MAKERERE UNIVERSITY

PHYSICOCHEMICAL CHARACTERISTICS OF YAM BEAN (*PACHYRHIZUS SPP*) SEED FLOUR

BY

ABBAS KISAMBIRA

BSc. FST (Mak)

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DECLARATION

I declare that this thesis that I hereby submit to the Directorate of Research and Graduate Training of Makerere University in partial fulfillment of the requirements for the award of the degree of Master of Science in Food Science and Technology of Makerere University is my original work and has not been submitted by me for a degree to any other university or institution of higher learning

Signed…….. Date……

ABBAS KISAMBIRA (BSc. FST)

This research thesis is submitted for examination with the approval of the following supervisors:

Signed…….. Date……

PROF. JOHN H. MUYONGA (PhD)

Signed…….. Date……

YUSUF B. BYARUHANGA (PhD)

DEDICATION

This work is dedicated to my parents Erias and Teddy Kisambira and my siblings: Dauda, Ibrahim, Janat, Mariam, Sowedi, Madina, Isaac, Bashir and Moses.

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Praise and thanks be unto the Almighty who has taught man by the pen and taught him that which he knew not.

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LIST OF ACRONYMS

AOAC - Association of Official Analytical Chemists

CIP - International Potato Centre

EC - Emulsion Capacity

ES - Emulsion Stability

FAO - Food and Agricultural Organization

FC - Foaming Capacity

FP - Flower Pruning

FS -Foam Stability

ITU - Inhibited Trypsin Unit

IVPD - *In Vitro* Protein Digestibility

LGC - Least Gelation Concentration

NaCRRI - National Crop Resources Research Institute, Namulonge

NAS - National Academy of Science

OAC - Oil Absorption Capacity

RVA - Rapid Visco Analyzer

RVU - Rapid Visco Units

SD- Standard Deviation

SDS -PAGE –Sodium Dodecyl Sulfate –Polyacrylamide Gel Electrophoresis

SE- Standard Error

UYB - Uganda Yam Bean

WAC - Water Absorption Capacity

WHO - World Health Organization

ABSTRACT

Yam bean (*Pachyrhizus spp*) is a robust legume crop native to South and Central America. The yam bean is mainly grown for its root tubers which are used as food. The yam bean seeds contain high amount of proteins and oil but are not consumed because they contain the toxic compound, rotenone. The aim of this study was to determine the physicochemical characteristics of yam bean seed flour as the first step towards identifying potential uses of the seeds.

Seeds from the three cultivated species of yam bean plants grown on-station at National Crops Resources Research Institute (NaCRRI), Namulonge- Uganda were collected from CIP- Uganda. The seeds were collected from ten selected accessions (2 accessions of *P. erosus*, 4 accession of *P. ahipa* and 4 accessions of *P. tuberosus*). The seeds were milled and a portion of the flour was defatted using hexane. Part of the defatted flour was used to prepare a protein isolate using the isoelectric precipitation method. The proximate composition of the whole seed (from the three species) and defatted yam bean seed flour from *P. erosus* was determined. The defatted flours and their protein isolate were also analyzed for water and oil absorption capacity, bulk density, protein solubility, least gelation concentration, emulsifying capacity, emulsion stability, foaming capacity and foam stability as well as pasting properties (for flour only). The *in vitro* protein digestibility of *P. erosus* seed protein was determined using the pepsin–pancreatin enzyme system. The protein was fractionated based on solubility in different solvents. The electrophoretic pattern (using SDS-PAGE under reducing and non-reducing conditions) of the defatted seed flours and their protein isolates from *P. erosus* was also determined.

The yam bean seeds were found to contain about 29.2-32.1% proteins, 31.3-33.0 % carbohydrates, 24.1-25.6 % crude fat, 7.5- 8.1% crude fiber and 3.4- 4.1% ash. The defatted *P. erosus* seed flour contained about 45.6- 48.8 % protein; 32.6-36.5 % total carbohydrate, 6.7 -7.1 % crude fiber and 6.0- 6.4 % ash and 5.2%crude fat.

Regarding functional properties, the protein solubility, least gelation concentration, water absorption capacity and oil absorption capacity of the defatted yam bean seed flours were found to

be 68.0-70.4%, 14%, 2.8-2.9% and 1.5% respectively. The defatted flour had about 35.7-36.0% emulsifying capacity, 33.2-33.5% emulsion stability, 42% foaming capacities and 25.1-25.8% foam stability. The defatted yam bean seed protein isolate had least gelation concentration of 8%, water absorption capacity 2.9-3 g/g, oil absorption capacity 0.8 g/g, protein solubility 80.4-81.6%, foaming capacity 37.0-37.2%, foam stability 73.4-74.1%, emulsion activity 12.9-14.7% and emulsion stability of 9.2%. The defatted yam bean seed flours had pasting temperatures of 80.0-81.3^{o}C, peak viscosity of 145-146 RVU, trough viscosity of 95.11-102.03 RVU, breakdown value of 43-51 RVU, setback value of 252 -258 RVU and final viscosity of 348-360 RVU.

Results of protein fractionation showed that albumins were the most dominant protein fraction (52.7-53.8%) followed by globulins (17.5-19.8%), glutelins (8.0-9.6%) and prolamines (2.6-2.7%). *In vitro* protein digestibilities of *P. erosus* for both raw and cooked samples were about 87.5 and 84.3%, respectively. The electrophoretic pattern of yam bean seed protein showed major bands corresponding to molecular weights of 35, 55 and above 85 kDa. These bands mainly represented albumins and globulins. Electrophoresis results indicated that there were no observable differences in the electrophoretic patterns of the two accessions of *P. erosus.* The results of this study indicated that yam bean seed flour and its protein isolate have potential for use in food and industrial applications like stabilization of foams and gel formation.

1 INTRODUCTION

1.1 Background

Leguminous plants are widely grown for their protein-rich seeds and are the second most important group of crop plants after cereals in terms of production (Forsyth, Ring, Noel, Parker, Cairns, Findlay, and Shewry, 2002). Legumes mainly constitute of tuberless plants and the part normally consumed are the pods. However, some leguminous plants, notably species of the genus *Pachyrhizus* (yam bean) which are native to South and Central America, have tuberous roots (Forsyth *et al.,* 2002). The name 'yam bean' is used to designate the species of the genus, in particular the three cultivated species which develop tuberous roots (Grüneberg, Freynhagen-Leopold, and Delgado-váquez, 2003).

Grown under very different ecogeographical conditions, the three cultivated species of yam bean include: *P. tuberosus* in South America (principally Bolivia, Peru, Equador, and Brazil), *P. erosus* (Jacatube or Mexican yam bean) in Central America and the Caribbean, and *P. ahipa* (ahipa) in the Andes of Bolivia as well as Northern Argentina (Forsyth *et al.,* 2002; Grüneberg, 2003). The National Academy of Sciences (NAS) (2006) reported the Spanish galleons to have carried the seed across the Pacific, and this productive, palatable, and nutritious legume subsequently spread through Asia and became a market-garden favorite from China all the way to India. During the last few years, yam bean has been introduced to Africa, with remarkable success in a number of West African countries (National Academy of Sciences, 2006). The crop has also been introduced in Central and East African countries including Uganda.

Zanklan, Ahouangonou, Heiko, Pawelzik, and Grüneberg, (2007) outlined the characteristics of yam bean which make it attractive to the small holder farmers and noted that the crop can grow without fertilizer because it exhibits an efficient symbiotic relationship with *Rhizobia*. The crop is also associated with *Mycorrhizae* (a fungus) which facilitate phosphorus uptake. From an ecological and socioeconomic perspective, the crop has become an integral part of sustainable land use systems.

The yam bean is regarded as under-exploited legume food resource attractive for agronomy as well as breeding (Grüneberg *et al.,* 2003). The crop is propagated by seeds with a high self-fertilization rate (Grüneberg *et al.,* 2003). As a root crop, it can provide high yields (above 50 MT/ha) and high yield stability and as a legume it provides more protein (3-5 times) than the traditional root crops (such as cassava and sweet potato) and has the capability to increase soil fertility (Santos, Cavalcanti, and Coelho, 1996). Grüneberg *et al.* (2003) reported that the use of yam bean as compared to traditional root crops is limited because of the usually low tuber dry matter usually between 12-22%. Breeding programs could be undertaken to address this limitation.

Because of the obvious competitive source/sink relationship between tuber growth and seed production (Sørensen *et al.,* 1997), all the flowers are removed, in both small scale and commercial production (Zanklan *et al*., 2007). Flower pruning increases the storage root production and it is only a few plants that are not flower pruned and left to produce seeds for propagation purposes (Zanklan *et al*., 2007). Sørensen *et al.* (1997) observed that unpruned plants show a much less attractive tuber formation than those reproductively pruned (Sørensen *et al.,* 1997). Zanklan *et al.* (2003) reported the average tuber yield ranging from 6 – 45 MT/ha, 21 - 81 MT/ha and 10 - 38 MT/ha for the Amazonian, Mexican and Andean yam bean, respectively. In a combined utilization of tubers and seeds, tuber yield ranged from 5-29 MT/ha, 10-49 MT/ha, 6- 27 MT/ha and seed yield from 1.5- 2.9 MT/ha, 3.5 to 4.6 MT/ha and 2.6 to 2.7 MT/ha for the Amazonian, Mexican and Andean yam bean, respectively (Zanklan *et al*., 2003). These results suggest that the relationship between tuber yield and seed yield is not an inverse one and it is possible to produce substantial yield of both.

The 1000-seed weight of yam bean is high (180-230 g) (Zanklan *et al*., 2007) compared to close relatives like peanuts. Santos *et al.* (1996) showed that yam bean seeds are high in protein (23%-28%), fat (27%) and carbohydrate content (27%) although the precise values may vary with species and growth conditions (Forsyth *et al.,* 2002). Despite the seeds having rich nutrient content, they are never consumed by people because they contain about 1% of toxic substance known as rotenone (Santos *et al.,* 1996; Zanklan *et al*., 2007). It is therefore important to

understand the composition and physicochemical properties of yam bean seed as the first step towards identifying potential uses of the seed.

1.2 Problem Statement

The challenge of providing adequate protein for the expanding world population is second only to the overall food problem (Pomeranz and Meloan, 1994). World demand for proteins is increasing and more plant protein is required from both conventional and new sources (Eltayeb *et al.*, 2011). According to Chavan, McKenzie, and Shahidi (2001), because of the inadequate supplies and shortage of proteins, there is a constant search for unconventional legumes as new protein sources for use in both food systems and for non food industrial applications. For successful use of legume proteins in food and non-food industrial applications, they should possess desirable functional properties (Sai-Ut, Ketnawa, Chaiwut and Rawdkuen, 2009). These functional properties of proteins affect their behavior in systems during processing, manufacturing, storage, preparation as well as consumption (Sai-Ut *et al.*, 2009; Butt & Batool, 2010).

Over the past 30 years, the use of concentrated and isolated proteins as well as flour from legume plant seeds has been on the increase because of greater knowledge of their functional properties, processing and nutritive value (El-Jasser, 2011). While historically, soy beans had a competitive advantage over other legume seeds, there is a need to develop other sources of concentrated and isolated plant proteins which ideally should be crops like the yam bean that are adapted to a wide range of conditions especially in the tropics and yields.

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 seeds have mainly been focused on the extraction of rotenone as a source of a natural insecticide. Information on yam bean seed composition and the physicochemical characteristics of the seed flour is rather limited. The seeds of yam bean have been reported to have high oil (20- 28%) and protein (23-34%) content. However, yam bean seeds are never consumed due to the presence of the toxic substance rotenone (Santos *et al.,* 1996; Grüneberg *et al.,* 1999; Zanklan *et al.,* 2007). If detoxified, the seeds could provide a protein and oil source for use in the food and allied industries (Biopact, 2007). Santos *et*

al. (1996) pointed out the potential value of yam bean seed meal for human consumption after the elimination of rotenone.

The lack of information on many basic aspects of underutilized crops such as the yam bean hinders their development and their sustainable utilization (Morales-Arellano, Chagolla-Lo´pez, Paredes-Lo´pez and Barba de la Rosa, 2001). Therefore the aim of this study was to determine the chemical composition and the physicochemical characteristics of yam bean (*Pachyrhizus spp)* seed flour and protein, as a first step towards identifying possible uses for the seeds.

1.3 The objectives of the study

1.3.1 Overall Objective

The overall objective of the study was to investigate the chemical composition and physicochemical characteristics of yam bean (*Pachyrhizus spp)* seed flour and protein

1.3.2 Specific objectives

1. To determine the proximate composition of seeds from the three cultivated yam bean species
2. To determine the functional and pasting properties of defatted yam bean (*P. erosus*) seed flour
3. To determine the physicochemical characteristics of yam bean (*P. erosus*) seed protein

1.4 Hypotheses

- There is no significant difference in the proximate composition of yam bean (*Pachyrhizus ssp*) seeds of the three cultivated species.
- There is no significant difference in the functional properties of the seed flour from the two accessions of yam bean (*P. erosus).*

1.5 Justification for the study

Yam bean production is spreading to different parts of the world and of recent it has been introduced in East Africa including Uganda. While yam bean tubers are safely used as food and can be incorporated in a variety of local dishes and feeding patterns, yam bean seeds are hardly utilized because of their content of the toxic rotenone. By understanding the properties of the yam bean seed, it would be possible to point to some possible uses of the seed. Since detoxification is feasible through oil extraction, yam bean seed may have both food and non-food industrial applications. An understanding of the physicochemical characteristics of yam bean seed flour and protein isolate is important for the assessment of the potential applications of these seeds. The use of the yam bean seeds would make the crop more valuable and therefore contribute to food security, environmental conservation as well as enhancing farmers' incomes.

2 LITERATURE REVIEW

2.1 Yam bean

2.1.1 The genus

The yam bean is generically known as *Pachyrhizus* which is derived from the Greek words *pachys* = thick (ened), and *rhiza* = root. It is placed taxonomically in the subtribe *Diocleinae*, tribe *Phaseoleae*, within the legume family (*Fabaceae*) (Sørensen, 1996). The genus contains three cultivated species; the Mexican yam bean (*P. erosus*), the Andean yam bean (*P. ahipa*) and the Amazonian yam bean (*P. tuberosus*) and two wild species; *P. panamensis* and *P. ferrugineus* (Zanklan, 2003).

All species in the genus have tuberous roots and are morphologically characterized by the presence of a "false beard" of short hairs along the dorsal (adaxial) side of the ovary, continuing almost to the base of the stigma along the incurved side of the style (Sørensen, 1996). The Mexican yam bean *(P. erosus*) is the only species which spread widely among the three cultivated species of yam bean (Sørensen, 1996). This may be attributed to the limited climatic adaptation of the other 2 species and/or possibly the historical progress of the Spanish and Portuguese conquest of Latin America and the general policy of destroying the traditional Andean agricultural systems (Sørensen *et al.*, 1997). *P. erosus* cultivars generally yield higher yield in most situations (Sørensen, 1996).

Among the Neotropical legume genera with edible tuberous roots, such as *Apios* Fabr., *Pachyrhizus* Rich. ex DC, *Pediomelum* Rydb. and *Vigna* Savi, the yam bean *(Pachyrhizus)* is the only one that is extensively cultivated, both as a garden crop, and, in the case of *P. erosus* (L.) Urban, also on a large scale for export. *P. erosus* is known by different local or indigenous names, including fan-ko (China), sankalu (India), sinkamas (Philippines), dolique tubereux or pais patate (French), Knollige Bohne (German) (National Academy of Sciences, 2006). *P. erosus* originated in Mexico and Central America, and is cultivated in Mexico, Guatemala, El Salvador and to a limited extent in Honduras.

It has been introduced to different pantropical regions, with notable success in Southeast Asia. The two other cultivated species, *P. ahipa* (Wedd.) Farodi and *P. tuberosus* have a South American distribution. At present, *P. ahipa* is only recorded as being cultivated, and the crop is grown by small communities located in the subtropical East Andean valleys of Bolivia and northern Argentina (Figure 2.1) (Sørensen, 1996).

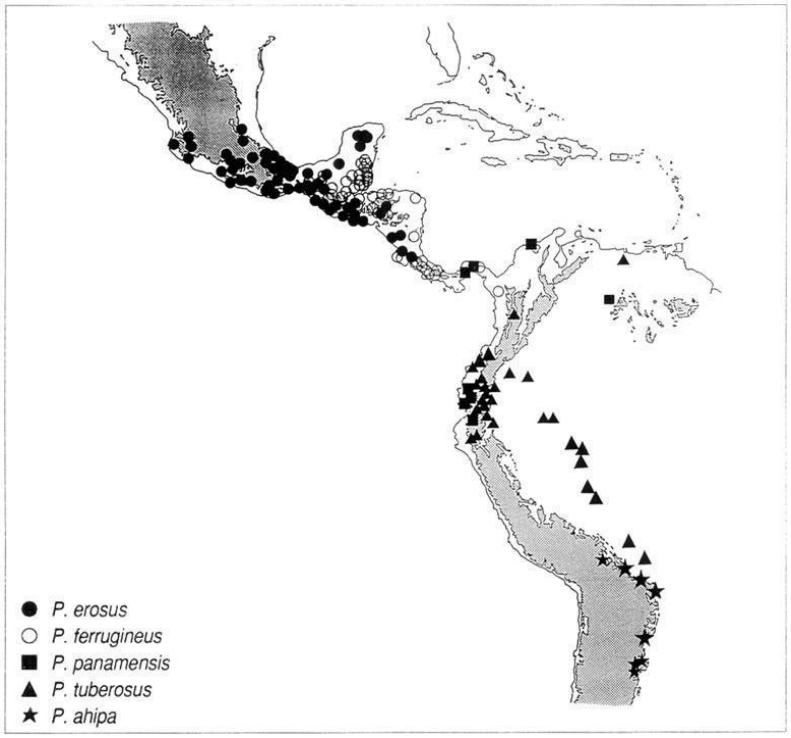

Figure 2.1: A map of South America showing the Neotropical distribution of the genus *Pachyrhizus* (both wild and cultivated species)
Source: Sørensen (1996).

According to Sørensen (1996) the genus *Pachyrhizus* is morphologically delimited by the presence of the following characteristics: vines or semi-erect herbaceous to somewhat lignified perennial plants with one or more tuberous roots; trifoliolate leaves with stipules and pinnately arranged leaflets with caducous stipels. Inflorescence is a complex to simple raceme and the flowers have a tubular calyx and a papilionaceous corolla.

The ovary has a basal crenate disc-formed nectarium, the recurving style is ciliated ventrally and the vertical surface of the stigma is subglobose. The straight legume is septate between the seeds, which are a square, more or less flattened, or a rounded kidney, shape. Colors range from olive green and deep maroon to black, or from black and white to mottled cream (Sørensen, 1996).

2.1.1.1 The Andean Yam Bean (*Pachyrhizus ahipa*)

According to Sørensen (1996) the local name of this species is *Ahipa* or *Ashipa*. It is distinguished morphologically from the other species by being an herbaceous plant with generally entire leaflets, short racemes, which are simple. The wing and keel petals are usually glabrous, but slightly ciliolated specimens have been reported (Sørensen, 1996). The morphological characters of the pods and seeds are also specific. The pods are only slightly dorsiventrally compressed (Figure 2.2). Both determinate and indeterminate growth habits exist in the species (Zanklan, 2003).

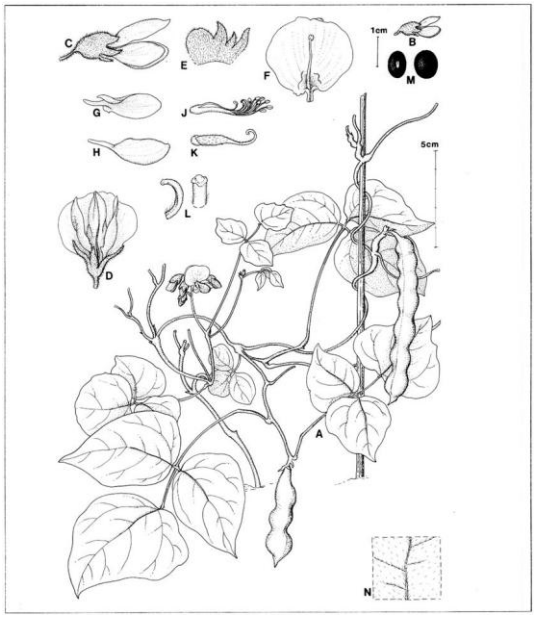

Figure 2.2: *Pachyrhizus ahipa*. A. Habit, 2/3 of natural size. B. Flower, natural size*. C. Flower, side view. D. Flower seen from underneath. E. Calyx opened. F. Standard with free median stamen. G. Wing. H. Keel. J. Stamens. K. Pistil with basal disc. L. Side and front view of style and subglobose stigma. M. Side and top view of seed. N. Section of adaxial leaf surface. (All parts from AC102, Prov. Tarija, and Bolivia).

Source: Sørensen (1996)

The Seeds of this species are black, lilac, maroon or black and white (cream) mottled, never olive-green or red; rounded kidney-shaped, never flattened and square, 9 x 10 mm. The 100-seed weight ranges from 17.3 to 41.2 g (Sørensen *et al.*, 1997). This species is furthermore unique in that both twining/trailing and semi-erect to short bushy erect growth habits are found, i.e. both determinate and indeterminate genotypes exist. Erect genotypes are 15-40 cm tall, semi-erect 30-60 cm and twining types 60 cm to several metres long (Sørensen *et al.*, 1997).

2.1.1.2 The Mexican Yam Bean (*Pachyrhizus erosus (L.) Urban*)

This cultivated yam bean species is found in Central America as well as South East Asia. The species is named Jicama in Mexico and bang kuang in Indonesia. It has a herbaceous vine with great variation in the outline of the leaflets, from dentate to palmate (Figure 2.3). Moreover the species is defined by the lack of hairs on the petals, the number of flowers (4-11) per lateral inflorescence axis by complex racemes. Morphological characters of the pods are also used to distinguish the species. A number of seed characters are also specific. These include the color, which ranges from olive-green to brown or reddish brown (Zanklan, 2003).

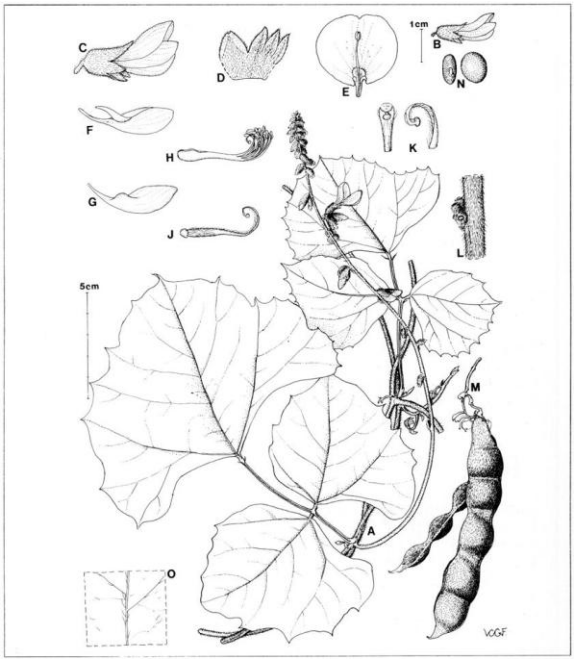

Figure 2.3: *Pachyrhizus erosus.* A. Habit, 2/3 of natural size. B. Flower, natural size. C. Flower, side view. D. Calyx opened. E. Standard with free median stamen. F. Wing. G. Keel. H. Stamens. J. Pistil with basal disc. K. Side and front view of style and subglobose stigma. L. Lateral axis of inflorescence. M. Mature legume. N. Side and top view of seed. O. Section of adaxial leaf surface. (All parts except legume from Abbott 404, GH; legume from grown from seeds from Oaxaca, Mexico).

Source: Sørensen (1996)

2.1.1.3 The Amazonian Yam Bean (*Pachyrhizus tuberosus*)

P. tuberosus is found cultivated in the tropical lowland to both slopes of the Andean mountain range as well as in the Caribbean. It has many cultivar groups with different local names such as *Ashipa, Jíquima*, and *Chuin*. *P. tuberosus* has a stem up to 7 m long and is the largest yam bean species. The pods are also larger than those of the other species and are conspicuously compressed between seeds (Figure 2.4). The seeds are black, black and white mottled or orange-red in color (Zanklan, 2003).

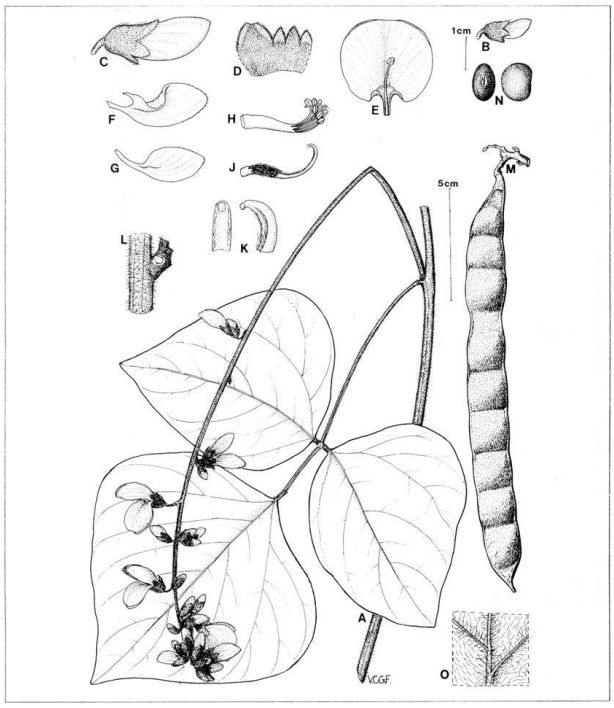

Figure 2.4: *Pachyrhizus tuberosus.* A. Habit, 2/3 of natural size. B. Flower, natural size. C. Flower, side view. D. Calyx opened. E. Standard with free median stamen. F. Wing. G. Keel. H. Stamens. J. Pistil with basal disc. K. Side and front view of style and subglobose stigma. L. Lateral axis of inflorescence. M. Mature legume. N. Side and top view of seed. O. Section of adaxial leaf surface. (A-L and O from Asplundh 15461, S; M from Lynch C43-25, BH; N from Clausen C43-25, BH).

Source; Sørensen (1996)

2.1.2 Yam bean seed production and utilization

Yam bean yields up to 6000kg/ha and has a high 1000- seed weight (180-230 g) (Grüneberg *et al.,* 1999) compared to other legumes like cowpea and peanut. Zanklan *et al.* (2003) has reported the yam bean pod size and thousand seed weight being comparable to those of *Phaseolus* beans. However, in both small-scale and commercial production, all the flowers are removed, as flower pruning (FP) increases storage root production. Only a few plants are not flower pruned and left to produce seeds, since the seed is the common method for propagation of the crop (Zanklan *et al*, 2007).

Yam bean seeds are generally not used as food by farmer and only a few are used for propagation. This is so because the seeds contain the toxic rotenone (about 1% seed weight) and usually farmers do flower pruning (removal of reproductive parts) in order to get rid of toxic seeds as well as increase tuber production (Zanklan *et al.,* 2007). Grüneberg *et al.* (1999) noted that currently, yam beans are exclusively grown on small scale for tuber production and the seeds remain as crop residuals on the field and suggested that the seeds could be used by farmers for small scale oil processing with additional by- products in form of protein meal and rotenone which could be used for biodiesel, animal feed and pesticide production respectively (Grüneberg *et al.,* 1999).

The young seed pods of *P. erosus* are sometimes eaten as a cooked vegetable, in a similar way as french beans, but cannot so be used as the seeds develop (Kay, 1987). The crushed pod of *P. tuberosus*, mixed with lard, is used in China to cure itch and seeds in powdered form are sometimes used as an insecticide or fish poison. In Indonesia, the pulverized seeds mixed with sulphur are used for the treatment of certain types of skin eruptions. One half seed may be taken as a laxative, but according to Kay (1987) if poisoning occurs coconut water can be taken to counteract it.

Santos *et al*. (1996) suggested that, if the rotenone could be removed, the seeds would provide a good protein food source and the seed oil can be of interest for the food industry with the potential of being an alternative to groundnut or cotton seed oil. The rotenone and rotenoids have insecticidal effects and can therefore be used in manufacture of insecticides (Zanklan *et al*, 2007).

2.1.3 Chemical and nutritional composition of yam bean seeds

Substantial work has been done on the chemical composition of yam bean seeds (Santos *et al.,* 1996, Grüneberg *et al.,* 1999, and Morale-Arellano, 2001). Grüneberg *et al.* (1999) studied seeds from 22 accessions of yam bean species of *Pachyrhizus ahipa* (14 accessions), *P. erosus* (5), *P.*

tuberosus (3) and found variations in oil and protein content, fatty acid composition of the seed oil and the total tocopherol content (Table 2.1).

The authors observed that that the oil and protein content of yam bean was comparable to that typical of soybean which contains about 34% protein and 19% oil and pointed out the high saturated fatty acid content of yam bean seed oil.

Table 2.1: Mean of oil and protein contents of yam bean seeds from the three cultivated species*

	Species / number of accessions			
	P. erosus **1996**	***P. tuberosus*** **1996**	***P. ahipa*** **1996**	***P. ahipa*** **1997**
Components	**5**	**3**	**37**	**38**
Oil content (%)	24.0	21.8	25.8	22.0
Protein content (%)	29.9	32.2	25.7	26.5
Palmitic acid (% total fatty acids)	27.8	24.2	29.2	29.3
Stearic acid (% total fatty acids)	4.5	5.2	5.5	5.4
Oleic acid (% total fatty acids)	25.4	26.6	23.1	23.7
Linoleic acid (% total fatty acids)	37.0	37.6	36.9	36.2
Linolenic acid (% total fatty acids)	1.3	2.0	1.6	1.6
Total tocopherol (mg/kg)	443.7	285.2	631.2	684.4

Means are for 83 samples of yam beans (*Pachyrhizus spp*.) grown in 1996 or 1997.

Source: Grüneberg *et al.* (1999)

Santos *et al.* (1996) analyzed *P. erosus* seeds on a wet basis for proximate composition, minerals, and protein fractions, antinutritional factors as well as rotenoids. The seeds showed a high content of protein (28.27 g/100 g), lipids (26.8 g/100 g), ash (4.58 g/100 g), crude carbohydrate (26.85 g/100 g), and moisture content (7.30 g/100 g).

Kay (1987) reported the following composition for the edible portion of the young seed pods for *P. erosus* (Philippines): water 86.4%, protein 2.6%, fat 0.3%, carbohydrate 10%, fiber 2.9%, ash

0.7%, calcium 121 mg/100 g, iron 1.3 mg/100 g, phosphorus 39 mg/100 g, vitamin A 575 IU/100 g, thiamine 0.11 mg/100 g, riboflavin 0.09 mg/100 g and niacin 0.8 mg/100 g.

Regarding the mature dry seeds, the composition was: water 6.7 %, protein 26.2 %, fat (oil) 27.3%, carbohydrate 20%, fiber 7%, and ash 3.64% (Kay, 1987). These results are almost similar to those reported by Grüneberg *et al*. (1999).

Despite the striking nutritional composition, the mature pods and seeds of yam bean are very toxic and contain a lot of toxic substances such as pachyrrhizine, pachyrrhizone,12-(A)-hydropachyrrhizone, dolineone, dehydropachyrrhizone, erosone, neodehydrorautenone, erosenone, erosenin, 12-(A)-hydroxylinenone, pachysaponin A & B as well as Rotenone ($C_{23}H_{22}O_6$) (Narongchai *et al.*, 2005).

Rotenone, which is the most important toxic substance in yam bean, is a colorless, crystalline solid, very soluble in organic solvents like alcohol and acetone, but is practically insoluble in water (Narongchai *et al.*, 2005; Hinson, 2000). Rotenone (Figure 2.5) has a molecular weight of 394.41 and melts at 165-166 ^{o}C (Hinson, 2000). Rotenone is generally unstable and degrades rapidly in water with the presence of light, heat, turbidity, shallow depth and low organic matter. Degradation of rotenone results in at least 20 degradation products, of which only one (6a β, 1 2α β –rotenone) is toxic (Hinson, 2000).

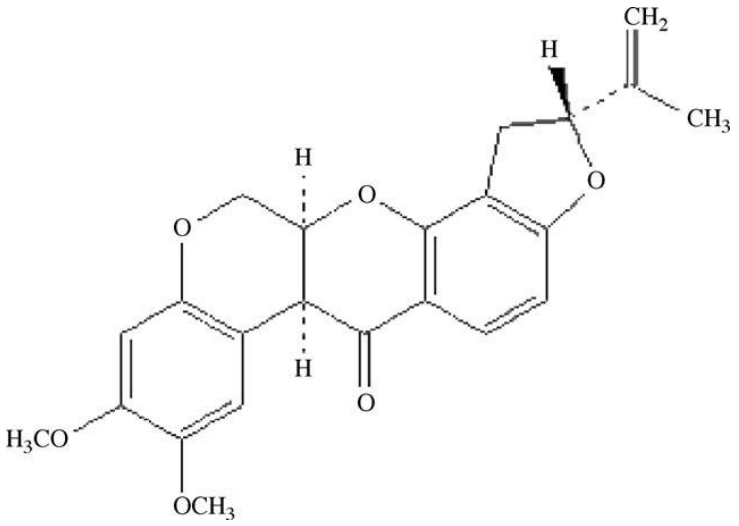

Figure 2.5: Chemical structure of rotenone molecule

Source: Sae-Yun *et al.* (2006)

According to Zanklan (2003) rotenone is an isoflavonoid with insecticidal and fungicidal properties. The seeds of yam bean show toxicity to several species of insects comparable to that of the rotenone-bearing roots of derris and loncho-carpus (Zanklan, 2003). The yam bean is therefore, of interest as a potential source of insecticidal material because of their ease of cultivation and harvesting (Norton & Hansberry, 1945). The yam bean seeds have been studied as a possible commercial source of a vegetable insecticide, since they contain 0.12-0.43% of rotenone, pachyrrhizone and pachyrrhizonic acid (Kay, 1987).

Rotenone is also formulated as pesticides and is used for fish killing to control their populations. However, these toxic effects have not been reported in exposed humans on ingestion and fatal cases of rotenone or yam bean toxicity are very rare in humans (Narongchai *et al.,* 2005). Rotenone is a potent inhibitor of mitochondrial electron transport, blocking nicotinamide adenine dinucleotide CoQ reductase (Complex I), which is the first step in electron transport thus the aerobic metabolism is disrupted, leading to lactic acidosis (Hung, *et al.*, 2007).

A study by Hung *et al.* (2007) on yam bean seed poisoning described five patients of yam bean seed poisoning in Taiwan, one of them life threatening. The patients presented with; peri-oral numbness, nausea and vomiting as well as severe abdominal cramping, diarrhea, vomiting and

flushing about 2 hours after eating a large quantity of the soup and the victim had difficulty breathing and lost consciousness one hour later (Hung *et al.,* 2007). Based on this case, it was concluded that yam bean seed poisoning can cause acute metabolic acidosis and altered mental status, which could be confused with acute cyanide intoxication from a cyanogenic glycoside-containing plant. However, Kay (1987) suggested that the toxic components in the seed can be eliminated by boiling the seeds in alcohol.

2.1.4 Yam bean seed protein and protein quality

The nutritional quality of legume protein is mostly determined by three different factors, namely: amino acid composition, protein digestibility and presence or absence of antinutritional factors (Maruatona, 2008).

High-quality proteins are those that contain all the essential amino acids at levels greater than the FAO/WHO/UNU reference levels, and a digestibility comparable to or better than those of egg-white or milk proteins (Smith, 2003a). Animal proteins are of better "quality" in terms of amino acid composition and digestibility than plant proteins (Smith, 2003a). Morales-Arellano *et al.* (2001) showed that Mexican yam bean seed protein amino acid composition had a better balance of essential amino acids than other seeds (Table 2.2) and concluded that yam bean seeds could be a good source of high nutritional quality protein.

Santo *et al.* (1996) reported *in vitro* protein digestibility of 74.4% for yam bean seed protein. This is quite satisfactory compared to other legume seed proteins which have in-vitro digestibility values as low as 50%. The high *in vitro* protein digestibility reported may be attributed to the low concentration of the antinutritional components in the yam bean (*P. erosus*) seeds (Santos *et al*., 1996) compared to other legumes.

Table 2.2 Amino acid composition of yam bean (*P. erosus*) seed and other seed flour (grams of amino acid /100g of crude protein)

Amino Acid	Yam Bean	Corn	Bean
Isoleucine	5.5	3.5	4.4
Leucine	7.8	12.3	7.6
Lysine	7.7	3.0	6.9
Methionine	1.8	2.0	1.0
Cysteine	3.5	2.3	0.7
Phenylalanine	5.7	4.4	5.4
Tyrosine	4.0	3.3	2.6
Threonine	4.2	3.3	4.2
Valine	6.5	4.3	5.2
Histidine	1.0	3.0	4.8

Source: Morales-Arellano *et al.* (2001)

2.1.5 Antinutritional factors in yam bean seeds and other legumes

Legume seeds including yam bean seeds are known to contain several antinutrtional components such as trypsin inhibitors, hemagglutinins, flatulence factors and glucosides (Gueguen, 1983). These antinutrtional factors limit protein digestibility and reduce the nutritional quality of legume protein (Maruatona, 2008).

Santos *et al.* (1996) reported yam bean seeds to contain tannins (10.2 mg/100 g), trypsin inhibitory activity (17.1 ITU), and low hemagglutinating activity (64-1 titer for rabbit erythrocytes with no specificity to human erythrocytes). However, Santos *et al*. (1996) noted that these antinutritional factors in the seeds are heat labile save for rotenone and present in low concentration thus not posing serious threat. The defatted Soybean meal is said to be richer than most of the usual legume seeds in trypsin inhibitors and hemagglutinating factors, containing about seven times more trypsin inhibitor activity and ten times more hemagglutinins activity than faba beans. However, these levels of antinutritional factors are generally decreased for soybean on heating the defatted flour (Gueguen, 1983).

Mugendi *et al*. (2010) noted that tropical grain legumes such as faba bean, kidney bean, jack bean and grass pea contain several anti-nutritional compounds, some of which have hepatotoxic and neurotoxic effects. To improve nutritional quality and effectively utilize grain legumes to their full potential as food, inactivation or removal of antinutritional factors by adopting economically viable processing techniques is required (Mugendi *et al*., 2010). Physical and biochemical methods used to process legume seeds include soaking, cooking, selective filtration, irradiation, enzymatic treatments, germination and fermentation.

2.2 Protein digestibility

Digestibility is a measure of the susceptibility of a protein to proteolytic enzyme action and therefore digestibility may be used as an indicator of protein bio-availability, an aspect of nutritional value of a protein (Byaruhanga, 2005). The digestibility of nutrients must be known in order to evaluate fully the significance of nutrient concentration (Kamara *et al*., 2009).

According to Gauthier *et al.* (1982), an important and frequently observed effect of food processing is the protein reduction in the nutrition quality and these changes may depend on the denaturation of the protein and reduction in availability by cross linking, racemization, degradation and formation of complexes with sugar and these may result in loss of digestibility. Therefore, when attempting to estimate protein quality, one of the first factors that must be evaluated is digestibility (Gauthier *et al.*, 1982). Gauthier *et al.* (1982) noted that the nutritional quality of the protein is related to its amino acid content and the capacity of digestive enzymes to liberate them thus methods using digestive enzymes have been tried to evaluate protein digestibility.

Protein digestibility would ideally be determined by conducting *in vivo* studies using humans. However, *in vivo* assays are expensive, time consuming and they raise a lot of ethical issues. Therefore, *in vitro* methods that are rapid and correlate well with *in vivo* studies would be valuable (Byaruhanga, 2005). *In vitro* methods for determining protein digestibility may involve the use of single or multiple proteolytic enzymes. The multiple enzyme systems include pepsin-pancreatin, pepsin-trypsin, and pepsin and α-chymotrypsin. Guimarães *et al*. (2012) reported that higher *in vitro* protein digestibility values were obtained with the pepsin–pancreatin enzyme system compared to those obtained using a single enzyme (pepsin). However, multiple enzyme systems are cumbersome due to the involvement of multiple digestions and washings (Gauthier *et al.*, 1982; Byaruhanga, 2005). Also, this may not correlate well with the single enzyme (pepsin) method (Byaruhanga, 2005). Although the nutritional quality of proteins must, in the final analysis, be established with feeding trials, *in vitro* methods of protein evaluation are useful in screening new protein foods and processing methods because of their rapidity (Akeson & Stahmann, 1964).

2.3 Functional properties of flour and protein

The term functionality in the context of food ingredient, is defined as any property, aside from nutritional attributes, that influences an ingredient‘s usefulness in food (Maruatona, 2008). Proteins play important roles in the functional properties of many foods and thus contribute to the quality and sensory attributes of many food products (Smith, 2003b). The ultimate success of using protein rich flours in food formulations depends largely upon their functional attributes and how they interact with other ingredients in the final product (Maruatona, 2008). Maruatona (2008) reported that the functional properties of flours, like soy flour, are generally due to their proteins; however, flours contain other components such as water-soluble carbohydrates, fiber and lipids that may also contribute to the overall effect observed.

Protein functional properties have traditionally been defined as physical and or chemical properties of proteins that affect their behavior in food systems during preparation, processing, storage and consumption (Wrolstad *et al.*, 2005). Protein functionality can be classified into three broad categories; (1) hydration properties such as solubility and water retention, (2) surface properties such as emulsification and foaming, and (3) protein-protein interactions such as gelation (Smith, 2003b; Wrolstad *et al.*, 2005). Functional properties of protein are important in food processing and product formulation and are affected by intrinsic factors of proteins such as molecular structure and size and many environmental factors including the method of protein separation, pH, ionic strength and the presence of other components in the food system (Nassar, 2008).

Functional properties are often the result of several physical or chemical reactions occurring simultaneously or sequentially in a food during processing and are not easily measured using a single physical or chemical test (Smith, 2003b). The physical and chemical properties that govern protein functionality include; size, shape; amino acid composition and sequence; net charge and distribution of charges; hydrophilicity/ hydrophobicity ratio; secondary, tertiary, and quaternary structures; molecular flexibility/rigidity; and the ability to interact with other components (Damodaran, 1996).

One protein in a food may be primarily responsible for the desired functional properties, or a group of proteins may be involved (Smith, 2003b). Since proteins possess a multitude of physical

and chemical properties, it is difficult to delineate the role of each of these properties with respect to a given functional property (Damodaran, 1996).

Although much is known about the physicochemical properties of several food proteins, Damodaran (1996) reported that prediction of functional properties from their molecular properties has not been successful. A few empirical correlations between molecular properties and certain functional properties in model protein systems have been established. However, behavior in model systems normally is not the same as behavior in real food products (Damodaran, 1996). This is attributable, in part, to denaturation of proteins during food fabrication. The extent of denaturation depends on pH, temperature, other processing conditions, and product characteristics. In addition, in real foods, proteins interact with other food components, such as lipids, sugars, polysaccharides, and minor components, and this modifies their functional behavior (Damodaran, 1996). Despite these inherent difficulties, considerable progress has been made toward understanding the relationship between various physicochemical properties of protein molecules and their functional properties (Damodaran, 1996).

3. RESEARCH

3.1 Chemical composition and functional properties of yam bean (*Pachyrhizus spp*) seed flour

Abstract

This work was aimed at determining the composition and functional properties of yam bean seeds from the three cultivated species of the crop. A total of 10 accessions (2 accessions of *P.erosus*, 4 accessions of *P. ahipa* and 4 accessions of *P. tuberosus*) were studied for chemical composition, pasting and functional properties (bulk density, least gelation concentration, water absorption capacity, oil absorption capacity, emulsifying capacity, emulsion stability, foaming capacity, foam stability and protein solubility). The results showed that yam bean seeds contained high nutrient levels with: 29.2-32.1 g/100 g proteins, 31.3-33.0 g/100 g carbohydrates, 24.1-25.6 g/100 g total fat, 7.5- 8.1g/100 crude fiber and 3.4- 4.1g/100g ash. The defatted *P. erosus* seed flour contained high protein (45.6- 48.8 g/100 g), total carbohydrates (32.6-36.5 g/100 g), crude fiber (6.7-7.1 g/100 g) and ash (6.0- 6.4 g/100 g) but lower crude fat (5.2/100 g). The defatted yam bean seed flour exhibited relatively high protein solubility (68.0-70.4%), least gelation concentration (14%) as well as water absorption capacity (2.8-2.9%) and oil absorption capacity (1.5%). The flour also exhibited emulsifying capacity of 35.7-36.0%, emulsion stability (33.2-33.5%), foaming capacities (42%) and foam stability (25.1-25.8%). With respect to pasting properties, the defatted yam bean seed flours exhibited pasting temperature of 80.0-81.3^{o} C, peak viscosity of 145.5-146.7 RVU, trough viscosity of 95.1-102.0 RVU, break down of 43.5-51.6 RVU, set back of 252.9-258.1 RVU and final viscosity of 348-360 RVU. In comparison with other legumes and protein foods, these results show that yam bean seeds have potential for use in food systems and industrial applications.

3.1.1. Introduction

Legume plants have been important sources of protein, starch, lipids, minerals vitamins and health promoting compounds. The seeds of yam bean play an important role in the traditional diets of many people in the world (Sai-Ut *et al*., 2009) and are a valuable basic material for food, animal feed and non food industrial applications.

Legume plants belonging to the genus *Pachyrhizus* are close relatives of soybeans and *phaseolus* bean (Bhat and Karim, 2009). The genus has three cultivated species (*P. erosus, P. ahipa and P. tuberosus*) and two wild species (*P. panamensis* and *P. ferrugineus*). The crop is mainly used for their tuberous storage roots as a food source. Yam bean plants have a high 1000 seed weight (180-230 g) and the seed yield of up to 5.2 MT/ha (Zanklan, *et al.*, 2007). Yam bean seed are rich in protein (25.7-32.2 g/100 g) and lipids (21.8-25.8) (Grüneberg, *et al.*, 1999) as well as carbohydrate (26.85 g/100 g) (Santos *et al.*, 1996). However, the use of yam bean seeds is limited mainly because of the presence of the toxin-rotenone (about 1% seed weight) (Santos, *et al*., 1996; Grüneberg, *et al.*, 1999).

Yam bean is an underutilized crop and was only recently introduced in Uganda in 2009. There is relatively limited information on properties of yam bean seeds. There is need to get more information and understand the characteristics of this new crop in East Africa for its optimal use and application. This study determined the composition of yam bean seeds from the three cultivated species of yam bean as well as functional properties of defatted yam bean seed flour from *P. erosus* species as a potential alternative source of starch and protein for food systems and industrial applications

3.1.2 Materials and methods

3.1.2.1 Sample collection

Yam bean seeds from the three cultivated species were otbtained from International Potato Center (CIP) which were from the harvest of the yam bean plants grown on station at National Crops Resources Research Institute (NaCRRI) –Namulonge in Uganda. Seed samples from a total of 10 selected accessions (Table 3.1.1) of the three cultivated species of *P. ahipa*, *P. tuberosus* and *P. erosus* were collected. The accesssions from each species were selected based on results from on station germplasm evaluation (yield).

Table 3.1.1: Samples of yam bean seeds that were collected from CIP

Species	**Selected accessions**			
P. ahipa	UYB 19	UYB 21	UYB 23	UYB 03
P. tuberosus	UYB 40	UYB 41	UYB 35	UYB 38
P. erosus	UYB 06	UYB 07		

Two accessions (UYB 06 and UYB 07) from the P. erosus species were selected for assessment of physicochemical characteristics and this was based on availability yam bean seeds.

3.1.2. 2 Sample preparation

The yam bean seeds were cleaned to remove extraneous matter and then milled to fine flour using hammer mill (Laboratory Mill 8", Christy Hunt Agricultural Ltd, England) fitted with 0.5 mm screen. The flour was then stored in a refrigerator at 4°C until use. The seed flour sample destined for the determination of functional properties was defatted by mixing with hexane at a flour/solvent ratio of 1:10 w/v, stirring for 24 hour and the solvent was separated by centrifugation (Fisher Scientific Centrific Model 225 Centrifuge, Made in USA) at 6500 rpm for 15 minutes followed by air drying of the flour at room temperature for 8 hours. The defatted flour was stored at 4°C until it was used.

3.1.2.3 Determination of proximate composition of yam bean seeds and defatted yam bean seed flours

The proximate composition of the flours was determined by AOAC methods (2000). Moisture content was determined using the electric oven method at 105ºC for 12 hours; crude protein content was determined by the Kjeldahl method using auto distillation unit (Foss Kjeltech^{TM} 8200, Type:10014901-Hilleroed, Denmark); crude fat was determined using the Soxhlet extraction method and total ash by dry ashing in an electric furnace at 550 ºC for 8 hours. Crude fiber was determined by the gravimetric method reported by Pomeranz and Meloan (1994) and the total carbohydrate was defined by difference (Santos *et al.*, 1996).

3.1.2.4 Functional properties of defatted yam bean seed flour

Bulk density

The bulk density of the defatted flour was determined according the method described by Butt & Batool (2010). Ten grams of sample were put into 100ml graduated cylinder and the cylinder was tapped 10 times on the laboratory bench for the sample to settle, compact and eliminate air pockets. The volume was noted and the bulk density expressed as g/cm^3.

Least Gelation Concentration

This was determined according to 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 minutes. Five milliliters each, of the dispersions were poured into 3 test tubes and heated to 100°C in a water bath for 1 hour 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).

Water and oil absorption capacities

Water and oil absorption capacities were determined according to the method described by Appiah *et al.* (2011) with modifications. One gram of each sample 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 (for both water absorption and oil absorption capacities) were agitated manually for 2 minutes, allowed to stand at 28°C for 30 minutes and then centrifuged (Fisher Scientific Centrific Model 225 Centrifuge, Made in USA) at 650 rpm for 20 minutes. 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 and its contents was weighed, the weight of water or oil absorbed by 1 g of flour or protein was calculated and expressed as water or fat absorption capacity.

Protein solubility

Protein solubility was determined according to the method of Butt and Batool (2010). The flour (0.25 g) was homogenized in 20 ml of 0.1M NaCl at pH 7 for 1 hour followed by centrifugation at 5200 rpm for 30 minutes. Nitrogen contents were determined in the supernatant and solubility was expressed as the percentage of total nitrogen of the original sample in the soluble fraction.

Emulsifying properties

Emulsifying properties (emulsifying capacity and stability) were determined according to the method reported by Butt & Batool (2010) with some modifications. Flour (1.8 g) was added to 25 ml of distilled water (pH 7) and dispersed at maximum speed in a blender (Moulinex –Optiblend 2000 Trio from China). Corn oil (12.5 ml) was added and blended at maximum speed for 1 minutes; the emulsion formed was equally divided into two 12 ml centrifuge tubes and centrifuged (Fisher Scientific Centrific Model 225 Centrifuge, Made in USA) for 5 minutes at 5200 rpm. 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 85^{o}C for 30 minutes and subsequently cooled to 25^{o}C prior to centrifugation.

$$Emulsion\ stability = \frac{\text{height of emulsified layer after heating} \times 100}{\text{height of total contents of tube}}$$

Foaming capacity and stability

The foaming capacity and stability were determined according to the method of Butt & Batool (2010). Yam bean seed flour was dispersed in distilled water to form 3% (w/v) dispersion. A portion (50ml) of the mixture was immediately transferred into a graduated cylinder and the volume recorded. This was followed by whipping the mixture using a warring blender (Moulinex – Optiblend 2000 Trio, China) for 4 minutes and volume after whipping was recorded. Foaming capacity was expressed as percentage volume change induced by whipping. The change in volume of foam after 60 minutes of standing at room temperature was recorded as foam stability.

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

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

3.1.2.5 Pasting properties of defatted yam bean seed (*P. erosus*) flour

The pasting properties of defatted yam bean seed flours were analyzed with a Series 4 Rapid Visco Analyzer (RVA) (Newport Scientific from Australia) with Thermocline for Windows software. The analysis was done using standard one profile. The flour suspensions (6.72 g in 25.28 ml H_2O) corrected to 14% moisture content were exposed to the following time/temperature sequence: 50ºC for 1 minute, heating from 50ºC to 95ºC at 12.16ºC/minutes, maintained at 95ºC for 2.5 minutes, and cooled from 95ºC to 50ºC at 11.84ºC/minutes rate. The apparent viscosity was expressed in RVU.

3.1.2.6 Statistical analysis

All experimental analyses in this study were done in triplicates. All the data analysis was done using SPSS version 16.0 Software. One way Analysis of variance (ANOVA) was performed to calculate significant differences in treatment means and Least Significant Difference (LSD) ($P<0.05$) was used to separate these means for the data from proximate analysis. The t test was performed for the rest of the data.

3.1.3 Results and discussion

3.1.3.1 Proximate composition of yam bean seed flour

The results of yam bean seed proximate composition (Table 3.1.2) showed that there were significant differences in composition of seeds from the three cultivated species save for crude fiber between *P. erosus* and *P.ahipa*. The differences in nutritional composition may be attributed to genetic and agronomic factors. These results for crude protein and total lipids content in this study are in agreement with those reported by Grüneberg *et al.* (1999) for all the three species. Grüneberg *et al.* (1999) reported *P. erosus, P. tuberosus* and *P. ahipa* to have a protein content of 29.9, 32.2 and 26.6%, and oil content of 24.0, 21.8 and 22.0%, respectively.

The results especially for the *P. erosus* in this study were only slightly higher than the ones reported by Santos *et al*. (1996) which may be attributed to the difference in the geographical location and agronomical practices.

Table 3.1.2: Proximate composition of yam bean seeds from the three cultivated species (g/100g) on dry matter basis

	Species/ No. of accessions		
	P. erosus	***P. ahipa***	***P. tuberosus***
Components	**2**	**4**	**4**
Moisture content	6.48^{c} (1.27)*	4.81^{a} (0.72)	5.97^{b} (0.71)
Crude protein	30.13^{b} (0.69)	29.23^{a} (2.07)	32.16^{c} (0.22)
Total fat	25.58^{c} (0.82)	25.04^{b} (0.82)	24.14^{a} (2.28)
Crude fiber	8.07^{b} (0.47)	7.48^{b} (0.65)	4.18^{a} (0.26)
Total Ash	3.36^{a} (0.04)	3.91^{b} (0.14)	4.12^{c} (0.25)
Total carbohydrates	32.10^{b} (0.39)	34.54^{c} (2.70)	28.67^{a} (5.53)

*Mean values in the same row with different superscript letters are significantly different ($P \leq 0.05$).*Values in parentheses are standard deviation (SD) of the respective means.*

The results in this study revealed that the levels of protein and oil of yam bean seed are high compared to that of other legumes like chick peas which was reported to have 24.2g/100g and 5.6g/100g for crude protein and total fat respectively (Sayar *et al.,* 2005), pea having 23.93, 3.12 g/100g for crude protein and fat and faba bean having 27.30 and 20.20g/100g for crude protein and fat respectively. The results in this study were close and almost similar to those reported for soybean showing protein and fat content of 42% and 20% respectively (Berk, 1992). Fernández-Quintela *et al*. (1997) reported soya bean protein and fat content of 36.69 and 22.43 g/100 g respectively. The total carbohydrate content of the yam bean seed, though higher than that reported for soybean (18.83 g/100 g) was lower than that of other legumes like pea (59.39 g/100 g) and faba beans (52.12 g/100 g).

The oil content in the yam bean seeds is only lower than that of *Arachis hypogaea* (peanut) among legumes (Santos *et al*., 1996). The high oil content of yam bean seed makes it a potential source of oil and with the growth of the biodiesel industry, the yam bean seed can be a potential source of

raw material (crude oil). Grüneberg *et al.* (1999) noted the vegetable fat with high concentrations of saturated fatty acids such as yam bean seeds are desired by the food industry, especially to avoid the need for hydrogenation and transesterification processes in the production of margarine and related products.

3.1.3.2 Proximate composition of defatted yam bean (*P. erosus*) flour

The proximate composition of defatted yam seed flour (Table 3.1.3) showed that there was no significant difference in the moisture content, total ash, crude fibre and residual total fat between the two accessions. However, the crude protein and total carbohydrates were significantly different. The defatted flours from the two accessions of *P. erosus* were characterised by high protein and carbohydrate content.

Table 3.1.3: Proximate composition of defatted yam bean (*P. erosus*) flour for the two accessions on dry matter basis

Component	Defatted yam bean seed flour	
	UYB06 (g/100g)	UYB07 (g/100g)
Moisture	$10.45^{a} \pm 0.11$	$10.47^{a} \pm 0.16$
Crude protein	$45.57^{a} \pm 1.20$	$48.78^{b} \pm 2.63$
Total fat	$5.16^{a} \pm 0.03$	$5.16^{a} \pm 0.06$
Crude fiber	$6.74^{a} \pm 0.03$	$7.13^{a} \pm 0.10$
Ash	$6.0^{a} \pm 0.03$	$6.38^{a} \pm 0.09$
Total Carbohydrates	$36.50^{b} \pm 1.11$	$32.55^{a} \pm 2.41$

The values in the table are means of triplicate determinations ± SD. Mean values in the same row with different superscript letters are significantly different (P≤0.05).

Defatting with hexane resulted in an apparent concentration of the protein and other nutrients in the flour. In view of the high content of storage proteins, the yam bean seed meal has potential to be used in a variety of potential industrial product applications such as adhesives, plastics as well as composites.

According to Guimarães *et al.* (2012) the direct use of defatted flours as functional ingredients play an important role in industries because of their lower production cost compared to that of protein concentrates (Guimarães *et at.*, 2012). However the use of these flours depends on their performance as functional ingredients and their behavior in particular food systems (Guimarães *et al.*, 2012).

3.1.3.3 Functional properties of the defatted yam bean seed flour from two accessions of *P. erosus*

The accession did not have any effect on the functional properties of yam bean seed flour (Table 3.1.4). This may be attributed to the fact that the two accessions are from the same species and cultivated under the same conditions.

Table 3.1.4: Functional properties of defatted yam bean seed flour for the two accessions of *P. erosus.*

Functional properties	Accession	
	UYB 06	UYB 07
Bulk density(g/cm3)	0.59 ±0.00	0.59 ±0.00
Least gelation concentration (%)	14.00 ±0.00	14.00 ±0.00
Water absorption capacity (g/g)	2.81 ± 0.02	2.90 ±0.04
Oil absorption capacity (g/g)	1.52 ±0.02	1.48 ± 0.02
Emulsion capacity (%)	35.70 ±1.40	36.02 ±2.80
Emulsion stability (%)	33.45 ±6.10	32.15 ±8.37
Foaming capacity (%)	42.00 ±2.00	42.00 ±3.46
Foam stability (%)	25.80 ±6.19	25.10 ±5.22
Protein solubility (%)	70.35 ±1.25	68.00 ±1.90

The values indicated in the table are means of triplicate determinations ± SD.

Bulk density

Bulk density depicts the behaviour of the material in dry mixes and is an important parameter that can determine packaging requirements of the product (Kamara *et al*., 2009). The bulk density of the defatted yam bean seed flours from the two accessions (UYB06 and UYB07) of *P. erosus* was 0.59 g/cm^3 and there was no significant difference in the bulk densities of the defatted flours from the two accessions (Table 3.1.4). This may be due to the fact that the samples may have had same particle size since the flours were prepared in the same way using the same equipment. Amadou *et al.* (2010) reported that the bulk density of the flour would vary with the particle size or fineness of the flour.

The bulk density result for the yam bean seed flour from this study were slightly higher compared to 0.55-0.62 g/cm^3 reported for tigernut (C*yoperus esculentus*) seed flour (Oladele and Aina, 2007), 0.53 g/cm^3 for defatted chick pea (Valim & Batistuti, 1998) and lower than 0.63 g/cm^3 for soybean protein meal (Amadou *et al.,* 2010). According to Valim and Batistuti (1998), bulk density can be used to predict the textural quality of the products.

Least Gelation Concentration

The Least Gelation Concentration (LGC) for the defatted yam bean seed flours of the two accessions (UYB 06 and UYB 07) was not significantly different (Table 3.1.4). Gelation is an aggregation of denatured protein molecules. The LGC results for yam bean seed flours were similar to the results reported for lupin seed flour (14%) (Khalid *et al*., 2012).

Gelation properties are related to water absorption capacities hence the high water absorption capacity recorded for the yam bean flours in this study could explain the good gel formation capacity. Adebowale *et al.* (2005) noted that gelation takes place more readily at higher protein concentration because of greater intermolecular contact during heating and that high protein solubility is always necessary for gelation. The relatively high LGC observed in the yam bean seed defatted flour may be a disadvantage in respect to the production of some products such as curd

since production of such products calls for materials with high gelation capacity like milk protein (casein).

Water and oil absorption capacity

Water and oil absorption capacities are useful indices of the ability of the protein in the material to prevent fluid loss from a product during food storage or processing (Kiosseoglou and Paraskevopoulou, 2011). The intrinsic factors that affect water binding properties of food flours with relatively high protein content relate to amino acid composition, protein conformation and surface polarity (Fekria *et al.*, 2012).

The defatted yam bean seed flours exhibited high water absorption capacity across the two accessions UYB 06 (2.81 ± 0.02 g/g) and UYB 07 flour (2.90 ± 0.04 g/g) and there was no significant difference between the WAC of the accessions of *P. erosus* (Table 3.1.4). These results are almost similar to those reported by Fekria *et al.* (2012) for the defatted ground nut which ranged from 3.03 to 3.07 ml/g for two groundnut varieties and higher than WAC values reported by Hussain *et al.* (2008) for defatted flax seed protein concentrate (2.2 g/g). However the results in this study were lower than WAC value reported by Valim and Batistuti (1998) for defatted chickpea flour (4.94 g/g). The variation in WAC of defatted yambean seed flour and other legumes may be attributed to the difference in protein structure and the presence of different hydrophilic carbohydrates. Flours with high WAC have more hydrophilic constituents such as polysaccharides as noted by Fekria *et al.* (2012).

The defatted yam bean seed flour showed moderate oil absorption capacity (Table 3.1.4) compared to other legume seeds. The observed OAC values were lower than the values reported for other legumes like chick pea flour (Valim and Batistuti, (1998) reported to have OAC values of and 2.60 g/g and defatted ground nut flour (Fekria *et al.*, 2012) reported to have OAC values of 2.87 and 2.93ml/g for two ground nut varieties. However the OAC values for flours in this study were higher than those reported for defatted flax seed flour (1.04 g/g) by Hussain *et al*. (2008). Fekria *et al.* (2012) reported that the variation in the OAC of the flours may be attributable to the presence of non- polar side chains of oil among the flours.

The high water absorption capacity of the defatted yam bean seed flour makes it desirable for use in meats, sausage, bread, mayonnaise and cakes. While the low OAC means the flour could be used as a coating in deep fat frying to reduce oil absorption by the fried food.

Interactions of water and oil with proteins are very important in food systems because of their effects on the flavor and texture of foods (Amadou *et al.,* 2010). Fekria *et al*. (2012) reported that the ability of the flour to absorb and retain water and oil may help to improve the binding capacity and enhance flavor retention, improve mouthfeel and reduce moisture and fat losses of extended meat products. Valim and Batistuti (1998) observed that extrusion cooking helps to enhance the oil absorption capacity of defatted legume flour probably due to the dissociation of the proteins which exposes non polar amino acid residues to interact with oil molecule.

Emulsifying capacity and stability

The formation and stability of emulsions is very important in food systems such as in mayonnaise and the emulsifying properties are usually attributed to the flexibility of solutes and exposure of hydrophobic domains (Fekria *et al.* 2012). The flours from the two yam bean accessions (UYB 06 and UYB 07) studied exhibited moderate emulsifying capacity (EC) values (35.70 and 36.02%) as well as emulsion stability (ES) (33.45 and 32.15%) respectively as compared to the conventional legumes with no significant differences between the accessions (Table 3.1.4). The EC and ES values recorded were higher than values reported by Fekria *et al.* (2012) for defatted groundnut flour from two varieties which ranged from 22.90 to 28.33 and 11.36-13.86% for emulsion activity and stability, respectively. However the results in this study for EC and ES were lower than those (51% and 50%) reported for defatted Baru nut flour respectively (Guimarães *et al.*, 2010). All these values are lower than those for egg york powder known for its excellent emulsifying characteristics with EC values of 74 % and ES value of 72 % (Ndife *et al.*, 2010).

Several natural and processed foods, such as milk, egg yolk, coconut milk, soy milk, butter, margarine, mayonnaise, spreads, salad dressings, frozen desserts, frankfurter, sausage, and cakes, are emulsion-type products where proteins play an important role as an emulsifier

(Damodaran,1996). Plant proteins normally have poor surface activity compared to animal protein such as ovalbumin and bovine serum albumin which are widely utilized for emulsification role in food systems.

The difference in the surface activity between the plant and animal protein is related to the difference in protein conformation (Damodaran,1996). Highly flexible molecules, such as caseins, can undergo rapid conformational changes once they are adsorbed at the interface, enabling additional polypeptide segments to bind to the interface while on the contrary, the rigid globular proteins, such as legume seed proteins, cannot undergo extensive conformational changes at the interface (Damodaran,1996).

Proteins with high emulsifying capacity such as egg york is good for products such as salad dressing, sausages, bologna, soups, confectionery, frozen dessert and cakes (Nassar, 2008). The results from this study show that the defatted yam bean seed flour due to its moderate emulsion capacity and stability may not be suitable for use in food system where formation and stabilization of emulsion is important such as in mayonnaise, cakes and frozen desserts among others unless modifications are made to the flour to improve on its surface active properties.

Foaming capacity and stability

The foaming capacity of a protein refers to the amount of interfacial area that can be created by the protein while foam stability refers to the ability of protein to stabilize against gravitational and mechanical stresses. Foam formation and stability are a function of the type of protein, pH, processing methods, viscosity and surface tension (Fekria *et al.*, 2012).

The defatted yam bean seed flour from the two accessions (UYB 06 and UYB 07) exhibited foaming capacity of 42 ± 2.00 and 42 ± 3.46% and foam stability of 25.80 ± 6.19 and 25.10±5.22% respectively (Table 3.1.4). The values for foaming capacity recorded in this study were higher than those reported for defatted ground nut flour (4 -4.20%) (Fekria *et al*., 2012) but lower than those reported for defatted Baru nut flour (69%) at pH 7 (Guimarães *et al*., 2010). On the other hand the values for Foam stability of yam bean seed flour were lower than those reported

for defatted ground nut flour (97-97.50%) (Fekria *et al*., 2012) and those of defatted Baru nut flour (35%) (Guimarães *et al*., 2010).

Egg white powder which is widely used for its excellent foaming characteristics has been reported to have higher FC values of 97.5% and FS values of 78.3 % (Ndife *et al.*, 2010). Result from this study indicate that the defatted yam bean seed flour has potential for application in food systems that require high percentage of porosity such as ice cream and cakes as well as in non food products as a foaming agent.

Protein solubility

Protein solubility is probably the most critical functional property since it affects other properties such as emulsification, foaming and gelation (Fekria *et al*., 2012). Protein solubility is influenced by many factors such as origin of the protein, processing conditions, pH, ionic strength as well as presence of other ingredients (Fekria *et al*., 2012).

Both accessions of *P. erosus* studied exhibited high protein solubility values of 68 and 70.35% respectively for UYB 06 and UYB 07 at pH 7 and there was no significant difference in the protein solubility of defatted flour in two accessions at $P \leq 0.05$ (Table 3.1.4). The Protein solubility values recorded for the yam bean seed flour in this study were higher than those reported by Valim and Batistuti (1998) (53.3±0.3%) for defatted chick pea flour and those reported by Fekria *et al*. (2012) for defatted groundnut flour (10 to 15% under the pH range of 6 to 8). Khalid *et al.* (2012) reported protein solubility of 80% for dehulled defatted cowpea flour at pH 7. Probably dulling enhanced the solubility of the protein in the dehulled defatted cowpea flour.

Industrial application of proteins such as in the production of fibres, adhesives, ingredients of coating, emulsifiers, food additives and different food products depend upon bringing proteineous materials into solution (Adebowale, 2008). Therefore knowledge of protein solubility is vital in selection of particular vegetable proteins for possible industrial applications. The high protein

solubility exhibited by *P. erosus* is an indicator that the flour may have potential for industrial applications.

3.1.3.4 Pasting properties of defatted yam bean (*P. erosus*) seed flour

The pasting temperature provides an indication of the minimum temperature required for cooking (Ikegwu *et al.,* 2010). The pasting temperatures of the two accessions of *P. erosus* (UYB 06 and UYB 07) were 79.9 and 81.33^{o}C respectively and were significantly different (Table 3.1.5 and Figure 3.1.1). Other studies have reported values of pasting temperatures for legumes ranging from 73^{o}C for defatted African walnut flour (Ndie *et al*, 2010), 81.63 ^{o}C for undehulled black bean flour (Akinjayeju & Ajayi, 2011) to values of 88.25^{o}C (Ikegwu *et al.,* 2010) for *Brachystegia Eurycoma* seed flour. The pasting temperature of the defatted yam bean seed flour was therefore within the range reported for other legumes.

Table 3.1.5: Pasting characteristics of defatted yam bean (*P. erosus*) flour for two accessions*

Sample	Peak Time/Min	Pasting Temp/^{0}C	Viscosity/RVU				
			Peak Viscosity	Trough Viscosity	Break Down	Final Viscosity	Setback
UYB 06	6.15^{a}	79.97^{a}	145.50^{a}	102.03^{a}	43.47^{a}	360.14^{a}	258.11^{a}
	±0.20	±0.06	±2.11	±2.14	±0.05	±5.55	±3.80
UYB 07	6.11^{a}	81.33^{b}	146.72^{a}	95.11^{b}	51.61^{b}	347.97^{a}	252.86^{a}
	±0.08	±0.46	±3.10	±0.83	±3.92	±10.46	±11.28

**Values are means of triplicate determinations± SD. Means in the same column with same superscript letter are not significantly different (P≤0.05).*

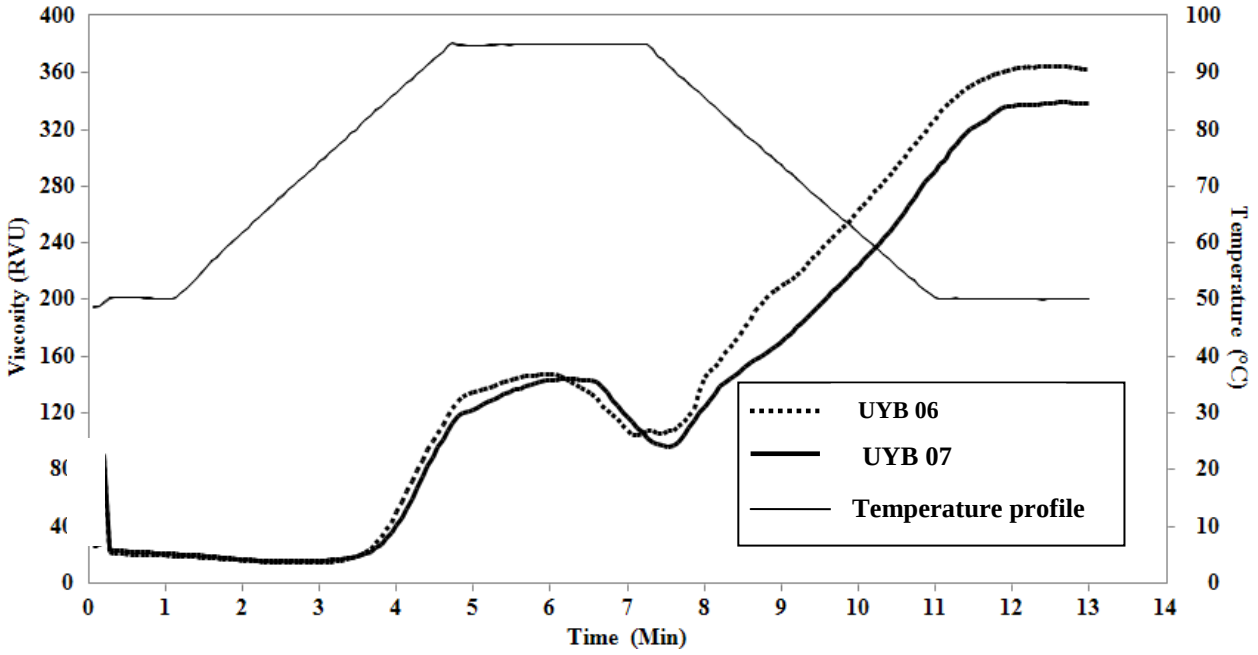

Figure 3.1.1: Pasting curves of defatted yam bean (*P. erosus*) seed flour from two accessions (UYB 06 and UYB 07) obtained from RVA analysis.

The peak viscosities of the defatted yam bean seed flour were mild (145.5 for UYB 06 and 146.72 RVU for UYB 07) and were lower than those of other legume seed flours like the defatted African walnut flour having 183.0 RVU (Ndie *et al.*, 2010) and undehulled black bean flour having 247 RVU (Akinjayeju & Ajayi, 2011) but higher than the values of 77.58 RVU reported for *Brachystegia Eurycoma* seed flour (Ikegwu *et al.*, 2010). The peak viscosity indicates the water binding capacity of the flour (Ikegwu *et al.*, 2010) and is often correlated with final product quality as well as providing an indication of the viscous load likely to be encountered by a mixing cooker. The relatively high peak viscosity exhibited by *P. erosus* flours compared to other legume flours is indicative that *P. erosus* flour may be suitable for products requiring high gel strength and elasticity.

The trough viscosity, which is the minimum value in constant temperature phase of the RVA profile, measures the ability of the paste to withstand breakdown during cooling. The defatted yam bean seed flour for the two accessions (UYB 06 and UYB 07) of *P. erosus* exhibited lower trough viscosities (102.03 and 95.11 RVU respectively) compared to that reported for corn flour (225.5

RVU) and *Brachystegia eurycoma* seed flour (229.25 RVU) as reported by Ikegwu *et al.* (2010). The break down viscosity of the defatted yam bean seed flour (43.47 and 51.61 RVU) was almost similar to those reported for *Brachystegia eurycoma* seed flour (53.8 RVU) and its starch (46.17 RVU) and slightly higher than values for corn starch (41.58 RVU) (Ikegwu *et al.*, 2010). The lower the break down in viscosity, the higher the ability of the flour to withstand heating and shear stress during processing (Ikegwu *et al.*, 2010). Therefore defatted yam bean seed flour should be able to withstand heating and shear processes without significant change in consistence.

The final viscosity which is the viscosity after holding cooked starch at 50^{o}C was almost twice the peak viscosity (Figure 3.1.1) and the following values 360.14 and 347.97 RVU for UYB 06 and UYB 07, respectively were recorded. Final viscosity is used to define the particular quality of the starch and indicate the stability of the cooked paste in actual use. It also shows the ability of the material to form various paste or gel after cooling. Low stability of starch paste is associated with high value of breakdown (Ikegwu *et al.*, 2010). The setback values of UYB 06 and UYB 07 were 258.11 and 252.86 RVU respectively and were not significantly different. These values are higher than those reported for corn starch (215.67 RVU) (Ikegwu *et al.*, 2010). The high the setback values indicate lower potential to resist retrogradation during cooling of the product made from the flour (Ikegwu *et al*., 2009). Setback involves retrogradation or re-ordering of the starch molecules and setback viscosity has been correlated with the texture of various products (Maziya *et al*., 2005). High setback is also associated with syneresis, or weeping especially during freeze/thaw cycles (Maziya *et al*., 2005). Substituted starches are commonly used where this presents a quality defect.

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3.1.4 Conclusion

The yam bean seeds from the three cultivated species have high protein, carbohydrate and fat content which could be exploited for use in food systems and industrial applications. This study also established that generally the seeds have significant differences in their proximate composition. The results of pasting and functional characteristics obtained indicate that defatted yam bean seed flour has useful technological characteristics for many food and industrial applications and these may include; manufacture of fillers, emulsifiers, stabiliser, paper, adhesives and bioplastics . Yam bean seed flour, however exhibited rather low emulsion and foaming capacities compared to other legume seed flours.

3.1.5 Reference

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3.2 The physicochemical characteristics of yam bean (*Pachyrhizus erosus*) seed proteins

Abstract

The aim of this study was to determine the physicochemical characteristics of the defatted flours and the protein isolates extracted from two accessions of yam bean (*P.erosus*) seeds. The flour was defatted using hexane and protein isolate was obtained by isoelectric precipitation. Protein fractionation was achieved through subjecting the defatted yam bean seed flours to successive extraction using different solvents (distilled water, Tris-HCl containing NaCl, 2-propanol, and NaOH). Functional properties of the yam bean protein isolates namely; bulk density, least gelation concentration, water absorption capacity, oil absorption capacity, emulsifying capacity, emulsion stability, foaming capacity, foam stability, protein solubility, *in vitro* protein digestibility as well as the protein electrophoretic patterns were determined. Protein recoveries of 93.6 and 93.1% were recorded for the defatted flour from the two accessions of *P. erosus* (UYB 06 and UYB 07) respectively. Albumins were found to be the most dominant protein fraction followed by globulins, glutelins and then prolamines, respectively representing; 52.7-53.8%, 17.5-19.8%, 8.0-9.6%, and 2.6-2.7%. With respect to the functional properties, the yam bean seed protein isolates exhibited least gelation concentration of 8%, WAC of 2.9-3.0 g/g, OAC of 0.8 g/g, protein solubility of 80.4-81.6%, foaming capacity of 37.0-37.2%, foam stability of 73.4-74.1%, emulsion activity of 12.9-14.7% and emulsion stability of 8.9-9.5%. *In vitro* protein digestibility results of *P. erosus* for both raw and cooked samples were 87.4- 87.7% and 84.3- 84.3% respectively. Regarding electrophoresis, major bands corresponding to molecular weights 31, 54 and above 93 kDa were observed, showing presence of albumins and globulins. Electrophoresis results indicated that there were no observable differences in the electrophoretic patterns for the two accession of *P.erosus*. From this study, it can be concluded the yam bean seed protein has potential for use in both food systems and industrial applications.

3.2.1. Introduction

Among the current needs of the food industry is the search for less expensive proteins for use as ingredients in several food processes. (Valim & Batistuti, 1998). Plant proteins are abundant, relatively inexpensive and widely recognized for their high nutritional value and excellent functional properties (Kamara *et al*., 2009). The use of plant proteins in the formulation of new food products, or in conventional foods as well as for non food applications has been the focus of much research in the recent years (Chavan *et al.,* 2000). The use of concentrated and isolated proteins from plant seeds has increased enormously because of the greater knowledge of their functional properties, processing and nutritive value as well as other industrial applications (Khalid *et al*., 2012). The functional properties of plant proteins have been exploited in a multitude of applications including for example, solubility in beverages, foaming in whipped toppings, and emulsification in processed meat. 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). Yam bean (*Pachyrhizus ssp*) has potential to contribute to the filling of shortage in protein supply because of its wide adaptation and high protein content.

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, 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. 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.

3.2.2 Materials and Methods

3.2.2.1 Sample collection and preparation

Yam bean seeds from two accessions (UYB 06 and UYB 07) of *P. erosus* species were collected from the harvest of the yam bean plants grown on-station at National Crops Resources Research Institute (NaCRRI) –Namulonge in Uganda by the International Potato Center (CIP) Uganda.

Preparation of defatted yam bean flour: A portion of the seeds were milled to fine flour (0.5 mm) using Hammer mill (8" laboratory mill Christy Hunt Agricultural ltd, England) and kept in refrigerator at 4°C until used. The whole seed flour samples were defatted with hexane (flour/solvent ratio of 1:10 w/v) and stirred for 24 hour. The solvent was eliminated by centrifugation (Fisher Scientific Centrific, Model 225 Centrifuge, USA) at 6500 rpm for 15 minutes and the meal was dried at room temperature and stored at 4°C until it was used.

Preparation of yam bean protein isolates: This was done using the method described by Sai-Ut *et al*. (2009) with some modifications. Dispersions of defatted yam bean seed flour (5%/ w/v) in distilled water were adjusted to pH 8 with 0.1N NaOH, shaken for 1 hour and then centrifuged at 5200 rpm for 15 minutes. The pH of the extract was adjusted to 4.5 with 1N HCl to precipitate the target proteins. The proteins were recovered by centrifugation (Centrifuge: Fisher Scientific. Model-225, USA) at 5200 rpm for 15 minutes, followed by removal of the supernatant by decantation. Protein curd was washed twice with distilled water and centrifuged at 5200 rpm for 10 minutes. The washed precipitate was then freeze-dried and referred to as "protein isolate"

Preparation of sample for electrophoresis: SDS-PAGE was run on both the cooked and uncooked defatted flour samples and its isolate. The cooked sample was prepared in the way that the defatted flour was suspended in the distilled water in the ratio of 1:10 (flour to water). The suspension was then heated to boil on Bunsen burner flame then simmered for one hour.

The remainder of the whole yam bean seeds was used for *in vitro* protein digestibility tests. A portion of the seeds were boiled in a pressure cooker for 30 minutes and the other portion tested raw.

3.2.2.2 Fractionation of yam bean (*P. erosus*) seed protein

Fractionation of protein was carried out according to the method of Osborne as reported by Morales-Arellano *et al.* (2001) with modifications. 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 hours at room temperature and centrifuged (Fisher Scientific Centrific, Model 225 Centrifuge, USA) at 6500 rpm for 15 minutes. The supernatant called albumin (a) was kept at 4°C until it was used. The pellet was resuspended with a solution of 50 mM Tris-HCl, pH 8, containing 0.1 M NaCl and stirred as before. The resulting supernatant was designated globulin ($g_{0.1}$). The pellet was extracted with 50 mM Tris-HCl, pH 8, containing 0.3 M NaCl. After centrifugation, the supernatant was called fraction globulin ($g_{0.3}$), and the pellet was resuspended with 70% aqueous 2-propanol, extracted under stirring for 3 hours, and centrifuged at 6500 rpm for 15 minutes. The resulting supernatant was designated the prolamin fraction (p), and the pellet was resuspended in a solution of 0.1 M NaOH; after centrifugation, the supernatant was designated the glutelins fraction (gt), and the pellet was called residue.

The protein content in each protein fraction was determined using the Kjeldahl method (AOAC, 2000). Nitrogen to protein conversion factor of 6.25 was used.

3.2.2.3 Assessment of functional properties of yam bean seed (*P. erosus*) protein isolate

Bulk density

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

Least Gelation Concentration

This was determined according to 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 maximum speed for 2 minutes. Five milliliters each, of the dispersions were poured into 3 test tubes and heated to 100°C in a water bath for 1 hour 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).

Water and oil absorption capacities

This was determined according the method described by Appiah *et al.* (2011). One gram of sample 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 minutes, allowed to stand at 28°C for 30 minutes and then centrifuged (Fisher Scientific Centrific Model 225 Centrifuge, USA) at 650 rpm for 20 minutes. 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 of water or oil absorbed by 1 g of flour or protein was calculated and expressed as water or fat absorption capacity.

Protein solubility

Protein solubility was determined according to the method of Butt & Batool (2010). The flour (0.25g) was homogenized in 20 ml of 0.1M NaCl at pH 7 for 1 hour followed by centrifugation (Fisher Scientific Centrific Model 225 Centrifuge, USA) at 5200 rpm for 30 minutes. Protein contents were determined in the supernatant and solubility was expressed as the percentage of total protein of the original sample in the soluble fraction.

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) and dispersed at maximum speed in a blender (Moulinex –Optiblend 2000 Trio, China). Corn oil (12.5 ml) was added and blended at high speed for 1 minutes; the emulsion formed was equally divided into two 12 ml centrifuge tubes and centrifuged (Fisher Scientific Centrific Model 225 Centrifuge, USA) for 5minutes at 5200 rpm. 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 85^{o}C for 30 minutes and subsequently cooled to 25^{o}C prior to centrifugation.

$$Emulsion\ stability = \frac{\text{height of emulsified layer after heating} \times 100}{\text{height of total contents of tube}}$$

Foaming Capacity and stability

The foaming capacity and stability were determined according the method of Butt & Batool (2010). Yam bean seed 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 warring blender (Moulinex –Optiblend 2000 Trio from China) at maximum for 4 minutes and volume after whipping was recorded. Foaming capacity was expressed as percentage volume change induced by whipping. The change in volume of foam after 60 minutes of standing at room temperature was recorded as foam stability.

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

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

3.2.2.4 *In-vitro* protein digestibility of yam bean (*P. erosus*) seed protein

The *in-vitro* protein digestibility was determined for both raw and cooked yam bean seed using pepsin–pancreatin enzymatic system (Chavan *et al.*, 2001). To achieve enzyme digestion, about 1 g of sample was suspended in 60 ml of 0.1M HCl containing 6 mg of pepsin, followed by gentle shaking for 15 minutes at 37^{o}C. The resulting solution was neutralized with 0.5 M sodium hydroxide 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 hours at 37^{o}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;

$$\%\ In\ vitro \text{ protein digestibility} = \frac{\text{A} - \text{B}}{\text{A}}$$

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

3.2.2.5 Electrophoretic pattern of defatted yam bean (*P. erosus*) seed flours and its protein isolates

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was 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 min-protean II Electrophoresis System (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^{o}C for 10 minutes. 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 hour. The gel was stained with 0.1% Coomassie Brilliant Blue R250 in methanol/acetic acid (40:10 v/v) solution. Destaining was achieved by washing the gel for 2 hours 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.

3.2.2.6 Statistical analysis

All experimental analyses in this study were conducted in triplicates. A paired-*t* test was performed for the *in vitro* protein digestibility data for raw and cooked samples. The rest of the data generated in this section were analysed using the *t* test to determine significant differences between treatment means at ($P<0.05$). All statistical analyses were conducted using SPSS/16.0 Software for windows.

3.2.3 Results and discussion

3.2.3.1 Fractionation of yam bean (*P. erosus*) seed protein

The results indicated that 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, then glutelins and prolamines in both accessions (Table 3.2.1). The proportions of the protein fractions were not significantly different for the two accessions (UYB 06 and UYB 07) of *P.erosus*.

The protein fractionation pattern observed is quite similar to 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 protein fractionation pattern for the yam bean seeds recorded was virtually similar to the one reported for amaranth seed protein with 33% albumins and 20% globulins (Leyva-Lopez *et a*l., 1995; Morale-Arellano *et al*., 2001).The results also showed that yam bean seed protein was different from that of other legumes such as soy bean (Morale-Arellano *et al*., 2001; Vasconcelos *et al.*, 2010), peas (Morale-Arellano *et al*., 2001), common bean (Chan and Phillips, 1994; Morale-Arellano *et al*., 2001) and mucuna seeds (Sridhar & Bhat, 2007) that have globulin as the dominant fraction.

Table 3.2.1: Protein recovery and composition in the different protein fractions of yam bean seeds (*P. erosus*).

Protein fraction	Amount /100 g Crude protein in the two yam bean (*P. erosus*) accessions*	
Accession	**UYB 06**	**UYB 07**
Albumin	53.8 ± 1.24	52.7 ± 1.67
Globulin (0.1M NaCl)	12.5 ± 3.02	11.5 ± 5.20
Globulin (0.3M NaCl)	7.3 ± 1.28	6.0 ± 1.52
Prolamin	2.7 ± 0.91	2.6 ± 1.71
Glutelin	8.0 ± 3.59	9.6 ± 6.05
Residue	9.4 ± 1.84	10.7 ± 2.21
Protein recovery	93.6 ± 0.55	93.1 ± 0.97

**All values are means of triplicate determinations ± SD.*

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 the protein extraction techniques and perhaps the treatment samples were subjected to. The insoluble proteins were recovered in the residues of UYB 06 and UYB 07 containing 9.4 and 10.7 g/100 g of protein for respectively. The insolubility of some protein can, in part, be attributed to the damage caused by the solvent hexane on the proteins (Morales-Arellano *et al.*, 2001). Chan & 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.

3.2.3.2 Functional properties of yam bean seed protein isolate from two accessions of *P. erosus*

There was no significant difference between the functional properties of the two accessions of *P. erosus* (Table 3.2.2). This is attributable to the fact that the two protein isolate were derived from seeds from two accessions belonging to the same species, cultivated in the same location.

Table 3.2.2: 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 ±0.00	0.59 ±0.00
Least gelation concentration (%)	8.00 ±0.00	8.00 ±0.00
Water absorption capacity (g/g)	3.00 ± 0.19	2.88 ±0.10
Oil absorption capacity (g/g)	0.79 ±0.05	0.78 ± 0.07
Emulsion capacity (%)	12.92 ±0.85	14.67 ±0.04
Emulsion stability (%)	9.48 ±0.42	8.90 ±0.40
Foaming capacity (%)	37.15 ±6.10	37.04 ±3.07
Foam stability (%)	74.12 ±0.76	73.37 ±1.81
Protein solubility (%)	81.64 ±1.25	80.37 ±0.43

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

Bulk density

The results of bulk densities for protein isolates from the two accessions were similar at 0.59 g/cm^3 (Table 3.2.2). The bulk densities for the protein isolates from the two accessions showed that the proteins from the accessions did not significantly differ from each other, an indication that the protein isolates had the same particle characteristics.

However the bulk densities of the isolate were lower than values reported for other legumes. Butt & 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 and final quality of the product.

Least gelation concentration

The least gelation concentration (LGC) of yam bean seed protein isolate from the two accessions (UYB 06 and UYB 07) was similar and gave a value of 8 % (Table 3.2.2). This indicates the minimum protein concentration at which a stable gel can be formed. The lower the LGC, the better the gelling ability of the protein. The LGC values recorded for yam bean seed protein isolates were lower than values reported for other legumes. Butt and Batool (2010) reported LGC of 14, 16, 16, and 18 % for protein isolates of pigeon peas, cow peas, mungbean and peas, respectively. The lower LGC observed for the yam bean seed protein isolate implies that yam bean seed protein isolate has better gelling ability as compared to some other legume crops.

Water and oil absorption capacity

Water and oil absorption capacity 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 results for water and oil absorption capacities revealed no significant difference between the two accessions of *P. erosus* (Table 3.2.2). The WAC and OAC results recorded were higher than those reported for other legumes. Butt & Batool (2010) reported WAC of 0.97, 1.38, 1.63, 1.52 g/g for pigeon pea, cow pea, mungbean, and cow pea protein isolates respectively. However WAC results observed in this study were almost similar to WAC results reported by Khalid *et al.* (2012) on cow pea (2.10 g/g) while for OAC, Khalid *et al*. (2012) reported higher value (1.90 g/g) for cow pea protein isolate than values recorded for yam bean seed protein isolate in this study.

Results of OAC for yam bean seed flour reported were lower than figures reported for other legumes protein isolates (1.68, 1.45, 1.13 and 1.40 g/g for pigeon pea, cow pea, mungbean, and peas protein isolates respectively) (Butt & Batool, 2010). Water absorption capacity values as low as 0.6 and as high as 4.9 g/g have been reported for protein isolates or concentrates prepared from pulses such as chick pea, pea, faba bean or lentil (Amodou *et al.*, 2010), showing that both the type of pulse and variety have an effect on the WAC. Kiosseoglou and Paraskevopoulou (2011) noted that the type of pulse 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.

The high water and oil absorption capacity of the yam bean seed protein isolate shows potential for use in meats, sausage, bread and cakes. Interactions of water and oil with proteins are very important in food systems because of their effects on the flavor and texture of foods (Amadou *et al.*, 2010).

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 (Terefe *et al*., 2011). The protein isolates from the two accessions (UYB 06 and UYB 07) exhibited low emulsifying capacity (12.92 and 14.67%) as well as stability (9.48 and 8.9%) with no significant differences between the accessions (Table 3.2.2). The EC and ES values recorded were lower than those values reported for other legume seed protein isolates. Butt & 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. 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, values higher than those recorded for the yam bean seed protein isolates in this study.

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 has potential for use in stabilised emulsions such as in paints and surface coatings upon modification to improve on the surface active properties.

Foaming capacity and stability

According to Butt & 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 (37.15 and 37.04%) and high foam stability (74.12 and 73.37%). There was no significant difference in foaming capacity and stability between the two accessions (Table 3.2.2). The results in this study especially foaming capacity were lower than the values (85 to 90%) reported by Eltayeb *el al*. (2011) for the Bambara protein isolate at the pH range of 6 to 7.5. However, these results for foaming capacity in this study were higher than the values reported for raw Mucuna bean protein isolate (about 12.5%) at pH 7 while for foam stability, Mucuna bean protein isolate was reported to have higher values (about 84.5%) (Eltayeb *et al*., 2011).

Amadou *et al.* (2010) reported that proteins have a tendency to denature and aggregate during whipping to show a large increase in the surface area in the liquid or air interphase and rapid conformational change as well as rearrangement at the interface. Foam stability requires formation of a thick, cohesive and viscoelastic film around each gas bubble thus foam stability is a function of the configuration of protein molecules.

The capacity of the proteins to form stable foams with gas by forming impervious protein films is an important property and it was likely due to the increased net charges on the protein, which weakened the hydrophobic interactions but increased the flexibility of the protein. This allowed the protein to diffuse more rapidly to the air-water interface to encapsulate air particles and then enhance foam formation (Amadou *et al.,* 2010).

Protein solubility

The protein solubility for the two yam bean accessions (Table 3.2.2) was relatively high. There was no significant difference in the protein solubility between the proteins isolates for the two accessions. The results for protein solubility of the yam bean seed protein isolates are almost similar to the solubility values (82 ± 4.47%) for pea (*Pisum sativum*) protein isolate and higher than results reported for mungbean protein isolate (72 ± 4.44%), cowpea (*Vigna unguiculata*) (65 ± 4.53%) and pigeon pea protein isolates (68 ± 3.09%) at pH 7 (Butt and Batool, 2010).

Protein solubility is usually affected by its hydrophilic and hydrophobic balance, depending on amino acid composition particular the protein surface (Butt & Batool, 2010). The high protein solubility of yam bean seed protein isolate may be due to the low number of hydrophobic residues and elevated charge as suggested by Butt & 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). Protein solubility has a close relationship with emulsifying properties and foaming properties (Kamara *et al.*, 2009). With respect to non food application, the high solubility yam bean seed protein isolates in water shows potential for its application in adhesive formulations.

3.2.3.3 *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 accessions exhibited considerably high IVPD (Table 3.2.3). There was no significant difference of yam bean IVPD between the two accessions. However, raw yam bean seed exhibited a significantly higher IVPD than cooked samples. The results recorded in this study were virtually similar to those reported by Sulieman *et al.* (2008) for lentil seeds for both raw (77.1-88.2%) and cooked (81.8-99.9%) samples for four different cultivars showing the same trend as observed in this study (cooking reducing protein digestility).

The IVPD values recorded in this study are relatively high compared to those for other legume seeds like cow pea seed protein with IVPD of 75.04% for raw and 76.69% for cooked protein (El-Jasser, 2010).

Table 3.2.3: *In vitro* Protein digestibility of Yam bean seeds from two accessions of *P. erosus*

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

**Each value in the table is the mean ± SD of triplicate determination on dry matter basis. Mean values in the column with same superscripts are not significantly different ($P \leq 0.05$). Mean values in the row with different superscripts are significantly different ($P \leq 0.05$).*

The IVPD protein digestibility results for both raw and cooked proteins were considerably higher than values reported by Santos *et al*. (1996) (74.7%) for the flour prepared from *P. erosus* yam bean seeds. Yam bean seed flour production entailed soaking, cooking, drying, milling, defatting and then drying (Santos *et al*. 1996). These processes may have negatively affected protein digestibility since processes like cooking and drying lead to protein denaturation.

The high IVPD results recorded in the current study may in part be attributed to the low concentration of the antinutritional components in *P. erosus* seeds. 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 of yam bean seed protein following thermal treatment. Heat causes oxidation of sulfhydryl groups and leads to interaction between acidic and basic residues that would be more resistant to proteases (Suleiman *et al.*, 2008).

The high *in vitro* protein digestibility of yam bean seed protein recorded in this study indicated that the seed has high quality protein compared to other legumes.

3.2.3.4 Electrophoretic pattern of yam bean seed protein of *P. erosus*

Electrophoretic pattern of the defatted yam bean seed flour and its protein isolate

Generally, there were no marked differences in the protein electrophoretic patterns of the flours and their protein isolates for the two yam bean accessions (Figure 3.2.1). However, differences were noticed in the patterns of the defatted flour and isolate for each accession with more bands observed for flour (10 visible bands) than the protein isolates (8 visible bands) under reducing conditions and fewer bands under non reducing conditions that is 8 to 9 visible bands for flour and 7 bands for isolate.

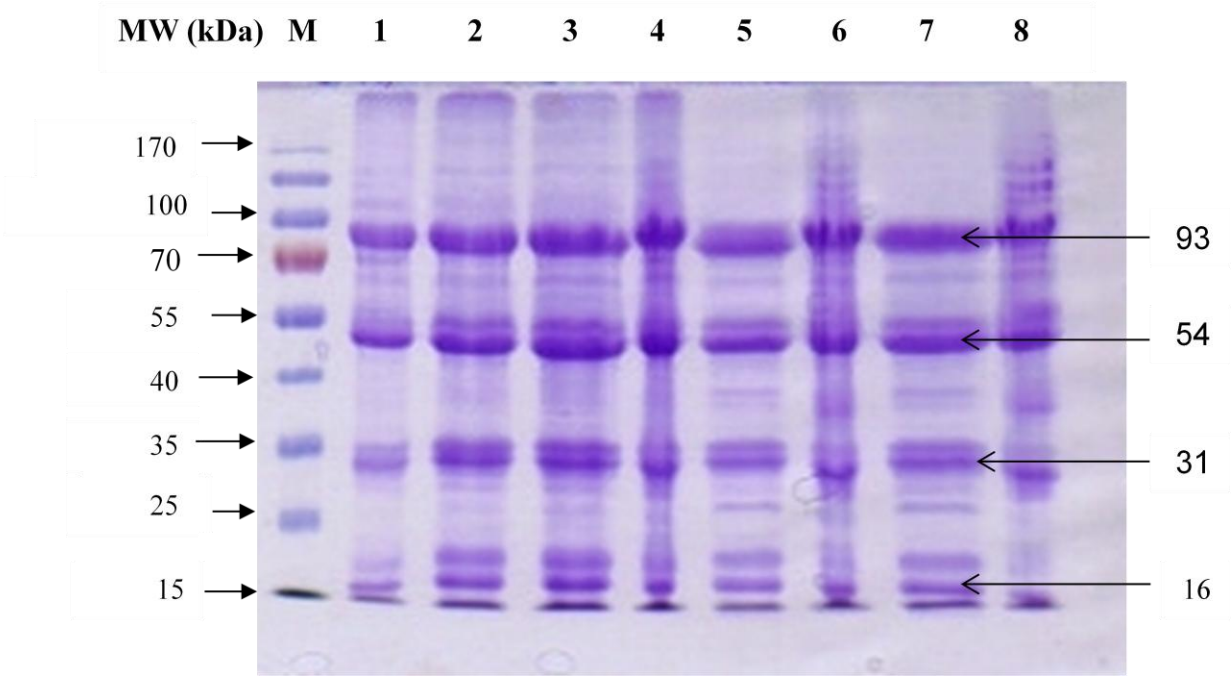

Figure 3.2.1: Electrophoretic pattern of yam bean seed flour and their protein isolates both under reducing and non reducing conditions. M: Standard protein marker, 1: UYB 06 isolate reduced, 2: UYB 06 isolate non reduced, 3: UYB07 isolate reduced, 4: UYB 07 isolate non reduced, 5: UYB06 flour reduced, 6: UYB 06 flour non reduced, 7: UYB 07 flour non reduced, 8: UYB 07 flour nonreduced.

Protein isolates of both yam bean accessions (UYB 06 and UYB 07) exhibited seven subunits each of apparent molecular weights ranging from 15 to above 85 kDa under reducing conditions. The main bands had molecular weights of 15, 31, 54, and 93 kDa. Under non reducing condition, fewer major bands (6 to 7 bands) were exhibited in the protein isolate of both accessions. The

defatted flours for the two accessions also showed the same pattern but had more subunits (10 subunits) in reduced state as compared to the protein isolates. These had apparent molecular weights ranging from 15 to above 100 kDa. The main polypeptides had molecular weights of 31, 54, 93 kDa. This may be attributed to the factor that some proteins may have been lost during preparation of the protein isolate.

The bands of proteins subunits under non reducing conditions (lanes; 2, 4, 6, and 8) for both isolates and flours were more aggregated (and thus less bands) than in their counterparts under reducing conditions (lanes; 1, 3, 5 and 7). This may be due to the effect of the secondary structures in the non reduced samples and upon reduction, the disulphide bond are broken producing peptides that separate into more bands. More bands were witnessed in the defatted flour samples than in their corresponding protein isolates and this may be attributed to loss of some proteins in the process of preparation of the protein isolate (Sai-Ut *et al*., 2009; Mugendi *et al.,* 2010).

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). The pattern is almost similar to that reported by Sai-Ut *et al*. (2009) for red kidney beans, navy beans and adzuki beans in absence of β mercaptoethanol where major polypeptides where reported with molecular weights ranging from 35 to 55 kDa.

The molecular weight of the different proteins influences their suitability for use in processes like protein texturisation 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).

Electrophoretic pattern of the raw cooked and cooked defatted yam bean flour

There were no marked observable differences in the electrophoretic pattern of similarly treated (cooked and raw) samples for the two accessions of *P. erosus* (Figure 3.2.2). However, marked

differences were noticeable between cooked and raw samples as well as between reducing and non reducing conditions.

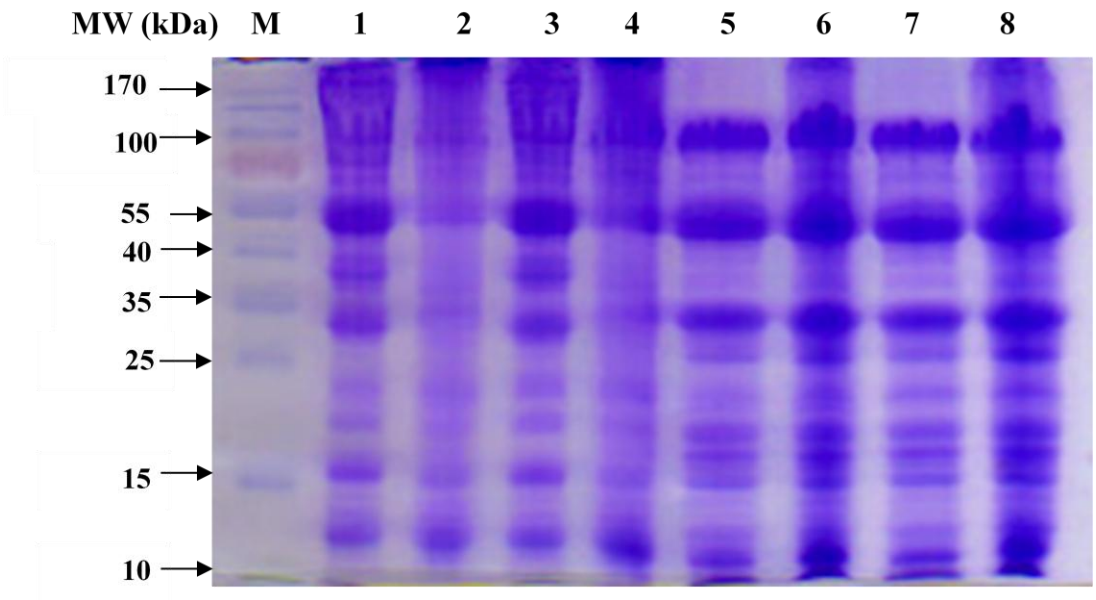

Figure: 3.2.2: Electrophoretic pattern of cooked and uncooked yam bean seed flour both in reducing and non reducing conditions. M: Standard protein marker, 1: UYB 06 uncooked-non reduced, 2: UYB 06 cooked non reduced, 3: UYB 07 uncooked nonreduced, 4: UYB 07 cooked non reduced, 5: UYB 06 uncooked reduced, 6: UYB 06 cooked reduced, 7: UYB 07 uncooked reduced, 8: UYB 07 cooked reduced.

It was also observed that bands from the raw samples were thicker than those for the cooked sample especially in the non reducing condition. This may be due to the aggregation of proteins on cooking. This may also be a reflection of the lower IVPD values recorded for raw yam bean seed flour than the cooked ones. The bands for both cooked and raw samples under reducing conditions were smaller and more in number.

This may be due to the fact that the reducing agent breaking down the disulphide bonds in the proteins producing smaller units and thus more proteins dissolve into solution creating more bands on the gel.

In raw samples under reducing conditions, the protein subunits were observed to be concentrated at the upper end of the lanes (lane 2 and 4) which upon reducing, the subunits are fragmented into more subunits as observed in the respective columns. The cooked samples had more and clearer protein subunits which were further enhanced in reducing conditions due to the breakdown of the quaternary and secondary structure to small subunits.

3.2.4 Conclusion

Yam bean seed protein seems to exhibit peculiar characteristics which can be exploited for food and industrial applications. Albumins fraction, which consists of biologically active protein, is the most dominant protein fraction in yam bean seeds. The yam bean seed protein isolates were shown to exhibit high gelation capacity, water and oil absorption capacities, foaming stability, nitrogen solubility and average emulsion properties. Yam bean seed protein showed higher *in vitro* protein digestibility than those for other legumes, suggesting superior nutritional value of the seeds. From the findings in this study, yam bean seed protein isolate have potential for use in food systems as ingredients and for other industrial applications. The isolates could be used in products requiring strong gel formation and for foam stabilization.

3.2.5 Reference

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4 GENERAL DISCUSSION

Proximate analysis of yam bean seeds from the three cultivated species revealed that the seeds are rich in proteins, fat and carbohydrates. The results indicated that *P. tuberosus* had the highest protein content followed by *P. erosus*. *P. erosus* had the highest fat content followed *P. ahipa* and with respect to total carbohydrate content, *P.ahipa* had the highest followed by *P.erosus*. This variation in the composition of the seeds across the species may be due to genetic factor since the seeds analyzed were derived from the same growth location. The high protein and oil content in yam bean seeds, makes them potential materials for utilization in both food systems such as composite flours and other technological applications such as in bio-composites, biodiesel and bioplastics industry. Given the problem of toxicity, non food applications offer more immediate opportunities than applications in food systems.

Generally the functional properties of defatted yam bean seed flours in this study are due to their proteins and other constituents such as water soluble carbohydrates, fiber and residual lipids. Bulk density, an attribute dependent on the fineness of the material, signifies the behavior of a product in dry mixes and is an important parameter in determining the packaging requirements (Amadou, *et al*., 2010). The defatted yam bean seed flour and its protein isolate exhibited bulk densities (Table 3.1.4 and 3.2.2) that were not significantly different. The results of the bulk density of both the defatted yam bean flour and its protein isolate were similar to that of rhizome flour (0.59 g/ml) (Shad *et al.*, 2011) and were virtually similar to the one reported for the Bambara flour (0.62 g/ml) and its protein isolate (0.56 g/ml) (Eltayeb *et al.*, 2011). Higher values of bulk density provide packing advantage (Shad *et al*., 2011).

Regarding Least Gelation Concentration, the defatted flours in this study showed higher values compared to the yam bean protein isolates. This may be attributed to higher protein concentration in the protein isolate relative to that that of flour. Adebowale *et al.* (2005) noted that gelation takes place more readily at higher protein concentration because of greater intermolecular contact during heating and high protein solubility is always necessary for gelation.

The results of this study are in tandem with the above statement, in which the isolate expectedly had lower LGC values (8%) and therefore, higher gelation capacity than the defatted yam bean flour (14%). These results are almost similar to LGC values reported for other legume flours and isolates (Butt and Batool, 2010; Khalid *et al.,* 2012). Defatted groundnut flour was reported to form strong and very strong gels at concentrations of 8 and 10% respectively (Fekria *et al.,* 2012). The higher LGC and therefore lower gelation capacity observed in the defatted yam bean seed flour in this study may be a disadvantage for use in production of some products such as curd compared to its protein isolate. The difference in the gelling properties of different legume flours may be due to variation in the relative ratios of different constituents such as proteins, lipids and carbohydrates. Flour for both accessions of yam bean in this study showed better gel forming capacity than those of other legumes. This may be due to relatively high protein content since gelling depends on protein concentration. Fekria *et al*. (2012) noted that protein conformations, disulfide linkages and hydrophobicity play significant roles in gelation. According to Jideani (2011), the ability of protein to form gels and provide a structural matrix for holding water, flavors, sugars and food ingredients is useful in food applications. The gelling property is important in comminuted sausage products and is the basis of many Oriental textured foods such as tofu (Jideani, 2011). Gelling is very useful in food systems such as puddings and sauces that require thickening and gelling (Fekria *et al.,* 2012).

With respect to WAC and OAC, both the defatted flours and their protein isolates in this study exhibited higher water and oil absorption capacities (Table 3.1.4 and 3.2.2) compared to the values reported for other conventional legumes (Khalid *et al*., 2012; Butt and Batool, 2010). WAC values as low as 0.6 and as high as 4.9 g/g have been reported (Amadou *et al.,* 2010) for protein isolates or concentrates prepared from pulses such as chick pea, cow pea, faba bean or lentil, and results from this study put yam bean seed flour and the protein isolate within the range. Interactions of water and oil with proteins are very important in food systems because of their effects on the flavor and texture of foods (Amadou *et al.,* 2010). The results in this study suggest that both the yam bean seed flours and their isolates may be used in meats, sausages, bread and cakes given the high WAC and OAC results recorded in the study. Results also indicate that both the flour and the protein isolate have potential for industrial applications.

Kiosseoglou & Paraskevopoulou (2011) noted that WAC and OAC data are useful for assessing the technological suitability of pulse protein material in food and technical applications.

The ability of proteins to form stable emulsions is important as it permits development of foods in which proteins and lipids are held together. Proteins being composed of charged, non-charged polar and nonpolar amino acids can serve as emulsifiers, able to interact with both water and oil in food systems (Amadou *et al.*, 2010). Results in this study (Table 3.1.4 and 3.2.2) revealed emulsion capacity of yam bean flour to be moderate and that for yam bean protein isolate to be poor compared to those of conventional legumes (Butt & Batool, 2010; Eltayeb *et al*., 2011; Fekria *et al*., 2012). Defatted yam bean seed flours exhibited higher EC values than those of the protein isolates and this may be attributed to the fact that flour has other biopolymers like polysaccharides which may, in part, contribute to the emulsifying activity of the flour. That notwithstanding, yam bean seed protein could be modified to make it more suitable for emulsification applications. The success of increasing the use of plant proteins in industrial applications can be influenced by chemical or physicochemical modifications (Askew, 2000). For non-food applications, protein additives and modifications do not need to be food compatible and therefore a much wider range of techniques is available to the chemist to manipulate protein properties, such as solubility, and improve their suitability for a wide range of industrial applications (Askew, 2000).

Foamability is a function of the configuration of protein molecules and the formation of protein based foams involves the diffusion of soluble proteins toward the air-water interface and rapid conformational change and rearrangement at the interface (Kamara *et al*., 2009). Foam stability requires formation of a thick, cohesive, and viscoelastic film around each gas bubble; hence foam stability is a function of configuration of protein molecules (Amadou *et al,* 2010). This happens through protein molecules forming continuous intermolecular polymers enveloping the air bubbles, since intermolecular cohesiveness and elasticity are also important for the production of stable foams (Kamara *et al.*, 2009). Proteins are denatured during whipping causing a large increase in the surface area in liquid/air interphase and rapid conformational change as well as rearrangement at the interface.

The yam bean seed flour in this study exhibited higher foaming capacity compared to the protein isolate (Table 3.2.2and 3.1.4). This may be due to the fact that the flour has number of other components like carbohydrates contributing to foam formation. Nonetheless, both the flour and the isolate showed inferior foaming properties compared to the egg white which is normally applied in products requiring high degree of porosity (Ndife *et al.*, 2010). Suitability of both the yam bean flour and its protein isolates as foaming agents can be achieved through modification of the yam bean seed protein.

Solubility of the protein is one of the critical functional attributes required for its use both in food and non food systems. Solubility directly influences other functional properties such as emulsion, gelation and foaming (Khalid *et al.*, 2012). The protein solubility results of yam bean seed flours and their protein isolate (Table3.1.4 and 3.2.2) at pH 7 showed that both the defatted flours and protein isolates demonstrated high protein solubility across the two yam bean accessions. There was a significant difference in the protein solubility of both flour and isolate samples from the two accessions, with the isolate exhibiting higher protein solubility. However there was no significant difference in the protein solubility of defatted flour and protein isolate in both accessions. The results of protein solubility of the yam bean seed flours and their isolates were comparable to the results reported by Khalid *et al.*, (2012) for dehulled defatted cowpea flour and its isolate (80% and about 76% respectively) at pH 7. The high protein solubility exhibited by both the defatted yam bean seed flours and their protein isolates in this study is reflective of the high *in vitro* protein digestibility values recorded for the yam bean seed protein and this makes them valuable materials for food and industrial applications. However the high protein solubility of both the defatted flour and its protein isolate can be significantly reduced by processing operation like extrusion cooking which has been reported to induce significant decrease in protein solubility (Valim & Batistuti, 1998).

The electrophoretic profile of yam bean seed flours and their protein isolate under non reducing conditions (Figure 3.2.1) revealed that the seed protein contained disulphide bond linked polypeptides and upon reduction with 2-macerptoethanol more bands were observed especially among the flours. This is in tandem with the results reported by Sathe *et al.* (2012) for the protein electrophoretic pattern of Inca peanut.

With respect to *in vitro* protein digestibility, the cooked sample showed lower digestibility than raw samples. Comparison of the electrophoretic pattern of the cooked and raw samples (Figure 3.2.2; lanes 5, 6, 7 & 8) showed that cooking may have caused more cross linking forming bigger oligomers and formation of complexes with other components in the sample. This phenomenon may explain the lower digestibility of cooked yam bean seed protein than the raw yam been seeds.

The pasting characteristics of flour determine their best use in food processing which, in most cases, depend on the botanic species of plants (Ndie *et al.,* 2010). The viscosity pattern is important to categorise the starch for end product recommendation (Ikegwu *et al.,* 2010). Results of pasting properties of the defatted yam bean seed flours in this study (Table 3.1.5 and Figure 3.1.1) indicated that the flours from the two accessions of *P. erosus* generally exhibited pasting properties comparing favorably with those of other legume flours. The pasting temperature of defatted yam bean seed flour (79.97-81.33^{o}C) was found to be lower than values reported for other materials such as corn starch (84.8^{o}C) and *Brachystegia eurycoma* flour (88.25^{o}C) (Ikegwu *et al.,* 2010). With respect to peak viscosity (145.50-146.72 RVU) and final viscosity (347.97- 360.14 RVU), the defatted yam bean flour exhibited lower values than those reported for corn starch (267.08 RVU, 441.17 RVU respectively) (Ikegwu *et al.,* 2010). The relatively lower peak viscosity and final viscosity values exhibited by the yam bean seed flour suggests that the flour may be suitable for products requiring low gel strength and elasticity (Ikegwu *et al.,* 2010).

The protein fractionation pattern of the yam bean (*P. erosus*) seed proteins was in agreement with the results reported by Morales-Arellano *et al.* (2001). The proportions of protein fractions give an insight into the plausible uses of such vegetable proteins (Akinhanmi *et al,* 2008). The study established albumins as the most dominant protein fraction in the yam bean seeds followed by globulins. These are mainly considered storage proteins (Ramakrishna, 2007). Albumins are considered to have a better amino acid profile compared to globulins (Wang *et al,* 2003) and these largely account for the biologically active proteins in the seed. This protein fractionation pattern was also reflected in the SDS electrophoretic pattern of the yam bean seed protein with major bands observed at molecular weights of 35, 55 and 85 kDa. Glutelin, which constituted 8 to 9.6% of the yam bean seed protein, is known to form large disulphide bonded aggregates which are susceptible to reduction when heated in presence of 2-mercapto ethanol. The observation of

numerous protein bands in the electrophoretic pattern under reduced conditions is attributable to changes in this protein group.

The yam bean seed protein exhibited high *in vitro* protein digestibility. This may be attributed to the high proportion of albumin protein fraction observed in this study. The high digestibility is also attributable to the low concentration of antinutritional factors as well as secondary metabolites in the seeds (Santos *et al*., 1996) compared to concentration of these substances in other legume seeds (Sridhar & Seena, 2006).

5 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

- Based on the results for the proximate composition of yam bean seeds, it can be concluded that the yam bean seeds are a good source of protein and oil. The study showed that there are significant differences in proximate composition of yam bean seeds among species with *P. tuberosus* exhibiting highest crude protein content, *P. erosus* showing the highest crude fat content and *P. ahipa* having the highest total carbohydrates content.
- Functional properties of both flour and the protein isolate showed that they have potential for use in food systems (as thickeners, coatings, fillers among others) as well as in a variety of industrial applications such as in production of adhesives, , , bioplastics, and biocomposites.
- Yam bean seed protein was found to have moderate emulsion and foaming capacities and therefore the protein may need to first undergo modification to make it suitable for applications which require much of these properties, for example in the production of mayonnaise, bread, cake and in other non-food application like in paint formulations.
- Albumins are the most dominant protein fraction in yam bean seed, which suggests that the seeds can be a good source of high nutritional quality protein since the fraction accounts for the biologically active proteins.
- Yam bean seeds protein have high *in vitro* protein digestibility which contributes to its protein quality and ultimately to its nutritional value.

5.2 Recommendations

- Research should be undertaken to establish the safety (residual rotenone content) of the defatted yam bean seed flour and its protein isolate if it is to be used for food applications and thus promote utilization for food and feed.
- There is need to explore cost effective means of detoxifying the yam bean seed. Alternatively, investigation on the possibility of developing rotenone free yam bean seeds through breeding could be undertaken. This will enhance full utilization of the crop.

- It is necessary to determine the performance of yam bean seed flour and the protein isolate as ingredients in food systems and/ or non food industrial application.
- Studies should be undertaken on the modification of yam bean seed protein to enhance specific functional properties like foaming and emulsifying capacities and stability.

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