International Food Research Journal 17: 357-366 (2010)

Nutritional quality and physicochemical properties of Mucuna bean (*Mucuna pruriens L.*) protein isolates

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Abstract: The potential for mucuna bean protein isolate (MBPI) application as functional ingredient in foods is unknown. In this study nutritional quality and physicochemical properties of MBPI were investigated. Bean samples were processed for L-dopa extraction in distilled water adjusted to pH 3.2 at 60°C for 48hr. MBPI was extracted at pH 9.0 and isoelectrically precipitated at pH 4.5. MBPI from raw and processed seed contained higher protein content (86.7 and 86.9% respectively) than soybean protein isolate (82.7%). Essential amino acids content of MBPI met FAO/WHO scoring pattern for 2-5 year-old. SDS-PAGE revealed four main polypeptide protein subunits of apparent MW of 11, 19, 36 and 98 kD in MBPI. MBPI exhibited high foam stability, emulsion activity and stability compared to soybean protein isolate. However, poor foam expansion, water and oil absorption capacity and dark colour (Hunter lab "***L***" value of 36.39) limits its potential as a functional food ingredient.

Keywords: Mucuna bean, protein isolate, functional properties, nutritional quality

Introduction

Mucuna bean is an underutilized tropical legume grown in Africa, South America and South Asia as a green manure/cover crop (Buckles, 1995; Ezeagu *et al.,* 2003). It is rich in protein (23-35%) and has a nutritional quality comparable to that of other pulses like soybean, rice bean and lima bean (Bressani, 2002; Gurumoorthi *et al.,* 2003). It has good potential as a cheap and alternate source of protein. Utilization of legume protein by the food industry, especially soybean protein has increased interest in seed protein research (Kinsella, 1979; Kim *et al.,* 1990). Legume proteins are used as ingredients primarily to increase nutritional quality and to provide a variety of functional properties, including desirable structure, texture, flavour, and colour characteristics in formulated food products. The proteins maybe characterized by assessing their chemical and physical properties. Functional properties of proteins reflect the physicochemical properties which in turn are a function of inherent primary composition and sequence of amino acids. Other factors such as temperature, pH and ionic strength also influence protein functionality. High quality protein ingredients providing both nutritional quality and functional properties are compatible with other ingredients under processing conditions and contain minimal antinutritional factors (Damodaran, 1997).

Protein functionality has been defined as any property, other than its nutritional value, that affects its utilization (Pomeranz, 1985), physicochemical properties that affect behaviour of a protein in food system during processing, storage and preparation and determine quality (Nakai and Powrie, 1981). Functional properties include among others: hydration, dispersibility, solubility and swelling; surface active properties such as emulsification, foaming and adsorption including fat binding; rheological properties such as gelation and texturization, sensory and kinesthetic properties (Nakai and Powrie, 1981). Functional properties of legume seed storage proteins have been studied as purified proteins or as protein isolates (Gueguen and Cerletti, 1994). Soybean protein is widely used in many foods as functional and

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nutritional ingredient in high protein foods including dairy foods, nutritional supplements, meat systems, infant formulas, nutritional beverages, cream soups, sauces and snacks and also as a protein source in milk replacers (Gandhi, 2009).

Foaming, gelation and electrophoretic characteristics of mucuna bean (*Mucuna pruriens*) protein concentrates have been studied by Adebowale and Lawal (2003) but information on mucuna bean protein isolate is scarce. The objectives of this study were to investigate the nutritional quality, physicochemical properties of protein isolates from raw and processed Mucuna bean.

Materials and Methods

Materials

Matured and dried seeds of mucuna bean were obtained from Kenya Agricultural Research Institute (KARI), Nairobi, Kenya. Seeds were sorted, cleaned and stored in plastic containers while damaged seeds were discarded. Beans were dehulled with a hammer mill and ground using a Waring commercial blender (Smart Grind, Black and Decker, Towson, MA, USA) to particle size of 1.00 – 1.70 mm.

Sample preparation

Bean samples (40gm) were soaked in distilled water in ratio of 1:20 (w/v) in a temperature controlled waterbath at 60°C for 48hr. The pH of soaking water was adjusted to 3.2 ± 0.2 using 18 N acetic acid and/ or 1 M NaOH solution. Treated samples were freeze dried (United Scientific, Virtis Bench Top freeze dryer, Gerdiner, NY) at -40 to -50°C. Raw and processed bean samples were separately ground into fine flour of particle size <0.5mm using the Fritsch Pulverizer, 02.102, Germany. Protein isolates were prepared from raw and processed bean samples following the method described by Johnson and Brekke (1983), as modified by El-Adawy (1996). Defatted bean flour (5%,w/v) was dispersed in distilled water, adjusted to pH 9.0 (Mettler Toledo, 320, pH meter) with 0.1M NaOH at 25°C, shaken for 1 hr and centrifuged at 8000 *g* for 15 min. Extraction was repeated, extracts combined and pH adjusted to 4.5 with 1M HCl to precipitate protein. Protein was recovered by centrifugation at 8000 *g* for 15 min, followed by decantation of supernatant. The acid precipitate was washed twice by further centrifugation at 8000*g* for 10 min, freeze dried, pulverized and stored until required.

Analytical methods

Amino acid profile

Amino acid profile of mucuna bean protein was determined using the Pico-Tag Amino Acid Analysis System (Waters Chromatography Div., Millipore Co., Milford, MA, USA) as reported by Bidlingmeyer *et al*. (1987). Samples were acid hydrolyzed, derivatized and subjected to HPLC analysis. Calibration was done using standard amino acid kit (Stock No. AA-S-18) from Sigma –Aldrich, Inc., Germany. Detection was at 254 nm wavelength using Detector Model 440, auto sampling by WISP 712, while identification and quantification was done using the software Millenium 32 Chromatograph (Waters Corp., Milford, MA, USA).

Crude protein

Mucuna bean was ground into fine flour (particle size diameter <0.5mm) and analyzed for crude protein according to AOAC (1990) method. Samples were analyzed in triplicate.

In vitro protein digestibility (IVPD)

This was determined by multi-enzyme method of Hsu *et al*. (1977). To a sample weight equivalent to 6.25 mg protein/ml, 10 ml of distilled water was added. A multi-enzyme (Sigma-Aldrich Inc, Germany) solution containing 1.6 mg trypsin, 3.1 mg chymotrypsin, and 1.3 mg peptidase per ml of distilled water was equilibrated to pH 8.0 and maintained in an ice bath. Samples were equilibrated to pH 8.0 and maintained at 37°C. A 1 ml aliquot of multi-enzyme solution was added to each sample, stirred for exactly 10 min and pH of enzyme hydrolysate measured. *In vitro* protein digestibility of sample was calculated using the following equation:

% digestibility = 210.464 – 18.103x

where x represents the pH after 10-minute incubation

Casein was run as a control to ensure enzyme activity.

L-Dopa content

L-Dopa was determined after acidic extraction of sample by the method reported by Siddhuraju and Becker (2005). The standard solution of L-Dopa concentration was 200 mg/ml. L-Dopa analysis was on a Pico-Tag C_{18}, 3.9 x 150mm column under the following conditions: injection volume 20 µl, flow rate: 1.0 ml/min, and column temperature of 27°C.

Functional properties

Water and oil absorption capacity.

This was determined by the method of Lin *et al.* (1974). Duplicate samples (0.5 g) and 5 ml deionized water (adjusted to pH 7.0), or 5 ml corn oil were stirred for 1 min in a graduated tube and allowed

to stand for 30 min at 25° C. Mixtures were then centrifuged at 3000*g* for 25 min. The volume of free liquid was measured and retained liquid expressed as ml of water or oil absorbed per gram of sample.

Whipping properties

Whipping properties (foaming capacity and foam stability) were determined according to method described by Kabirullah and Willis (1982). A sample of 50 ml of protein suspension (1% protein adjusted to pH 7.0), in duplicate was blended in a homogenizer at 12000 rpm for 1 min. The blend was immediately transferred into a 100-ml graduated cylinder. After standing for 30 minutes, the volume of foam was determined.

$$\text{Foam capacity (\%)} = \frac{(\text{Volume after whipping} - \text{Volume before whipping}) \times 100}{\text{(Volume before whipping)}}$$

$$\text{Foam stability} = \frac{(\text{Volume after standing} - \text{Volume before whipping}) \times 100}{(\text{Volume after whipping} - \text{Volume before whipping})}$$

Emulsifying properties

Emulsifying properties (emulsifying capacity and emulsion stability) were determined according to the method of Yasumatsu *et al.* (1972) with modifications (Wang and Kinsella, 1976). A protein sample (0.7 g) was added to 10 ml of distilled water (pH 7) and dispersed at low speed (12000 rpm) in a homogenizer (Ultra-Turrax T25, 1Ka, Janke and Kunkel, Germany). Corn oil was added and blended at high speed (20000 rpm) for 1 min; the emulsion formed was equally divided into two 12 ml centrifuge tubes and centrifuged for 5 min at 200*g*. Emulsion capacity was calculated as follows:

$$\text{Emulsion capacity (\%)} = \frac{\text{(height of emulsified layer)} \times 100}{\text{(height of total contents of tube)}}$$

Emulsion stability was determined in a similar way to that of emulsion capacity except that the emulsion was initially heated in a water bath at 80°C for 30 min and subsequently cooled to 25°C prior to centrifugation.

$$\text{Emulsion stability (\%)} = \frac{\text{(height of emulsified layer)} \times 100}{\text{(height of total contents of tube)}}$$

Gelation properties

This was determined according to the method described by Coffmann and Garcia (1977). Sample dispersions of 4, 6, 8, 10, 12, and 14% (w/v) were prepared in distilled water, adjusted to pH 7.0 and mixed in a Waring Blender (Smart Grind, Black and Decker, Towson, MA, USA) at the highest speed for 2 min. Dispersions were poured into test tubes in 5 ml aliquots (3 test tubes for each concentration), heated to 100°C in a water bath for 1 hr and cooled to 4°C in an ice bath. The lowest concentration at which all dispersions in triplicate formed gels that did not collapse or slip from inverted tubes was reported as the Least Gelation Concentration (LGC).

Colour

Colour was determined using a colour difference meter (Spectrophotometer, NF 333, Nippon, Denshoku, Japan). Measured values were expressed as *L, a, b* units where *L*= lightness, +*a* = redness, -*a* = greenness, +*b* = yellowness, -*b* = blueness, and $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{½}$. The ΔE refers to the total difference between sample and standard (commercial soybean protein isolate - Sigma-Aldrich Inc, Germany).

Nitrogen solubility

Protein solubility was determined in pH range of 2.0 –10.0 according to AACC method 46-23 (1983) with some modifications. Each sample of 100 mg was accurately weighed into 50 ml centrifuge tubes and dispersed in 20 ml of water. The pH was adjusted to six different levels (pH 2.0, 4.0, 5.0, 6.0, 8.0, and 10.0) using 0.1 M NaOH or 0.1 M HCl solutions. Dispersions were shaken using a mechanical shaker (Flow Laboratories, Titertek, Type DSG-304, Germany) at a setting of 6 for 1 hr at 25°C, and pH checked and adjusted regularly. Dispersions were then centrifuged at 8000*g* for 15 min, supernatants analyzed for nitrogen and reported as nitrogen solubility index (NSI). NSI was expressed as percentage of total nitrogen of original sample that was present in the soluble fraction.

Molecular weight of isolated proteins

Molecular weight of isolated proteins was determined using Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method by Laemmli (1970). Polyacrylamide slab gels of 12% concentration were used. Electrophoresis was carried out using a Mini-Protean II Electrophoresis System (Bio-Rad, Richmond, CA, USA) based on the instruction manual supplied. Samples were prepared in a buffer containing 10mM Tris-HCl, 2.5% sodium dodecyl sulfate, 5% β-mercaptoethanol, 20% glycerol and 0.01% bromophenol blue. Samples were denatured by heating at 100° C for five min. A 4µl aliquot of each sample was loaded onto gel for protein separation. Appropriate standard protein molecular weight markers (LabAid, PageRuler Unstained

Protein ladders No. SM0661, MW range, 10-200kDa, Fermentas, Life Sciences) were run concurrently with the samples and used to estimate apparent molecular weight of the different fractions detected. Gels were stained in Coomassie Blue R-250. Gel images were taken using Bio Rad Versa Doc Imaging System, Model 300, and results analyzed by the software, Bio Rad laboratories Quantity one 4.4.1, The Discovery Series, 1998, CA, USA.

Statistical analysis

The data was exported from excel and analyzed using SPSS Version 11.5, SPSS Inc., Chicago , IL USA . Statistical differences between means were compared using paired T-test. Amino acid data was subjected to one way analysis of variance (ANOVA) and post hoc Tukey B test. Differences in means were considered statistically significant at $p < 0.05$. Values were expressed as means ± SD.

Results and Discussion

Amino acid composition

Amino acid composition of mucuna bean protein isolates (MBPI) and raw mucuna bean protein are presented in Table 1. Levels of essential amino acids (EAAs) threonine, phenylalanine- and tyrosine, valine, methionine- and cysteine, isoleucine, leucine, lysine and tryptophan in raw MBPI were 34.49, 86.51, 42.21, 15.80, 38.87, 62.28, 57.67 and 5.16 while those in processed MBPI were 33.49, 87.57, 39.82, 16.80, 38.90, 62.72, 59.49 and 5.11 mg/g, respectively. Protein isolates met the recommended EAAs requirements by FAO/WHO/UNU (1985) for 2-5 yr old pre-school children except for sulphur amino acids (methionine and cysteine) and tryptophan. Content of sulphur amino acids and tryptophan in raw and processed MBPI was 15.80, 5.16 and 16.80 5.11 mg/g, respectively. They are the limiting amino acids in MBPI. Protein isolates contained significantly more tyrosine and phenylalanine (86.51, 87.57 mg/g) and isoleucine (38.87, 38.90 mg/g) than the recommended values (63.00, 28.00 mg/g), respectively. Compared to soybean protein isolate (SBPI) whose EAAs content is higher than FAO/WHO/UNU (1985) reference requirements, utilization of processed MBPI in foods would require complementation from other food ingredients for the limiting amino acids. Raw and processed MBPI crude protein content (86.70% and 86.90%) was significantly ($P<0.05$) higher than for commercial SBPI (82.70%) (Table 2). The *in vitro* digestibility (IVPD) for raw and processed MBPI was significantly ($P<0.05$) higher than for SBPI. In addition, digestibility of processed MBPI was significantly ($P<0.05$) higher than for raw MBPI. Processing mucuna bean for removal of L-dopa did not alter protein content but it improved digestibility of the protein. The IVPD for raw and processed MBPI was 81.39% and 87.67%, respectively compared to that of SBPI (63.65%). The L-Dopa content of raw MBPI (0.79%) was significantly ($P<0.05$) higher than for processed MBPI (0.07%). Processing mucuna bean reduced L-dopa content of protein isolate to the recommended levels of 0.1% dry matter (Teixeira *et al.,* 2003).

Physicochemical properties

Functional properties of raw and processed MBPI, raw mucuna bean flour and SBPI are shown in Figures 1-3. Oil and water absorption capacity for processed MBPI (1.13 and 1.43 ml/g, respectively) was significantly ($P<0.05$) lower than values obtained for SBPI (1.57 and 10.0 ml/g, respectively). Foam stability values for processed MBPI (93.75%) was significantly ($P<0.05$) higher than that obtained for SBPI (28.79%). Emulsion capacity for raw and processed MBPI (54.39, 55.56%, respectively) was significantly ($P<0.05$) higher than values obtained for SBPI (40.96%). In addition, emulsion stability for processed MBPI (64.50%) was significantly ($P<0.05$) higher than for both raw MBPI (49.37%) and SBPI (42.97%). MBPI formed gels at 18-20% concentration while SBPI did not gel even at 22% concentration.

Emulsion stability is important in food emulsions as it indicates the capacity of emulsion droplets to remain dispersed without separation by creaming, coalescing and flocculation (Damodaran, 1997). Unfolding of proteins at oil and water interfaces plays a significant role in formation and stability of emulsions. Other factors such as adsorption kinetics, interfacial load, decrease of interfacial tension, rheology of the interfacial film and its surface hydrophobicity also affect emulsion properties (Das and Kinsella, 1990). MBPI exhibited slightly better emulsion properties than SBPI at pH 7.0. However, processed MBPI exhibited average emulsion properties (emulsion capacity of 55.56% and stability of 64.5%). This could be attributed to protein denaturation during isolation (Damodaran, 1997). Heat treatment impairs emulsifying properties of proteins (Voutsinas *et al.,* 1983). Heat denaturation of protein during processing modifies functional properties. It may decrease solubility and emulsion properties but improve water holding capacity and oil absorption. However, denaturation that partially opens up globulin structure or controls unfolding of polypeptides generally increase accessibility

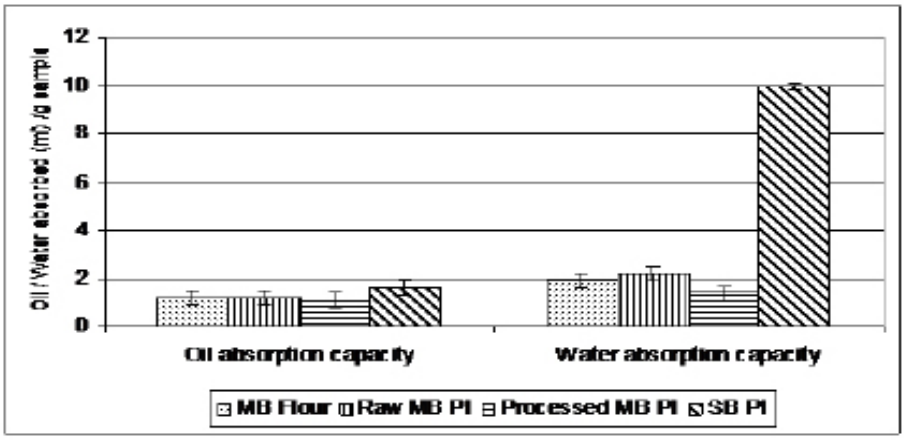

Figure 1. Oil and water absorption capacity of mucuna bean flour (MB flour), protein isolate (MBPI) and soybean protein isolate (SBPI). Values are means of triplicate determinations.

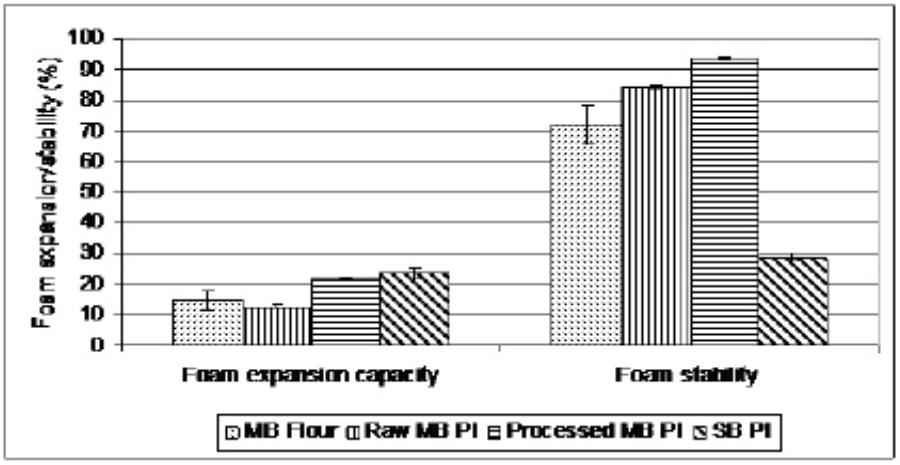

Figure 2. Foam expansion and stability of mucuna bean flour (MB flour), mucuna bean protein isolate (MBPI) and soybean protein isolate (SBPI). Values are means of triplicate determinations.

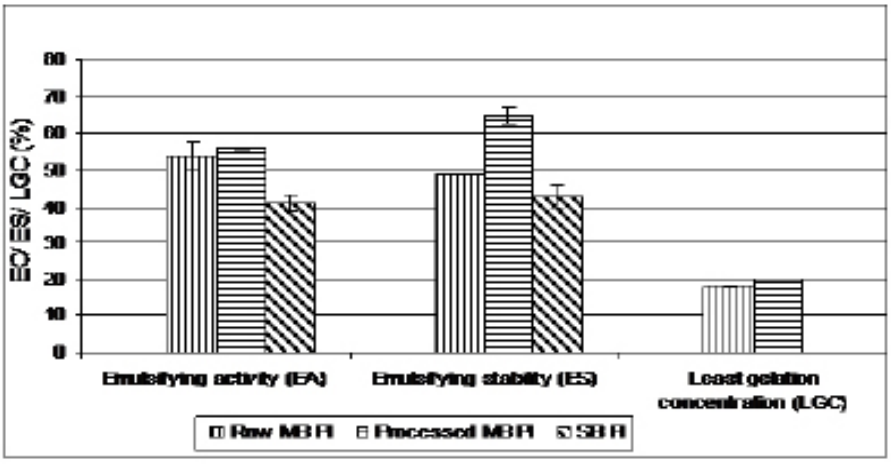

Figure 3. Emulsion capacity and stability and least gelation concentration of mucuna bean protein isolate (MBPI) and soybean protein isolate (SBPI). Values are means of triplicate determinations.

of buried reactive sites of molecules. Controlled cleavage of disulfide bonds improves protein functionality while increased unfolding enhances accessible hydrophobicity of protein (Voutsinas *et al.,* 1983). According to Nakai (1983), emulsification of proteins is influenced by solubility and surface hydrophobicity. The ability of protein to gel and provide a structure for holding water, flavours, sugars and food ingredients is useful in food applications.

In food foams, foaming performance depends on the ability of the continuous phase to include air (foam capacity) and also retain it for specific period of time (foam stability) (Prins, 1988). At pH 7, foam capacity and stability of processed MBPI was higher compared to SBPI. However, foam capacity (35.17%) for MBPI was low though with high stability (93.75). Low foam capacity for MBPI could be attributed to poor solubility at pH 7.0. Ragab *et al.* (2004) reported similar observations on cowpea protein isolates whose foaming capacity depended on pH. Protein solubility depends on pH and determines foam properties. Damodaran (1997) also reported that ability of protein to reduce surface tension upon adsorption affects foam formation. According to Hettiarachchy *et al.* (1996), ability to form stable foam depends on sufficient intermolecular (protein-protein) interaction and thus degree of cohesion. High foam stability of MBPI denotes a capacity to effectively reduce interfacial tension and favorable molecular orientation at the oil and water interface.

Fat and water absorption of protein are important functional properties in foods because fats and oils act as flavour retainers and contribute to mouthfeel (Kinsella, 1979). Oil absorption capacity (OAC) for MBPI (1.13 ml/g) was lower than that reported for cowpea and pigeon pea protein isolates at pH 8.5 (1.67, 2.45ml/g, respectively) (Mwasaru *et al.,* 1999) and chickpea (2.08 and 3.96 ml/g) (Kaur and Singh, 2007). Poor OAC of MBPI suggests decreased hydrophobic residues on the protein surface compared to other protein isolates. Water absorption, holding or binding capacity (WAC) may be defined as ability of food material to hold its own and added water during application of forces and heating (Zayas, 1997). Processed MBPI exhibited significantly lower WAC (1.43ml/g) compared to SBPI (10.0ml/g) at pH of 7.0. It is also lower than that reported for protein isolates from great northern bean (2.73 ml/g) (Sathe and Salunkhe, 1981), and chickpea (2.08-3.96 ml/g) (Kaur and Singh, 2007). However, WAC for MBPI was higher than that reported for cowpea and pigeon pea isolates at pH 8.5 (0.85, 0.84ml/g respectively) (Mwasaru *et al*., 1999). Low water absorption capacity of MBPI may be attributed to decreased charge on mucuna protein. Changes in environmental factors such as pH affect protein ionization and magnitude of net charge on protein molecules influencing attractive and repulsive forces within the protein and its ability to associate with water. Water absorption is due to its dipolar nature so that proteins with more charged amino acids tend to absorb more water than proteins with uncharged amino acids (Damodaran, 1997).

Hunter (***L, a, b***) values of SBPI (standard), MBPI and dehulled mucuna bean flour are shown in Table 3. The ***L*** (lightness) and $^{+}$**b** (yellowness) values for processed MBPI (36.39, 8.30) were significantly ($P<0.05$) lower than for both mucuna flour (88.56, 11.30) and SBPI (94.40, 13.56). However, the $^{+}$**a** (redness) value for MBPI (0.47) was significantly ($P<0.05$) higher than for SBPI (0.24) but lower than for mucuna flour (1.07). Total colour difference (***ΔE***) between SBPI and MBPI (58.25) was significantly ($P<0.05$) higher than for mucuna flour (6.07). Processed MBPI was very dark as evidenced by low "***L***" value (36.39) compared to SBPI and mucuna flour. Higher colour difference has been associated with protein isolates obtained by isoelectric precipitation (Paredes-Lopez *et al.,* 1991) and oven drying of the isolate during processing. The dark colour of MBPI could be a major disadvantage in its potential application in food systems.

Nitrogen solubility is the most important functional property influencing other properties such as emulsification, gelation, and foaming thereby determining behaviour of proteins in food systems. Higher nitrogen solubility generally improves emulsifying properties. Nitrogen solubility index (NSI) of MBPI was low (less than 45%). Solubility of MBPI exhibited typical bell-shaped curves (Figure 4). For raw and processed MBPI, minimum nitrogen solubility was at pH 4.0-5.0 and two maxima at pH 2 and 10.0, respectively. Processed MBPI exhibited higher solubility at pH values below isoelectric point. At isoelectric point of protein, net charge is zero and molecules associate resulting in decreased solubility (Zayas, 1997). Increased solubility at low and high pH may be attributed to decreased protein-protein interaction owing to the charged nature of proteins outside their isoelectric point. Similarly, Ragab *et al*. (2004) reported a minimum nitrogen solubility for cowpea protein isolates at pH 4 and 5 and increased solubility at low and high pH. Variations in functional properties especially on solubility of commercially prepared protein isolates have been attributed to heat treatment given during manufacturing that may cause protein denaturation (Bejosano and Corke, 1999). Low solubility of MBPI implies limited functional properties and hence use in food systems.

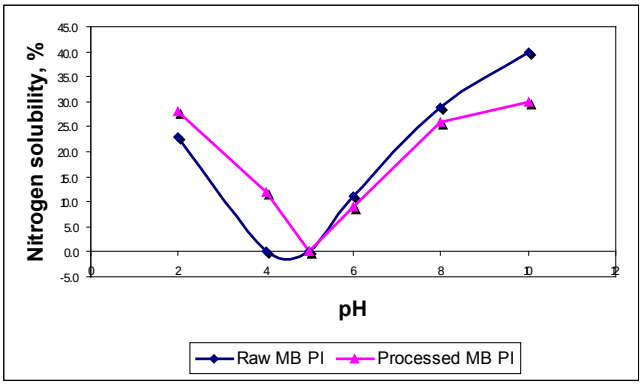

Figure 4. Nitrogen solubility of raw and processed mucuna bean protein isolates (MBPI). Values are means of duplicate determinations.

It could be attributed to increased hydrophobic interactions that promote protein-protein interactions thereby decreasing solubility. Protein denaturation during processing and storage is the major intrinsic factor that affects nitrogen solubility and thus, the functional properties of proteins (Hettiarachchy *et al*, 1996) and has been measured in terms of loss in solubility (Nakai, 1983). Protein solubility is also determined by behavior of the globulin fraction of seed protein. Legume seeds contain mainly globulin and albumin fraction (83%) of seed proteins, of which globulin alone accounts for 62% (Janardhanan and Lakshmanan, 1985). Vijayakumari *et al*. (2002) reported high globulin concentration (9–16.7%) followed by albumin (4.9–6%), glutelins (1.3–2.9%), and prolamin (0.8–2%) in mucuna bean.

Electrophoresis (SDS-PAGE) of raw mucuna bean protein and protein isolates showed a distribution pattern of protein subunits in Figure 5. Raw mucuna bean protein exhibited seven subunits of apparent molecular weights of 11, 19.8, 26, 33, 36, 45 and 46 kDa. The main bands had apparent molecular weights of 11, 19.8, 45 and 46 kDa. MBPI comprised of several subunits with varying molecular weights ranging from relatively low (11kDa) to high (98 kDa). Processed MBPI exhibited nine subunits with a similar pattern for molecular weight except that the bands corresponding to 25, 42, 61, 84 kDa were missing. Raw MBPI exhibited thirteen subunits with apparent molecular weights of 11, 18.7, 19, 25, 27, 36, 42, 45, 46, 54, 61, 84 and 98 kDa, respectively. The major bands for the two protein isolates were similar with apparent molecular weights of 11, 18.7, 19, 45 and 46 kDa. Four main polypeptide protein units of apparent molecular weight of 11, 19, 36 and 98 kDa were present in protein isolates and raw mucuna bean protein. Processed MBPI had fewer subunits compared to raw MBPI indicating that some

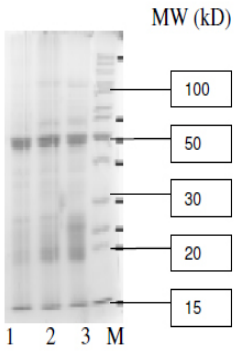

Figure 5. SDS-PAGE of (1) raw mucuna bean protein; (2) processed bean protein isolate; (3) raw bean protein isolate, and (M) molecular weight markers.

protein subunits may have been lost during processing of bean for reduction of L-Dopa. In addition, raw mucuna bean protein had fewer subunits compared to either of the protein isolates. This means that there was fragmentation of bigger polypeptide chains into smaller subunits during protein isolation process. Jesse (2000) characterized seed protein fractions from seven varieties of mucuna beans grown in Nigeria using SDS-PAGE electrophoresis and reported no varietal differences in the band patterns except in the number, size, and intensity of bands between albumin and globulin fractions. Adebowale and Lawal (2003) reported presence of five protein subunits of apparent molecular weight of 200, 116, 82, 63, and 59 kDa of mucuna bean protein concentrates. Similar range of molecular weight protein subunit distribution has also been reported for several other legumes (Gueguen and Cerletti, 1994).

Conclusion

In conclusion, processed mucuna bean protein

isolate has good nutritional quality. It has a high protein content and *in vitro* digestibility compared to soybean protein isolate. It exhibited high foam stability and average emulsion properties. However, the isolate exhibited poor oil- and water absorption, foaming capacity and low nitrogen solubility. From the findings, mucuna bean protein isolate could only be used in food systems as a functional ingredient after modification through physical, chemical, or enzymatic methods to improve functional properties. Notwithstanding, mucuna bean protein isolate has potential as a source of protein in foods.

Acknowledgements

The authors are grateful to Legume Research Network Project, Kenya Agricultural Research Institute, Nairobi, Kenya, for providing mucuna bean and partly funding this research; University of Pretoria, Department of Biochemistry where processing of mucuna bean, protein and L-Dopa analysis was done and Department of Science and Technology, ZA; Tropical Soils Biology and Fertility Institute, and Kenyatta University for funding this study.

References

American Association of Cereal Chemists. 1983. Approved methods of AACC. St. Paul, MN: The Association.

AOAC 1990. Official methods of Analysis, 15th edition. Association of Official Analytical Chemists, Washington, DC.

Adebowale, K. O. and Lawal, O.S. 2003. Foaming, gelation and electrophoretic characteristics of mucuna bean (*Mucuna pruriens*) protein concentrates. Journal of Food Chemistry 83: 237–246.

Bejosano, F. P. and Corke, H. 1999. Properties of protein concentrates and hydrolysates from Amaranthus and Buckwheat. Industrial Crops and Products 10: 175–183.

Bidlingmeyer, B.A., Cohen, S.A., Tarvin, T. L. and Frost, B. A. 1987. New, rapid, high sensitivity analysis of amino acids in food samples. Journal of Association of Official Analytical Chemists 70 (2): 241-247.

Bressani, R. 2002. Factors influencing nutritive value in food grain legumes: Mucuna in comparison to other grain legumes. In: Flores, M., Eilittä, M., Myhrman, M., Carew, L. and Carsky, R. (Eds); Mucuna as a Food and Feed: Current Uses and the Way Forward, p 164-188, Proceedings of an international workshop held April 26-29, 2000 in Tegucigalpa, Honduras.

Buckles, D. 1995. Velvetbean: A "new" plant with a history. Economic Botany 49 (1): 13-45.

Coffmann, C. W. and Garcia, W. W. 1977. Functional properties and amino acid composition of a protein isolate from mung bean flour. Journal of Food Technology 12: 473-484.

Damodaran, S. 1997. Amino acids, peptides and proteins. In Fennema, O. R. (Ed). Food Chemistry, 3rd edition, p. 312-425. New York: Marcel and Dekker.

Das, K. P. and Kinsella, J. E. 1990. Stability of food emulsions: Physicochemical role of protein and nonprotein emulsifiers. Advances in Food and Nutrition Research 34: 144-149.

El-Adawy, T. A. 1996. Chemical, nutritional and functional properties of mung bean protein isolate and concentrate. Menofiya Journal of Agricultural Research 21: 657 –672.

Ezeagu, I. E., Maziya-Dixon, B. and Tarawali, G. 2003. Seed characteristics and nutrient and antinutrient composition of 12 Mucuna accessions from Nigeria. Journal of Tropical and Subtropical Agroecosystems 1: 129-140.

FAO/WHO/UNU. 1985. Energy and protein requirements; In: Report of joint FAO/WHO/UNU Expert Consultation. WHO Technical Report Series 724, Geneva, Switzerland: World Health Organization.

Gandhi, A. P. 2009. Quality of soybean and its food products. International Food Research Journal 16: 11-19.

Gueguen, J. and Cerletti, P. 1994. Proteins of some legume seeds: soybean, pea, fababean and lupin. In Hudson, B.J.F. (Ed), New and Developing Sources of Food Proteins, p. 145-193. New York: Chapman and Hall.

Gurumoorthi, P., Pugalenthi, M. and Janardhanan, K. 2003. Nutritional potential of five accessions of a South Indian tribal pulse, *Mucuna pruriens* var utilis II. Investigations on total free phenolics, tannins, trypsin and chymotrypsin inhibitors, phytohaemagglutinins, and *in vitro* protein digestibility. Journal of Tropical and Subtropical Agroecosystems 1: 153-158.

Hettiarachchy, N. S., Griffin, V. K. and Gnanasambandmam, R. 1996. Preparation and Functional Properties of a Protein Isolate from Defatted Wheat Germ. Cereal Chemistry 73 (3): 363-367.

Hsu, H. W., Vavak, D. L., Satterlee, L. D. and Miller, A. 1977. A multienzyme technique for estimating protein digestibility. Journal of Food Science 42: 1269–1273.

Janardhanan, K. and Lakshmanan, K.K. 1985. Studies on the pulse, *Mucuna utilis*: Chemical composition and antinutritional factors. Journal of Food Science and Technology 22: 369–371.

Jesse, M. 2000. Characterization of the seed proteins of velvet bean (*Mucuna pruriens*) from Nigeria. Journal of Food Chemistry 68: 421–427.

Johnson, E. A. and Brekke, J. 1983. Functional properties of acylated pea protein isolates. Journal of Food Science 48: 722 –725.

Kabirullah, M. and Willis, R. B. H. 1982. Functional properties of acetylated and succinylated sunflower protein isolate. Food Technology 17: 235-249.

Kaur, M. and Singh, N. 2007. Characterization of protein isolates from different Indian chickpea (*Cicer arietinum* L.) cultivars. Journal of Food Chemistry 102: 366-374.

Kim, S. Y., Park, P. S. W. and Rhee, K. C. 1990. Functional properties of proteolytic enzyme modified soy protein isolate. Journal of Agriculture and Food Chemistry 38: 651-656.

Kinsella, J. E. 1979. Functional properties of soy proteins. Journal of American Oil Chemists Society 56: 242-245.

Kinsella, J. E., Damodaran, S. and German, B. 1985. Physicochemistry and function of oilseed proteins. In Altschul A. M. and Wilcke, H. L. (Eds), New Protein Food, p. 156-162. New York: Academic Press.

Kinsella, J. E., Rector, D. J. and Philips, L. G. 1994. Physicochemical properties of proteins: Texturization via gelation, glass and film formation. In Yada, R. Y., Jackman. R. L. and Smith, J. L. (Eds). Protein Structure-Function Relationships in Foods, p. 1-21. New York: Chapman and Hall.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

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

Mwasaru, M. A., Muhammad, K., Bakar, J. and Cheman, Y. B. 1999. Effect of isolation technique and conditions on the extractability, physiochemical and functional properties of pigeon pea (*Cajanus cajan*) and cow pea (*Vigna unguiculata*) protein isolates. II. Functional properties. Journal of Food Chemistry 67: 445–452.

Nakai, S. 1983. Structure-function relationship of food proteins. Journal of Agriculture and Food Chemistry 31: 676-683.

Nakai, S. and Powrie, W.D. 1981. Modification of protein for functional and nutritional improvement. In Pomeranz, Y. and Munck, L. (Eds). Cereals – A Renewable Resource: Theory and Practice, p 217. St Paul, MN. American Association of Cereal Chemists.

Paredes-Lopez, O., Ordorica-Falomir, C. and Olivares-Vasquez. M. R. 1991. Chickpea Protein isolates: phytochemical, functional and nutritional characteristics. Journal of Food Science 56: 726-729.

Pomeranz, Y. 1985. Functional Properties of Food Components. New York: Academic Press,

Prins, A. 1988. Principles of foam stability. In Dickinson, E. and Stainsby, G. (Eds). Advances in Food Emulsion and Foams. p. 91-123. London: Elsevier Applied sciences.

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

Sathe, S. K. and Salunkhe, D. K. 1981. Studies on trypsin and chymotrypsin inhibitory activities, hemagglutinating activity, and sugars in Great Northern bean (*Phaseolus vulgaris* L.). Journal of Food Science 46: 626-629.

Shewry, P. R., Napier, J. A. and Tatham, A. S. 1995. Seed storage proteins: Structures and biosynthesis. Plant Cell 7: 945–956.

Siddhuraju P. and Becker K. 2005. Rapid reversed-phase high performance liquid chromatographic method for the quantification of L-Dopa (L-3,4-dihydroxyphenylalanine): non-methylated and methylated tetrahydroisoquinoline compounds from Mucuna beans. Journal of Food Chemistry 91: 275–286.

Teixeira, A. A., Rich, E. C. and Szabo, N. J. 2003. Water extraction of L-Dopa from Mucuna bean. Journal of Tropical and Subtropical Agroecosystems 1: 159-172.

Vijayakumari K., Smitha K. B. and Janardhanan K. 2002. Biochemical characterization of the tribal pulse, *Mucuna utilis* Wall ex. Wight Seeds. Journal of Food Science and Technology 39: 650–653.

Voutsinas, L. P., Cheung, E. and Nakai, S. 1983. Relationships of hydrophobicity to emulsifying properties of heat denatured proteins. Journal of Food Science 48: 26-32.

Wang, L. C. and Kinsella, J. E. 1976. Functional properties of novel proteins: alfafa leaf protein. Journal of Food Science 47: 286-292.

Yasumatsu, K., Sawada, K., Moritaka, S., Toda, J. and Ishii, K. 1972. Whipping and emulsifying properties of food proteins. Canadian Institute of Food Science and Technology Journal 16:185-190.

Zayas, F. J. 1997. Foaming properties of proteins. In Zayas, J. F. (Ed). Functionality of proteins in food, p. 260–309. Berlin: Springer.