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Nutritional Potential of Bambara Bean Protein Concentrate

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Abstract: Bambara bean protein concentrate was prepared under optimum conditions previously determined. Concentrate yield (27.12%) and protein yield (84.35%) were high. The concentrate had 70.85% protein and 13.15% lipid. Water-soluble compounds were removed in abundance from the flour, during the protein extraction. Bambara bean protein concentrate had similar levels of Fe and Zn as the flour. Antinutritional factors were significantly ($p < 0.05$) reduced in the concentrate. The concentrate showed significantly ($p < 0.05$) higher protein digestibility than the flour and had balanced amino acids contents with respect to the FAO/WHO pattern. Lysine and leucine were the predominant essential amino acids. The protein extraction process removed sulphur- and tryptophan-rich proteins. This affected the chemical score (26.80%), protein digestibility corrected amino acid score (25.40%), essential amino acid index (60.44%) and calculated biological value (54.18%) of the protein concentrate, which were lower than those of the flour.

Key words: Bambara bean, protein concentrate, antinutritional factors, protein quality

INTRODUCTION

Intense efforts are currently made in the search of cheap protein sources with good nutritional and functional properties, to attenuate the problem of protein malnutrition widely spread in developing countries (Siddhuraju *et al.*, 1996). In this regards, many studies have reported that many food grain legumes consumed in Africa are very important sources of nutrients, especially proteins and excellent sources of complex carbohydrates (Minka *et al.*, 1999; Minka and Bruneteau, 2000). The grain legumes also contain lipids with a good proportion of polyunsaturated fatty acids (Glew *et al.*, 1997; Onwuliri and Obu, 2002). Bambara bean [*Vigna subterranea (L.) verde*], one of these grain legumes, is widely cultivated in west and central Africa. Its grains are used to prepare many popular dishes and contain 16-25% protein rich in lysine, threonine and leucine (Borget, 1989; Minka and Bruneteau, 2000). The utilization of Bambara bean grain for food is limited by the presence of antinutritional factors, such as trypsin and chymotrypsin inhibitors, phytates, nitrates and cyanogens (Nwokolo, 1987; Okafor *et al.*, 2002). Traditional processing techniques such as soaking, pouting or cooking have limited effects on the improvement of protein quality of grain legumes (Vijayakumari *et al.*, 2007; Shimelis and Rakshit, 2007). Nevertheless, techniques employed for extracting protein therefrom are known to be effective in the elimination of the above antinutrients (Mwasaru *et al.*, 1999; Rangel *et al.*, 2004). In addition, protein extracts have superior functional properties and are more effectively used in the formulation of foods, as compared to the grain flour (Neto *et al.*, 2001).

Isoelectric precipitation is the most common technique for extracting protein in the food industry. Protein extraction from grain legumes involves various combination of physicochemical parameters to give different concentrate and protein yields. Our previous study reported the optimum combination of these parameters for the preparation of protein concentrate from Bambara bean seed flour (Mune *et al.*, 2010). In the present study, we investigate the chemical composition and the nutritive value of the concentrate in view of its possible use in the food industry as a nutritional ingredient.

MATERIALS AND METHODS

Bambara bean seeds were purchased from Mokolo market (Yaoundé, Cameroon). The seeds were hand-picked and stored in polyethylene bags in the refrigerator ($\sim 4^{\circ}\text{C}$) until used.

Preparation of Bambara bean flour: Bambara bean seeds were washed and rinsed in deionized water at room temperature ($25 \pm 2^{\circ}\text{C}$). They were dried in an air convection oven at 50°C for 72 h and then cracked and dehulled. The dried seeds were ground into flour, passed through a $150 \mu\text{m}$ mesh sieve, stored hermetically in polyethylene bag in a refrigerator at about 4°C .

Preparation of Bambara bean protein concentrate: Bambara bean protein concentrate was prepared by the isoelectric precipitation method as described by Mune *et al.* (2007). An aliquot (10 g) of Bambara bean flour was mixed with 100 mL of NaCl solution (0.17 M) and stirred

at 35°C for 150 min. The pH was adjusted to 8.99 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 methods (1990). Dietary fibre was analyzed 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 anthrone 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 (1982). 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 concentration from 40-200 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 (trihydroxymethan) 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 Panasiuk *et al.* (1998). The Trypsin Inhibitory Unit (TIU) was expressed as that producing the inhibition of 1 mg pure trypsin.

Alpha-Amylase inhibitor activity: Alpha-Amylase inhibitory activity was determined based on the method described by Bhandari *et al.* (2008), after extraction of alpha-amylase inhibitors as described by Lonstaff and Mc Nab (1991). The assay mixture contained 2 mL of inhibitory solution, 50 µL of porcine pancreatic alpha-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 min. The reaction was stopped by addition of 6 mL ethanol 90% (v/v) and the test tubes plunged in and ice bath for 10 min. 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 alpha-amylase solution. A control set was prepared in which inhibitory solution was deleted from the assay mixture. The alpha-amylase Inhibitory Unit (AIU) was defined as one unit of alpha-amylase activity (1 µmol maltose/min) inhibited.

Amino acids: Amino acids were determined using a BECKMAN 6300 amino acid analyzer 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.*, 2001a,b) and pepsin-pancreatin (Akeson and Stahman, 1964) enzymatic systems. 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 2 h reaction in the dark at ambient temperature was used for calculating reactive lysine. Absorbance measurements were performed using a Spectronic Model 601 spectrophotometer (Milton Roy company, Rochester, NY, 14625, USA).

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:

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

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

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

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

Statistical analysis: Results are expressed as mean value \pm standard deviation of three different determinations, except for amino acid contents. The data were statistically analyzed 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 Bambara bean protein concentrate: The chemical composition of Bambara bean flour is presented in Table 1. The high protein content (22.78%) of the flour shows that this grain legume is a good raw material for the preparation of protein concentrate. For viable commercial exploitation, the extraction of protein may have to be carried out concomitantly with starch which is the major macromolecule (52.94%), in order to ensure economic feasibility.

Table 1: Chemical composition of Bambara bean flour and protein concentrate (dry weight basis)^a

	Flour	Protein concentrate
Nutritional factor (g/100 g)		
Moisture	6.45 \pm 0.24b	8.92 \pm 1.29a
Crude protein	24.78 \pm 0.74b	70.85 \pm 0.41a
Non-protein nitrogen	2.00 \pm 0.05a	ND ^b
Protein nitrogen	22.78 \pm 0.74b	70.85 \pm 0.41a
Crude lipid	5.90 \pm 0.18b	13.15 \pm 0.63a
Fibre (NDF)	5.35 \pm 0.33a	1.82 \pm 0.11a
Starch (by difference)	52.94 \pm 1.94a	11.25 \pm 1.12a
Total sugars	7.15 \pm 0.32a	0.89 \pm 0.00a
Reducing sugars	0.36 \pm 0.60b	0.81 \pm 0.06a
Ash	3.88 \pm 0.37a	3.86 \pm 0.08a
Minerals (mg/100 g)		
Iron	16.64 \pm 5.26a	20.05 \pm 1.84a
Zinc	6.78 \pm 1.68a	7.97 \pm 0.18a

^aMeans followed by different letters (a-b) in the same line are significantly ($p < 0.05$) different. ^bND: below detection row limit

Bambara bean protein concentrate represented 27.12% of the weight of the flour and contained 84.35% of the total protein. The protein extraction yield was considerably higher than those obtained for cowpea (36.7-53.5%), pigeon pea (35.1-58.1%) or beach pea (67.9-77.3%) (Mwasaru *et al.*, 1999; Chavan *et al.*, 2001a,b). Incomplete recovery of protein may in part be due to the loss of acid-soluble proteins during isoelectric precipitation, or the retention of protein in the residue, due to complexation with other seed material. Chew *et al.* (2003) showed that alkaline extraction resulted in solubilization of 87% of sweet lupin protein, of which 59% was recovered by isoelectric precipitation. Ma (1983) reported that the residue of protein extraction from oat contained 10-20% of the total protein and Sumner *et al.* (1981) noted that 8% of the total field pea protein remained in the residue after alkaline extraction.

Chemical composition: The chemical composition of Bambara bean protein concentrate is presented in Table 1. The protein content (70.85%) was less than those reported by Lawal *et al.* (2007) (79.43%) and Yusuf *et al.* (2008) (81.50) and was in the same range as those obtained for oat (67.9-74.0%) and sweet lupin (67.1-75.1%) protein concentrates (Ma, 1983; Chew *et al.*, 2003). The relatively low protein content of Bambara bean concentrate could be mainly explained by its high crude lipids content (13.15%). Sanchez-Vioque *et al.* (1999) and Chavan *et al.* (2001a,b) observed that lipids, particularly those of a polar nature, were extracted together with protein by weak alkaline solutions and concentrated with the protein fraction. The fibre content of Bambara bean concentrate (1.82%) was in the same range as those for pigeon pea (1.25-2.83%) and cowpea (1.54-1.81%) (Mwasaru *et al.*, 1999). Sugars and low molecular weight water-soluble nitrogen compounds, are generally eliminated in abundance during the protein extraction process. Non-protein nitrogen was not detected in Bambara bean concentrate, which also contained significantly ($p < 0.05$) lower total soluble sugars (0.89%) than the corresponding flour (7.15%). The non reducing sugar (difference between total sugars and reducing sugars) content of the bambara bean concentrate (0.08%) was significantly ($p < 0.05$) lower than that of the flour (6.79%). Since flatulence-causing oligosaccharides such as raffinose, stachyose and verbascose are non-reducing, it could be concluded that these sugars were largely removed during the protein extraction process. Minka and Bruneteau (2000) demonstrated their existence in Bambara bean seed. Furthermore, Phillips and Abbey (1989) showed that Bambara bean seed contained raffinose (4.37 mg/g). Non-significant ($p > 0.05$) difference of ash content was observed between Bambara bean flour (3.88%) and protein concentrate (3.86%). Minerals, particularly divalent metals were probably associated to proteins in the concentrate. This association could have some nutritional importance, since minerals such as iron and zinc are rare in foods and particularly needed by children and pregnant women (Besancon, 1999). Bambara bean concentrate had appreciable amounts of iron (20.85 mg/100 g) and zinc (7.97 mg/100 g) contents.

Amino acid composition: The amino acid compositions of Bambara bean flour and protein concentrate are presented in Table 2. In terms of essential amino acids, Bambara bean flour and protein concentrate were rich in lysine and leucine and poor in tryptophan and total sulphur amino acids. The lysine and leucine contents were respectively 7.33 and 9.05% for Bambara bean flour, 6.98 and 9.71% for Bambara bean concentrate. Glutamic acid (14.74-15.56%) and aspartic acid (12.37-

Table 2: Amino acid composition of Bambara bean flour and protein concentrate (g/16 g N)

Amino acid	Flour	Protein concentrate	FAO/WHO (1991)
Histidine	3.82	3.47	1.9
Isoleucine	4.31	4.39	2.8
Leucine	9.05	9.71	6.6
Lysine	7.33	6.98	5.8
Thréonine	4.44	3.94	3.4
Tryptophane	0.98	0.53	1.1
Valine	5.16	5.09	3.5
Méthionine	0.55	0.10	
Cystéine	0.55	0.57	
Total sulfur amino acids	1.10	0.67	2.5
Tyrosine	1.61	2.21	
Phénylalanine	4.89	5.43	
Total aromatic amino acids	6.50	7.64	6.3
Total essential amino acids	38.87	38.95	33.9
Aspartic acid	12.37	12.81	
Serine	6.93	6.81	
Glutamic acid	14.74	15.56	
Proline	5.08	4.94	
Glycine	7.32	6.91	
Alanine	7.29	7.06	
Arginine	3.56	3.49	
Total non-essential amino acids	61.13	61.05	
Leucine/isoleucine ratio	2.10	2.21	2.36

12.81%) were the major non-essential amino acids. These results are common with most vegetable protein such as those of chickpea and lupin (El-Adawy *et al.*, 2001; Sanchez-Vioque *et al.*, 1999). Bambara bean protein concentrate had higher levels of total aromatic amino acids, leucine and isoleucine than the corresponding flour. The remaining essential amino acids were in lower contents in the concentrate than in the flour. Lower lysine and sulphur amino acids in the concentrated protein compared to the flour was also observed with beach pea and pigeon pea (Chavan *et al.*, 2001a; 2001b; Ant'Anna *et al.*, 1985). This was probably due to the high loss of albumins, which are rich in lysine, cystine and methionine (Clemente *et al.*, 1998; Chavan *et al.*, 2001a,b). Bambara bean flour and protein concentrate had higher total essential amino acids than the FAO/WHO (1991) reference pattern. Except for tryptophan and total sulphur amino acids, Bambara bean flour and protein concentrate satisfied the FAO/WHO (1991) requirements. For the nutritional use of the Bambara bean concentrate, it may be particularly interesting to complement its protein with those of cereals which are rich in tryptophan and sulphur amino acids (Youssef, 1998; Cheffel *et al.*, 1985). The leucine/isoleucine ratios of Bambara bean flour (2.10) and protein concentrate (2.21) were in the 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.

Table 3: Antinutritional factors and *in vitro* protein digestibility of Bambara bean flour and protein concentrate^a

Materials	Antinutritional factors				<i>In vitro</i> protein Digestibility (%)	
	Polyphenol (mg/100 g)	Phytate (g/100 g)	Alpha-AI ^b (AIU/g)	Ti ^c (TIU/g)	Pepsin-Trypsin	Pepsin-Pancreatin
Bbf	311.66±4.69a	0.71±0.01a	1.11±0.12a	6.32±0.23a	70.19±0.42b	74.53±3.11b
Bbc	232.53±1.15b	0.55±0.05b	1.14±0.13a	3.19±0.12b	91.57±0.97a	94.76±0.56a

^aBbf, Bambara bean flour; Bbc, Bambara bean protein concentrate; alpha-AI, alpha-amylase inhibitor; TI, Trypsin Inhibitor; Mean in the same column followed by different letters (a-b) are significantly ($p < 0.05$) different.

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

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

Antinutritional factors and *in vitro* protein digestibility:

Antinutritional factors and *in vitro* protein digestibility of Bambara bean flour and protein concentrate are presented in Table 3. Polyphenolic compounds can interact with proteins and reduce their digestibility, as well as alter amino acid availability and functional properties (Lin *et al.*, 1974). However, they may also be beneficial due to their strong antioxidant activity (Matthaus, 2002). In practice, low levels of polyphenol are therefore desirable. Bambara bean protein concentrate was significantly ($p < 0.05$) lower in polyphenolic content (232.53 mg/100 g) than the corresponding flour (311.66 mg/100 g), the difference being about 25.39%. Similarly, a 22.54% reduction in phytate content was observed in Bambara bean concentrate compared to the flour. Phytates influence the nutritional and functional properties of grain legumes and their derivatives by forming complexes with proteins, amino acids and trace minerals (Reddy and Salunkhe, 1981). Inactivation of trypsin and alpha-amylase inhibitors by an adequate heat treatment will be required before utilization of Bambara bean concentrate as nutritional ingredient. In fact, alpha-amylase inhibitor activity was not significantly ($p > 0.05$) different in Bambara bean flour and protein concentrate and half of trypsin inhibitor activity of the flour was present in the protein concentrate.

In vitro protein digestibility was evaluated with pepsin-trypsin and pepsin-pancreatin enzymatic systems (Table 3). Bambara bean protein concentrate showed significantly ($p < 0.05$) higher protein digestibility (91.57-94.56%) than the corresponding flour (70.19-74.53%) for both enzymatic systems. The pepsin-pancreatin protein digestibility was significantly ($p < 0.05$) higher than the pepsin-trypsin one, for Bambara bean flour and protein concentrate. This could be due to the more efficient action of pancreatin which contains amylases and lipases in addition of proteases. Mune *et al.* (2007) showed *in vitro* protein digestibility of Bambara bean flour was 71.58% with a multienzyme system comprising of trypsin, chymotrypsin and peptidase. Sanchez-Vioque *et al.* (1999) reported that *in vitro* digestibility of chickpea protein isolates (95.6-96.1%) was higher than that of the flour (76.2%), with a multienzyme system. Chavan *et al.* (2001a,b) noted that

in vitro digestibility of beach pea protein isolate was 80.6-82.6% with pepsin-trypsin enzymatic system and 78.6-79.2% with pepsin-pancreatin system. Improvement in protein digestibility was mainly attributed to the denaturation of proteins and the reduction of antinutritional factors such as trypsin inhibitors, phytates and polyphenols. Sanchez-Vioque *et al.* (1999) showed that the dissociation of globulins, probably due to the action of pH, facilitated their accessibility to digestive proteases. Richardson (1991) reported that protease inhibitors are albumins and that most of them are removed during protein concentrate preparation.

Nutritional parameters and available lysine: Protein digestibility and availability of amino acids to the body are determining factors in protein quality assessment. Cheftel *et al.* (1985) reported that the nature and amino acids contents of a dietary protein, determined the efficiency with which an organism could use the protein. Our results on nutritional parameters and available lysine of Bambara bean flour and protein concentrate are presented in Table 4. The chemical score of Bambara bean protein concentrate (26.40%) was lower than those of concentrated proteins from cowpea (82%) and sweet lupin (47.14-48.8%) (Rangel *et al.*, 2004; El-Adawy *et al.*, 2001). Based on chemical score, the first and second limiting amino acids were respectively total sulphur amino acids and tryptophan for Bambara bean flour and protein concentrate. These amino acids were also limiting for beach pea and were respectively first and third limiting amino acids for bitter and sweet lupin (Chavan *et al.*, 2001a,b; El-Adawy *et al.*, 2001). Protein efficiency ratio of Bambara bean concentrate (3.51-3.71) was higher than those reported for beach pea (2.75-2.81) and lupin protein isolates (2.22-2.32) (Chavan *et al.*, 2001a,b; El-Adawy *et al.*, 2001). Essential Amino Acid Index (EAAI) of Bambara bean protein concentrate (60.44%) was in the same range as that reported for cowpea protein isolate (64%) (Rangel *et al.*, 2004). Higher chemical score, Protein Digestibility Corrected Amino Acid Score (PDCAAS), EAAI and Biological Value (BV) of Bambara bean flour compared to the protein concentrate, was probably explained by the high reduction of tryptophan and sulphur amino acids contents.

Table 4: Nutritional parameters and available lysine of Bambara bean 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			
Bbf	44.00	Cys + Met (44%)	Trp (89.09%)	32.79	68.85	3.20	3.47	63.35	0.90±0.03b	
Bbc	26.80	Cys + Met (26.80%)	Trp (44.18%)	25.40	60.44	3.51	3.71	54.18	1.85±0.10a	

^aBbc, Bambara bean protein concentrate; Bbf, Bambara bean 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

A significant (p<0.05) improvement of available lysine content was observed in Bambara bean protein concentrate (1.85 g/16 g N), in comparison with the corresponding flour (0.90 g/16 g N). This was probably due to the elimination of flatulence-causing sugars and polyphenols during the protein extraction process. The available lysine value of Bambara bean concentrate was much lower than the value given by the amino acid analysis (6.98 g/16 g N). The inaccessibility of lysine residues to Orange 12 was mainly explained by the maillard reaction, occurred during storage of grain legume after harvest, since the sun-drying of grain legumes for their better conservation was economical and then widely used by farmers.

Conclusion: The results obtained in this study show that Bambara bean protein concentrate had a chemical composition and nutritional quality comparable to those of other grain legume protein concentrates. The elimination of antinutritional factors during treatment of Bambara bean flour and the increase of protein digestibility in the resultant protein concentrate, improved its nutritional potential. Hence, Bambara bean protein concentrate could be used as a source of low-cost protein in nutritional applications, for the benefit of low-income populations of developing countries.

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