Fee L, Grimsley G, Gray T (1995). How to measure and predict the molar absorption coefficient of a protein. *Protein Science* 4: 2411-2423.
- Panasik R, Amarowicz OR, Kostyra H, Sijtsma L (1998). Determination of α -amino nitrogen in pea protein hydrolysates: a comparison of three analytical methods. *Food Chemistry* 62(3): 363-367.

- Rangel A, Saraiva K, Schwengber P, Narciso MS, Domont GB, Ferreira ST, Pedrosa C (2004). Biological evaluation of a protein isolate from cowpea (*Vigna unguiculata*) seeds. *Food Chemistry* 87: 491-499.
- Reddy NR, Salunkhe DK (1981). Interaction between phytate, protein and minerals in whey fractions of black gram. *Journal of Food Science* 46: 564-567.
- Sanchez-Vioque R, Clemente, A, Viogue J, Bautista J, Millan F (1999). Protein isolates from chickpea (*Cicer arietinum* L.): chemical composition, functional properties and protein characterization. *Food Chemistry* 64: 237-243.
- Shahidi F, Chavan UD, Bal AK, McKenzie DB (1999). Chemical composition of beach pea (*Lathyrus maritimus* L.) plant parts. *Food Chemistry* 64: 39-44.
- Shahidi F, Naczki M (1989). Effect of processing on the content of condensed tannins in rapeseed meals. *Journal of Food Science* 54: 1082-1083.
- Simpson HC, Lousley RS, Greekie M, Hockaday TDR, Carter RD, Mann JI (1981). A high carbohydrate leguminous fibre diet improves all aspects of diabetes control. *Lancet* 1: 1-4.
- Singleton VL, Rossi JA (1965). Colorimetry of total phenolic with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16: 144-158.
- Spackman DM, Stein WH, Moore S (1958). Automatic recording apparatus for use in the chromatography of amino acids. *Analytical Chemistry* 30: 1190-1206.
- Stone FE, Hardy RW, Spinelli J (1984). Autolysis of phytic acid and protein in canola meal (*Brassica spp.*), wheat bran (*Triticum spp.*) and fish silage blends. *Journal of Science of Food and Agriculture* 35: 513-519.
- Tharanathan RN, Mahadevamma S (2003). Grain legumes – a boon to human nutrition. *Trends in Food Science and Technology* 14(12): 507 – 518.
- Thompson DB, Erdman JW (1982). Structural model for ferric phytate: implication for phytic acid analysis. *Cereal Chemistry* 59: 525.
- Waller G, Feather M (1983). *The maillard reactions in foods and nutrition*. Washington, D. C.: American Chemical Society.
- Yusuf AA, Ayedun H, Sanni L O. (2008). Chemical composition and functional properties of raw and roasted Nigerian benniseed (*Sesamum indicum*) and Bambara groundnut (*Vigna subterranean*). *Food Chemistry* 111(2): 277-282.

