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Physicochemical Characteristics of Yam Bean (*Pachyrhizus erosus*) Seed Proteins

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Abstract

This study sought to determine the physicochemical and functional properties of yam bean (*Pachyrhizus erosus*) seed proteins. *Pachyrhizus erosus* seeds from two accessions (UYB 06 and UYB 07) were milled into flours and then defatted. A portion of the defatted flour was used for production of protein isolates and protein fractions. The physicochemical and functional properties, *in vitro* digestibility and electrophoretic pattern of the flour and protein isolate were determined. The results showed that albumins (53.3%) were the dominant protein fraction followed by globulins (18.7%), glutelins (8.8%) and prolamins (2.7%). Regarding functional properties, the *Pachyrhizus erosus* seed protein isolates exhibited 8% of least gelation concentration, water absorption capacity of 3.0 g g^{-1}, oil absorption capacity of 0.8 g g^{-1}, protein solubility of 81.0%, foaming capacity of 37.1%, foam stability of 73.8%, emulsion activity of 13.8% and emulsion stability of 9.2%. *In vitro* protein digestibility of the raw and cooked beans was 87.6% and 84.3%, respectively. The electrophoretic pattern of *Pachyrhizus erosus* protein showed major bands corresponding to molecular weight 13.3, 15, 29.8, 54.4 and above 84.7 kDa. The results, suggest that *Pachyrhizus erosus* seed protein has potential for use in both food and non-food applications such as films and coating.

Keywords: *Pachyrhizus erosus* seed protein, yam bean, functional properties, electrophoretic pattern, protein fractionation and *in vitro* protein digestibility

1. Introduction

Over the past 30 years, the use of concentrated and isolated proteins from plant seeds has increased enormously because of their increased use in industrial applications such as films and coatings (Gennadios et al., 1994) and the greater knowledge of their functional properties, processing and nutritive value (Khalid et al., 2012). While historically, soy beans had a competitive advantage over other legume seeds as a source of protein for industrial use, there is a need to explore and develop other sources of plant proteins. New protein sources could help address the limitations of soy protein and increase the diversity of sources. Crops like the yam bean (*Pachyrhizus ssp*) offer such opportunities since they are adapted to a wide range of conditions especially in the tropics, are high yielding and have high protein content.

The functional properties of plant proteins have been exploited for a multitude of applications including for example, solubility in beverages, foaming in whipped toppings, and emulsification in processed meat, paint and ink among others. This has resulted into an ever increasing demand for plant protein ingredients with improved processing and functional characteristics (Kamara et al., 2009). There has been a constant search for unconventional legumes as new protein sources to fill supply gaps (Chavan et al., 2001). The seeds of yam bean (*Pachyrhizus ssp*) offer unexploited source of protein.

Yam bean crops have mainly been grown for tuber production as a source of food while the use of yam bean

seeds have not been used as food because they contain a toxin – rotenone. The yam bean seeds have high oil (20 - 28%) and protein (23 - 34%) content. However the seeds have mainly been used for the extraction of rotenone as a source of a natural insecticide. If detoxified, the yam bean seeds could provide a protein source for use in the food and non-applications such as edible and or biodegradable films and coatings (Gennadios et al., 1994). Santos et al. (1996) pointed out the potential value of yam bean seed meal for human consumption after the elimination of rotenone.

The final success of utilizing plant proteins as additives depends greatly upon the favorable characteristics that they impart to foods (Khalid et al., 2012; Kamara et al., 2009). In order to develop plant protein for use as food ingredients and other applications, their physicochemical and functional properties have to be evaluated (Chavan et al., 2001). Therefore, the relationship between protein quality and processing parameters that affect the functional performance of protein products is worthy of extensive investigation. Information on physicochemical characteristics of yam bean seed protein is rather scarce. This study was therefore, aimed at determining the physicochemical and functional characteristics of yam bean seed proteins with a view to explore its potential for use in food systems as well as industrial applications.

2. Materials and Methods

2.1 Sample Collection and Preparation

P. erosus seeds from two accessions (2.0 kg each) identified with Ugandan codes UYB 06 and UYB 07 and corresponding to Internatioanl Potato Center (CIP) germplasm codes 209017 and 209018, respectively were collected from yam bean plants grown on-station at National Crops Resources Research Institute (NaCRRI) – Namulonge in Uganda .

2.1.1 Preparation of Defatted Yam Bean Flour

A portion (2.0 kg) of the seeds was milled to fine flour (0.5 mm) using Hammer mill (8" Laboratory Mill Christy Hunt Agricultural Ltd Suffolk England). Flours were stored at 4 °C for a maximum of 12 hours before use. The whole seed flour samples were defatted with hexane (flour/solvent ratio of 1:10 w/v) and stirred for 24 h using a magnetic stirrer (Heidolph Instruments GmbH, Schwabach, Germany). The solvent and the defatted flour were separated by centrifugation at 3400 × G for 15 min using a centrifuge (225; Fisher Scientific, Pittsburg, PA, USA). The supernatant was poured away; the meal was collected and dried at 24-25 °C before storing at 4 °C for further use.

2.1.2 Preparation of Yam Bean Protein Isolates

Yam bean seed protein isolate was prepared following the method described by Sai-Ut et al. (2009). Dispersions of defatted yam bean seed flour in distilled water (5%, w/v) were adjusted to pH 8.0 with 0.1N NaOH, shaken for 1 h and then centrifuged at 5200 rpm for 15 min using a centrifuge. The pH of the extract was adjusted to 4.5 with 1N HCl to precipitate the target proteins. The proteins were recovered by centrifugation using Centrifuge at 3000 × G for 15 min, followed by removal of the supernatant by decantation. Protein curd was washed twice with distilled water and centrifuged again at 3000 × G for 10 min. The washed precipitate was then freeze-dried using a freeze dryer (Alpha 1-4 LOC Christ *Martin Christ* Gefriertrocknungsanlagen GmbH *Osterode am Harz Germany*) at -35 °C for 24h, 0 °C 24h and 20 °C 4h. The freeze dried material was referred to as "protein isolate"

2.2 Fractionation of P. erosus Seed Protein

Fractionation of protein was carried out according to the method of Osborne as reported by Morales-Arellano et al. (2001). Samples of defatted yam bean flour from two accessions of *P. erosus* were suspended in distilled water in the ratio of 1:10 w/v and stirred for 3 h at room temperature and centrifuged using a centrifuge (225; Fisher Scientific, Pittsburg, PA, USA) at 3400 × G for 15 min. The supernatant called albumin was kept at 4 °C until used. The pellet was re-suspended with a solution of 50 mM Tris-HCl, pH 8.0, containing 0.1 M NaCl and stirred as before. The resulting supernatant was designated globulin. The pellet was extracted with 50 mM Tris-HCl, pH 8.0, containing 0.3 M NaCl. After centrifugation at 3400xG for 15 min, the supernatant was called fraction globulin, and the pellet was re-suspended with 70% aqueous 2-propanol, extracted under stirring for 3 h, and centrifuged at 3400 × G for 15 min. The resulting supernatant was designated the prolamin fraction, and the pellet was re-suspended in a solution of 0.1 M NaOH; after centrifugation at 3400 × G for 15 min, the supernatant was designated the glutelins fraction, and the remaining pellet was called residue. The protein content in each protein fraction was determined using the Kjeldahl method (Association of Analytical Chemists, 2000). Nitrogen to protein conversion factor of 6.25 was used.

2.3 Determination of Functional Properties of P. erosus Protein Isolate

2.3.1 Bulk Density

The bulk density of protein isolate was determined according the method described by Butt and Batool (2010). Ten grams of sample were put into 100 mL graduated cylinder and tapped several times (minimum, 10 times) on the laboratory bench for the sample to settle. The volume was noted and density expressed as g/cm^3.

2.3.2 Least Gelation Concentration

Least Gelation Concentration was determined using the method described by Mugendi et al. (2010). 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 (Moulinex – Optiblend 2000 Trio, China) at the highest speed for 2 min. Five milliliters each, of the dispersions were poured into 3 test tubes and heated to 100 °C in a water bath for 1 h 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).

2.3.3 Water and Oil Absorption Capacities

Water and oil absorption capacities were determined according the method described by Appiah et al. (2011). One gram of protein isolate was mixed with 10 mL distilled water (for water absorption capacity determination) or refined corn oil (for oil absorption capacity determination) in a pre-weighed 20 mL centrifuge tube. The water and oil slurries were agitated manually for 2 min, allowed to stand at 28 °C for 30 min and then centrifuged at 3400xG for 20 min. The clear supernatant was decanted and discarded. The adhering drops of water or oil in the centrifuge tube were removed with cotton wool and the tube was weighed, the weight in grams of water or oil absorbed by 1 g protein isolate was calculated and expressed as water or fat absorption capacity.

2.3.4 Protein Solubility

Protein solubility was determined according to the method of Butt and Batool (2010). The protein isolate (0.25 g) was homogenized in 20 mL of 0.1M NaCl at pH 7.0 for 1 h followed by centrifugation using a Centrifuge at 5200 rpm for 30 min. Protein contents in the supernatant was determined and expressed as a percentage of total protein of the original sample.

2.3.5 Emulsion Capacity and Stability

Emulsifying properties (emulsifying capacity and stability) were determined according to the method reported by Butt and Batool (2010). Protein isolate (1.8 g) was added to 25 mL of distilled water (pH 7.0) and dispersed at maximum speed in a blender. Corn oil (12.5 mL) was added and blended at high speed for 1 min; the emulsion formed was equally divided into two 12 mL centrifuge tubes and centrifuged using a Centrifuge (Model 225) for 5 min at 5200 rpm. Emulsion capacity was calculated as follows:

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

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 85 °C for 30 min and subsequently cooled to 25 °C prior to centrifugation.

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

2.3.6 Foaming Capacity and Stability

The foaming capacity and foam stability of yam bean seed protein isolate were determined according the method of Butt and Batool (2010). Protein isolate was dispersed in distilled water to form 3% (w/v) dispersion. A portion (50 mL) of the mixture was immediately transferred into a graduated cylinder and the volume recorded. This was followed by whipping the mixture using a blender at maximum speed setting for 4 min and volume after whipping was recorded. Foaming capacity was expressed as percentage volume change induced by whipping. The percent change in volume of foam after 60 min of standing at room temperature was recorded as foam stability.

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

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

2.4 In-vitro Protein Digestibility of P. erosus Seed Protein

The *in-vitro* protein digestibility for both raw and cooked yam bean seed was determined using pepsin–pancreatin enzyme method described by Chavan et al. (2001). About 1 g of sample was suspended in 60 mL of 0.1M HCl at pH of 1.0 containing 6 mg of pepsin, followed by gentle shaking for 15 min at 37 °C. The resulting solution was neutralized with 0.5 M sodium hydroxide to pH 7.0 and treated with 16 mg of pancreatin from porcine pancreas, (activity equivalent to 4×US pharmacopeia) in 30 mL of phosphate buffer (0.1 M, pH 8.0). The mixture was then shaken for 24 h at 37 °C in water bath shaker (3G86GB Grant Cambridge England). The undigested solid was separated by filtration using glass wool (about 0.5 g) under suction from a vacuum pump and washed twice with 10 mL distilled water. The protein content in the undigested solid and initial protein content of both cooked and raw samples was determined using the Kjeldahl method (AOAC, 2000). *In vitro* protein digestibility was expressed as percentage as indicated below:

$$\text{In vitroprotein digestibility } (\%) = \frac{\text{A-B}}{\text{A}}$$

Where; A= % protein in the samples before digestion, and B = % protein after enzyme digestion

2.5 Electrophoretic Pattern of Defatted Yam Bean (P. erosus) Seed Flours and Its Protein Isolates

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was run on both the cooked and raw sample of both the defatted flours and the protein isolate. The cooked samples were prepared in such a way that the defatted flour was suspended in the distilled water in the ratio of 1:10 (w/w, flour: water) and was heated to boil Bunsen burner flame then simmered for 1 h.

SDS-PAGE was carried out according to the method of Laemmli (1970) with and without 2-mercaptoethanol (2-ME). A separating gel of 10% and acrylamide stacking gel of 4% were used. Electrophoresis was carried out using a Bio-Rad vertical Electrophoresis System (Min-protean II cell Bio-Rad Richmond CA USA). The protein samples were mixed with the sample buffer (0.5 M Tris-HCl, pH 6.8, and 10% (w/v) SDS, 20% glycerol and 1% bromophenol blue) at the ratio of 1 to 1 in presence and absence of 5% 2-mercaptoethanol. The samples were denatured by heating at 85 °C for 10 min. A 10 µl aliquot of each sample was loaded onto the gel for protein separation. Electrophoresis was conducted at a constant voltage of 200 V for 1 h. The gel was stained with 0.1% Coomassie Brilliant Blue R250 in methanol/acetic acid (40:10 v/v) solution. De-staining was achieved by washing the gel for 2 h with the same solution but without the dye and then overnight with a solution of acetic acid/methanol (7:5 v/v). Gel images were taken using a scanner. A standard protein molecular weight marker (Thermo Scientific, Pageruler prestained protein ladder, MW range; 10-170 kDa) was run concurrently with the sample and used to estimate apparent molecular weight of the different fractions detected.

2.6. Statistical Analysis

All experimental analyses in this study were conducted in triplicates. One way analysis of variance (ANOVA) was performed to determine significant differences among treatment means at ($P<0.05$) and a paired *T*-test was performed for the *in vitro* protein digestibility data for raw and cooked samples. All statistical analyses were conducted using SPSS/16.0 Software (IBM Corporation).

3. Results and Discussion

3.1 Fractionation of Yam Bean (P. erosus) Seed Protein

A protein recovery rate of 93.1 and 93.6 g per 100 g proteins for UYB 07 and UYB 06, respectively was recorded. Albumins were the most dominant protein fraction recorded, followed by globulins, glutelins and prolamins in both accessions (Table 1). The proportions of the protein fractions were not significantly different between the two accessions (UYB 06 and UYB 07) of *P. erosus*.

The protein fractionation pattern observed in this study is in agreement with the results reported by Morales-Arellano et al., (2001) for *P. erosus* where albumins were reported as the major fraction (31.0-52.1%) followed globulins (27.5-30.7%) with a protein recovery of 99.8-99.9%. The results also showed that yam bean seed protein was different from that of other legumes such as soy bean (Vasconcelos et al., 2010), peas, common bean (Chan & Phillips, 1994; Morales et al., 2001) and mucuna seeds (Sridhar & Bhat, 2007) that have globulin as the dominant fraction.

Table 1. Protein recovery and content of different protein fractions of two accessions (UYB 06 and UYB 07) of yam bean seeds (*P. erosus*) †

Protein fraction	G 100 g^{-1} of crude protein in the defatted yam bean (*P. erosus*) seed flour	
	UYB 06	UYB 07
Albumin	$53.8^{a‡} \pm 1.24$	$52.7^{a} \pm 1.67$
Globulin (0.1M NaCl)	$12.5^{a} \pm 2.02$	$11.5^{a} \pm 1.20$
Globulin (0.3M NaCl)	$7.3^{a‡} \pm 1.28$	$6.0^{a} \pm 1.52$
Prolamin	$2.7^{a} \pm 0.91$	$2.6^{a} \pm 1.71$
Glutelin	$8.0^{a} \pm 1.59$	$9.6^{a} \pm 2.05$
Residue	$9.4^{a} \pm 1.84$	$10.7^{a} \pm 2.21$
Protein recovery	$93.6^{a} \pm 0.55$	$93.1^{a} \pm 0.97$

† All values are means of triplicate determinations ± SD.

‡Means values in the same row with different superscript letters are significantly different ($P \leq 0.05$).

Santos et al. (1996) reported glutelin as the most dominant protein fraction in yam bean seed protein (*P. erosus)* with globulins, albumins and prolamins reported at 28.8, 16.3 and 7.0%, respectively, contrary to the results of this study. This disparity may be due to the differences in soil fertility and climatic conditions where the crops were grown. Castle and Randall (1987) and Malik et al. (2012) demonstrated that soil fertility can affect grain protein composition. Besides, there can be variability in grain legume germplasm collections, segregating populations, mutant populations, and cultivated varieties (Burstin et al., 2011). Insoluble proteins were recovered in the residues of UYB 06 and UYB 07 containing 9.4 and 10.7 g/100 g of protein, respectively. The insolubility of some protein could, in part, be attributed to the damage caused by the solvent hexane on the proteins (Morales-Arellano et al., 2001). Chan and Phillips (1994) reported that the relative proportion of each protein fraction in the seed strongly affects the nutritional and functional quality of the total seed protein. Therefore, yam bean seed protein having albumin as a dominant protein fraction is indicative of good quality protein in the seed for human and animal nutrition.

3.2 Functional Properties of Yam Bean Seed Protein Isolate From Two Accessions of P. erosus

The results of the various functional properties of yam bean seed protein isolate from two accessions are indicated in Table 2. There was no significant difference between the functional properties of the two accessions of *P. erosus* studied (Table 2).

Table 2. Functional properties of yam bean seed protein isolates from the two accessions of *P. erosus* †

Functional properties	Accession	
	UYB 06	UYB 07
Bulk density(g cm^{-3})	$0.59^{a‡} \pm 0.00$	$0.59^{a} \pm 0.00$
Least gelation concentration (%)	$8.00^{a} \pm 0.00$	$8.00^{a} \pm 0.00$
Water absorption capacity (g g^{-1})	$3.00^{a} \pm 0.19$	$2.88^{a} \pm 0.10$
Oil absorption capacity (g g^{-1})	$0.79^{a} \pm 0.05$	$0.78^{a} \pm 0.07$
Emulsion capacity (%)	$12.92^{a} \pm 0.85$	$14.67^{a} \pm 0.04$
Emulsion stability (%)	$9.48^{a} \pm 0.42$	$8.90^{a} \pm 0.40$
Foaming capacity (%)	$37.15^{a} \pm 6.10$	$37.04^{a} \pm 3.07$
Foam stability (%)	$74.12^{a} \pm 0.76$	$73.37^{a} \pm 1.81$
Protein solubility (%)	$81.64^{a} \pm 1.25$	$80.37^{a} \pm 0.43$

† All values in the table are means of triplicate determinations ± SD.

‡ Means values in the same row with different superscript letters are significantly different ($P \leq 0.05$).

3.2.1 Bulk Density

The bulk densities of the isolate in this study were lower than values reported for other legumes. Butt and Batool (2012) reported bulk densities of 0.71 and 0.68 g cm^{-3} for proteins isolates of cowpea and pea, respectively. Bulk density is known to affect the packaging requirements of the product after processing.

3.2.2 Least Gelation Concentration

The least gelation concentration (LGC) indicates the minimum protein concentration at which a stable gel can be formed - low LGC, is associated with high gelling ability of the protein. The LGC values recorded for yam bean seed protein isolates in this study (Table 2) were lower than values reported for other legumes. These results suggest that yam bean seed protein isolate have better gelling properties than protein isolates of other legumes. Butt and Batool (2010) reported LGC of 14, 16, 16, and 18 % for protein isolates of pigeon peas, cow peas, mung bean and peas, respectively.

3.2.3 Water and Oil Absorption Capacity

Water absorption capacity (WAC) and oil absorption capacity (OAC) represent the amount of water and oil, respectively, that can be bound per unit weight of the protein material and constitutes useful indices of the ability of the protein to prevent fluid leakage from a product during food storage or processing (Kiosseoglou & Paraskevopoulou, 2011). The WAC results recorded in this study (Table 2) were higher than those reported for other legumes suggesting high water absorption capabilities for yam bean. Butt and Batool (2010) reported WAC of 0.97, 1.38, 1.63, and 1.52 g g^{-1} for pigeon pea, cow pea, mung bean, and cow pea protein isolates, respectively. Khalid et al. (2012) reported WAC of 2.10 g g^{-1} for cow pea.

Results of OAC for yam bean seed flour reported in this study (Table 2) were lower than figures reported for other legumes protein isolates, suggesting that yam bean seed protein isolate would absorb less oil in a frying process. Butt and Batool (2010) reported OAC value of 1.68, 1.45, 1.13 and 1.40 g g^{-1} for pigeon pea, cow pea, mung bean, and pea protein isolates, respectively. Khalid et al. (2012) reported OAC of 1.90 g g^{-1} for cow pea. The differences between the WAC and OAC of protein isolate from yam bean and other legumes can be attributed to both species and variety (Burstin et al., 2011). Kiosseoglou and Paraskevopoulou (2011) noted that the type of legume notwithstanding, it appears that the technique employed for protein recovery may also influence the water absorption capacity value, citing an example of the protein material obtained by isoelectric precipitation from pea and chick pea exhibiting higher water binding ability than those prepared by ultra filtration.

Interactions of water with proteins are important in food systems because of their influence on the food product texture and succulence (Amadou et al., 2010). The high WAC and moderate OAC of the yam bean seed protein isolate would allow moisture and oil retention which suggests potential for its use in meats, sausage, bread and cakes to improving product texture succulence.

3.2.4 Emulsion Capacity (EC) and Stability (ES)

Emulsion capacity reflects the ability of a protein to aid the formation of an emulsion, while emulsion stability reflects the ability of the protein to impart strength to emulsion for resistance to stress (Zayas & Lin, 1989). The protein isolates from the two accessions (UYB 06 and UYB 07) exhibited relatively lower emulsifying capacity as well as stability values (Table 2) than those reported for other legume seed protein isolates. Butt and Batool (2010) reported emulsion activity values of 49.5, 47.5, 41.1 and 45.5% and emulsion stability of; 83.3, 52.2, 21.0 and 43.2% for pigeon, cowpea, mung bean, and pea protein isolates, respectively. Also Eltayeb et al. (2011) reported emulsion capacity of about 54% for mucuna bean protein isolate and emulsion stability of about 48% at the pH of 7.0.

Nassar (2008) noted that proteins with high emulsifying capacity are good for salad dressing, sausages, bologna, soups, confectionery, frozen dessert and cakes. However, results in this study indicate that yam bean seed protein isolate can only be used as an emulsifier possibly with modification of its properties.

3.2.5 Foaming Capacity and Stability

According to Butt and Batool (2010), foaming properties are used as indices of whipping characteristics of protein isolates. The protein isolates from the two accessions (UYB06 and UYB07) exhibited moderate foaming capacity and high foam stability (Table 2). The foaming capacity results for yam bean seed protein isolate in this study were lower than those (85 to 90%) reported by Eltayeb el al. (2011) for the Bambara protein isolate at the pH range of 6.0 to 7.5. However, for the foaming capacity of yam bean seed protein isolate in this study were higher than those reported for raw Mucuna bean protein isolate (about 12.5%) at pH 7.0 while for foam stability,

Mucuna bean protein isolate was reported to have higher values (about 84.5%) (Eltayeb et al., 2011). During whipping to form foam, proteins denature and aggregate to exhibit an increase in the surface area at the liquid and air interface which involves rapid conformational change and rearrangement. Foam stability requires formation of a thick, cohesive and viscoelastic film around each gas bubble, which is a function of the configuration of protein molecules (Amadou et al., 2010).

3.2.6 Protein Solubility

The protein solubility for the two yam bean accessions (Table 2) was relatively high. The results for protein solubility of the yam bean seed protein isolates are almost similar to the solubility values (82%) for pea protein isolate and higher than results reported for mung bean protein isolate (72%), cowpea (65%) and pigeon pea protein isolates (68%) at pH 7.0 (Butt & Batool, 2010). Protein solubility is usually affected by its hydrophilic and hydrophobic balance, depending on amino acid composition in particular at the protein surface. The high protein solubility of yam bean seed protein isolate in this study can be attributed to the low number of hydrophobic residues and elevated charge Butt and Batool (2010). Protein solubility is an important prerequisite for food protein functional properties and it is a good index of potential applications of proteins (Kamara et al., 2009). With respect to non food application, the high solubility of yam bean seed protein isolates in water shows potential for its application in water based adhesive formulations.

3.3 In-vitro Protein Digestibility (IVPD)

Protein digestibility is one of the major determinants of the nutritional quality of protein and influences bioavailability of amino acids (Sridhar & Bhat, 2007). Both cooked and raw samples of the two yam bean accessions in this study exhibited considerably high IVPD (Table 3). The protein digestibility values in this study were higher than those reported for *P. erosus* flour by Santos et al. (1996). Yam bean seed flour production by Santos et al. (1996) entailed soaking, cooking, drying, milling, defatting and then drying. These processes may have negatively affected protein digestibility since processes like cooking and drying cause protein cross linking and lead to protein denaturation which ultimately affects protein digestibility. In addition, they were also higher than 75.04% for raw and 76.69% for cooked cow pea protein reported by El-Jasser (2010). The digestibility results were in agreement with those reported by Sulieman et al. (2008) for raw (77.1-88.2%) and cooked (81.8-99.9%) lentil seeds. However, raw yam bean seed exhibited a significantly higher IVPD than cooked samples.

Table 3. *In vitro* protein digestibility of yam bean seeds from two accessions of *P. erosus*[†]

Accessions	% *in vitro* protein digestibility	
	Raw	**Cooked**
UYB06	$87.65^{b‡} \pm 1.60$	$84.32^{a} \pm 1.50$
UYB07	$87.35^{b} \pm 1.21$	$84.25^{a} \pm 1.65$

[†] All values in the table are means of triplicate determinations ± SD.

[‡] Means values in the same row with different superscript letters are significantly different ($P \leq 0.05$).

The high IVPD results recorded in the current study may in part be attributed to the lower concentration of the antinutritional components in *P. erosus* seeds compared to other grain legumes. Santos et al. (1996) reported that *P. erosus* seeds contained low levels of tannins (10.2 mg/100 g) and trypsin inhibitory activity (17.1 ITU) compared to other legumes. The lower IVPD in cooked samples may be due to aggregation and cross linking of yam bean seed protein following thermal treatment. Heat causes oxidation of sulfhydryl groups to form disulfide bonds and also leads to interaction between acidic and basic residues that would be more resistant to proteases (Duodu et al., 2003; Suleiman et al., 2008). The electrophoresis results Figure 2 in this study seem to confirm this supposition due to the increase in the number of high molecular weight proteins bands exhibited on cooking and their decrease under reducing conditions. The high *in vitro* protein digestibility recorded in this study indicated that yam bean seed protein has good nutritional quality.

3.4 Electrophoretic Pattern of Yam Bean Seed Protein of P. erosus

3.4.1 Electrophoretic Pattern of the Defatted Yam Bean Seed Flour and Its Protein Isolate

Figure 1 shows the electrophoretic pattern of yam bean seed flour and their protein isolates both under reducing

and non-reducing conditions. The results show that the proteins of yam bean seed flour were in five major band categories namely 100-170, 70-95, 40-55, 25-25 and 15-20 kDa both under reducing and non-reducing conditions. The bands that showed high intensity appeared at 93, 54, 31 and 16 kDa. The electrophoretic pattern recorded in this study for both the defatted yam bean seed flours and their protein isolates is typical of yam bean seed protein as reported by Morales-Arellano et al. (2001). Although yam bean accessions did not show any difference in the electrophoretic pattern, the defatted flours (lanes 6 and 8) of both accessions showed intense bands of 100-170kDa under non-reducing condition. However, these bands were not visible under reducing conditions (lanes 5 and 7). The 10-170kDa bands were lightly visible in protein isolates from both yam bean seed accessions under reducing and non-reducing conditions (lanes 1-4). These results suggest the presence of disulphide linked high molecular weight protein aggregates that are cleaved to smaller bands on reduction with mercaptolethanol. In addition, the results further suggest that the 100-170 kDa proteins were either not extracted or were lost during the preparation of the protein isolates (Sai-Ut et al., 2009; Mugendi et al., 2010). Leyva et al. (1995) working on amaranth proteins reported that processes such as defatting can influence the electrophoretic pattern of proteins.

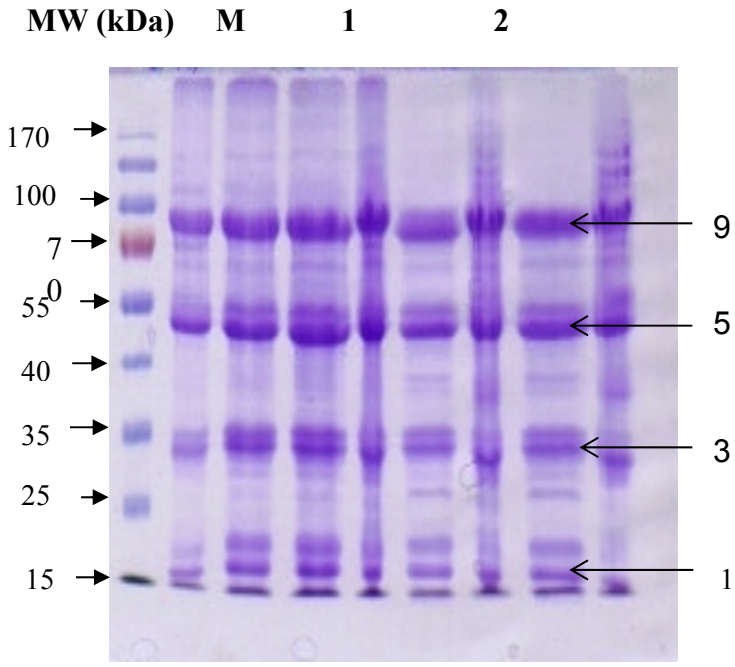

Figure 1. Electrophoretic pattern of yam bean seed flour and their protein isolates both under reducing and non-reducing conditions

M: Standard protein marker, 1: UYB 06 isolate reduced, 2: UYB 06 isolate non-reduced, 3: UYB07 isolate reduced, 4: UYB 07 isolate non-reduced, 5: UYB06 flour reduced, 6: UYB 06 flour non-reduced, 7: UYB 07 flour reduced, 8: UYB 07 flour non-reduced

The molecular weight of the different proteins influences their suitability for use in processes like protein texturization with proteins of molecular weights in the range of 10 to 50 kDa preferred for the purpose (Belitz et al., 2009). Proteins less than 10 kDa are weak fiber builders while those with molecular weight higher than 50 kDa are disadvantageous due to their high viscosity and the tendency to gel in alkaline pH range (Belitz et al., 2009). A substantial number of protein bands were within the range 10-50 kDa (Figure 1) suggesting that yam bean seed protein is potentially good for texturization.

3.4.2 Electrophoretic Pattern of The Raw Cooked and Cooked Defatted Yam Bean Flour

Figure 2 shows electrophoretic pattern of cooked and uncooked yam bean seed flour both in reducing and non-reducing conditions. Electrophoresis showed the five major band categories as discussed for Figure 1. In

general, the intensity of the bands of 100 kDa and above was higher while that of bands less than 35 kDa was lower in the cooked (lanes 2 and 4) as compared to the uncooked samples (lanes 1 and 3) under non-reducing conditions. Under reducing conditions, the intensity of the bands of 100 kDa and above decreased while that of the lower kDa increased (lanes 6 and 8). The results suggest heat induced disulphide linked protein aggregation. A similar heat induced protein aggregation has been reported in Kafirin proteins (Duodu et al., 2003). This result agrees with the reduced IVPD recorded on cooking of yam bean seed flour in this study.

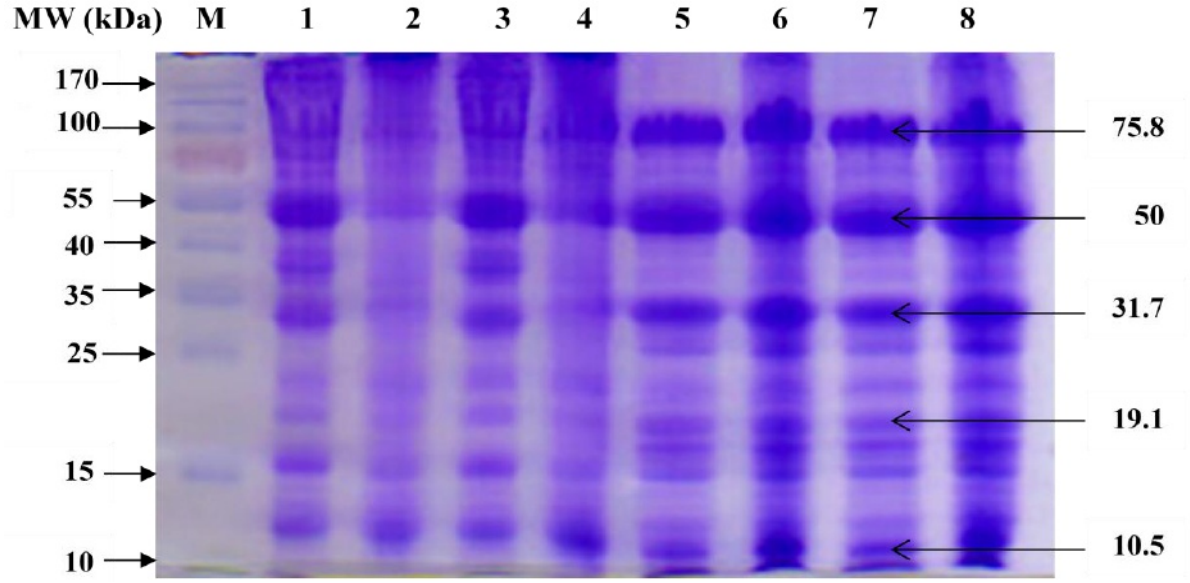

Figure 2. Electrophoretic pattern of cooked and uncooked yam bean seed flour both in reducing and non-reducing conditions

M: Standard protein marker, 1: UYB 06 uncooked non-reduced, 2: UYB 06 cooked non-reduced, 3: UYB 07 uncooked non-reduced, 4: UYB 07 cooked non-reduced, 5: UYB 06 uncooked reduced, 6: UYB 06 cooked reduced, 7: UYB 07 uncooked reduced, 8: UYB 07 cooked reduced

4. Conclusion

The yam bean seed protein isolates exhibited good gelation capacity, water absorption capacity, foam stability, and nitrogen solubility, properties which can be exploited for food and non-food applications. These properties can further be enhanced by modification of the protein. Albumins fraction, which consists of biologically active protein, is the most dominant protein fraction in yam bean seeds. The protein digestibility of yam bean seed is high even though it reduces on cooking.

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