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THESIS

Topic:

DETERMINATION OF COMPOSITIONAL CHARACTERISTICS, FUNCTIONAL PROPERTIES AND CLUSTER ANALYSIS OF LIMA BEAN ACCESSIONS (*PHASEOLUS LUNATUS*)

By:

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DECLARATION

I declare the thesis is as a result of my own original research conducted under the supervision of Dr. (Mrs.) Antonia Y. Tetteh and Dr. Jacob Agbenorhevi. The references to specific authors has been duly acknowledged and cited. The study has not been presented for another certificate in other University or elsewhere.

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ABSTRACT

Demand for alternative sources of protein foods have increased due to population growth especially in developing countries. Lima beans are underutilized legumes of wide importance for human and livestock nutrition. Thirty-one lima accessions have been identified in Ghana but information on their nutritional composition, genetic variability, storage protein content and functionalities is scarce. This research was carried out to determine the proximate composition and mineral content of ten lima bean accessions and to investigate the storage protein content, functional properties of the flour and on this basis classify the genotypes into groups for genetic improvement. A wide variability in seed size, seed weight, nutrient composition, storage protein content and functional properties was observed. Mean seed length ranged from 10.2 to 23.0 mm. Hundred seed weight varied from 34.2 to 138.5 g. Nine of the accessions belonged to the Andean gene pool while one was of a Meso-American origin. The range values of protein, fat, ash, fiber, and carbohydrate contents on dry weight basis was 20.5 to 24.6 %, 0.6 to 1.7 %, 3.29 to 6.7 %, 2.93 to 9.6 %, and 47.0 to 58.1 %, respectively. Mean mineral contents were 893 mg/100 g for potassium, 212 mg/100 g for calcium, 4.9 mg/100 g for iron, and 207.5 mg/100 g for phosphorus. Potassium and phosphorus was the most abundant mineral in lima beans. With regard to storage proteins, globulins were present at 19.27-61.88 % and albumins at 10.53-57.67 %. Glutelins and prolamins occurred at least concentrations of 6.68-35.77 % and 3.13-16.94 %, respectively. Lima bean flour possessed high water and oil binding capacities of 1.00-2.33g/g and 1.01-1.55g/g, respectively. However, emulsion and foaming capacities were low in comparison to soybean and cowpea. Correlation coefficients among the four groups of traits were mostly weak and nonsignificant except between bean size, hundred seed weight, and storage protein content. UPGMA cluster analysis based on seed size, proximate, storage proteins

and functional properties clustered the ten accessions into five main groups. Principal component analysis revealed that the first six principal components accounted for 95 % of the variation in the data. Size and weight, protein, fat and carbohydrate, albumin, prolamin, glutelin and the functional properties, water and oil binding capacities, swelling capacity and bulk density were the most important traits that contributed to the variance.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

1.0 Background

Lima bean (*Phaseolus lunatus* L.) is the second most important species of the grain legume *Phaseolus*, the family encompassing the common bean and runner bean. Lima bean originated from the region of Guatemala, Mexico and Peru and is now commonly cultivated in tropical, subtropical climates and warm temperate climates. Introduced to West and Central Africa by the Portuguese explorers in the 16th century, the bean now forms the major food legume in eastern and southern Africa (Aghkhani *et al*., 2012).

The largest producer of lima beans is the U.S.A. where over 40,000 acres are cultivated annually (Kee *et al.,* 2004). The value of lima bean production in the U.S.A. in 2005 was $355/ton for a total value of $6,475,000 (Scuse and Chris, 2006). In Africa, about 120,000 to 200,000 hectares are devoted to lima bean cultivation in the sub-humid and humid regions indicating the need for its maximum utilization (Nwokolo, 1996). In Ghana lima bean is fourth in importance after cowpea, groundnut and bambara groundnut whereas in Nigeria it is second in importance to cowpea (Asante *et al*., 2008). In West Africa, lima bean is an underutilized legume.

Two botanical varieties occur, namely var. *silvester*, the wild relative, and var. *lunatus* as the landraces (Baudet, 1977; Baudoin, 2006). By means of seed size, biochemical characters and molecular markers, lima bean is divided into two gene pools (Gutiérrez Salgado *et al*., 1995; Nienhuis *et al*., 1995; Caicedo *et al*., 1999; Fofana *et al*., 2001), the small-seeded or Mesoamerican type which arose from a single domestication event in central–western Mexico and the large-seeded or Andean gene pool which was

domesticated in the Andes of southern Ecuador–northwestern Peru as revealed by analyses of genetic distances and haplotype networks (Motta-Aldana *et al*., 2010). Beside these two forms, intermediary types arising from introgression between the two gene pools have been reported (Nienhuis *et al.,* 1995; Lioi *et al.,* 1998).

A worldwide stable supply of nutritionally balanced food is of fundamental importance. It is estimated that over 842 million people in the world today are undernourished and/or malnourished (FAOSTAT, 2014) of which a large percentage are in developing countries. The nutritional value and price of underutilized legumes make them good candidates for use in fighting hunger and malnutrition particularly in children and pregnant women in developing countries (Coulter *et al*., 1988; Chel-Guerrero *et al*., 2002; Arinathan *et al*., 2003).

Lima bean has a rich source of protein (21 – 26 %) and high carbohydrate (55 - 64 %), low fat (1.0 – 2.3 %) and fiber levels (3.2 – 6.8 %), high levels of minerals such as potassium, zinc, calcium and iron, and low levels of sodium and phosphorus (Kay, 1979; Sathe *et al.,* 1984; Oshodi and Aletor, 1993; Kizito, 2010). However, lima bean contains some anti-nutrient factors such as lectins, trypsin inhibitors, phytates and oxalates (Ologhobo and Fetuga, 1982; Fasoyiro *et al.,* 2006**)** which can be reduced by modern and traditional food processing methodologies including soaking, germination, dehulling, cooking and fermentation (Honke *et al.,* 1998; Fasoyiro *et al.,* 2006).

By 2050, the world will need to feed two billion additional people. Legumes especially grain legumes are critical crops in developing countries. They are nutrient dense, staple foods that help ensure food and nutritional security. They also provide healthy food

processing aids for emulsification, foaming, texturization, and other functionalities required for food manufacture. Legume flour has been used as protein sources in food based on its functional properties (Kaur *et al.,* 2007; Kaur *et al.,* 2009).

Functional properties refer to physical and chemical properties of food or food component, which affect utilization (Zayas, 1997). They include solubility, water and binding capacity, emulsion capacity, foam capacity, gelation, swelling power and so on (Onimawo and Akubor, 2005). A comparative assessment of chemical and functional properties of lima bean consumed in Nigeria demonstrated high water absorption capacity, foam capacity ,and least gelation concentration (Obiakor- Okeke, 2014) making them suitable for use in food product manufacture such as soups and gravies (Chel-Guerrero *et al*., 2002).

Breeding activities on lima bean in Ghana is new, and began with collection and regeneration of thirty-one landraces from four eco-geographical regions by Dr. James Y. Asibuo of the Crop Research Institute of the Center for Scientific and Industrial Research, Fumesua, in 2013, and is currently in the pure line selection stage and testing of dry bean composition. Asante *et al.* (2008) evaluated sixteen phenotypic and seed protein characteristics on 31 accessions of lima bean assembled in Ghana. The ten accessions of lima beans selected for this project are those that have received agronomic improvement in terms of nutritional composition, early maturity, disease resistance and yield but have not yet been released. There is lack of information on the nutritional properties such as proximate composition and minerals. Similarly, information on storage protein fractions and functional properties for possible incorporation into food product manufacture is not available.

Knowledge of the genetic relationships among accessions is useful to the plant breeder because it permits organization of germplasm into groups for maximizing genetic gain during hybridization. Despite the availability of reports on proximate composition and storage protein content of lima bean of worldwide (Kathirvel and Kumudha, 2011; Sathe *et al.,* 1984; Chel-Guerrero *et al*., 2002), similar information on lima bean in West Africa, a geographical region characterized by extreme climates and a myriad of biotic and abiotic constraints is scanty.

In recent years, plant breeders target improvement in grain and seed composition of cultivars in order to offer a diversity of crops for many applications. Classification of lima beans based on nutritional composition and storage protein would enhance variety improvement opportunities for the breeder and determine their functionalities for food product development.

Many studies have been carried out to characterize lima bean collections from many parts of the world and to assign to two major historic gene pools on the basis of morphometric traits, isozyme analysis and DNA polymorphism. Asante *et al.* (2008) evaluated sixteen phenotypic and seed protein characteristics on 31 accessions of lima bean assembled in Ghana. UPGMA cluster analysis of phenotypic data and seed protein profile classified the accessions into six and three groups, respectively, providing evidence that different set of traits can reveal variation in grouping.

Lioi *et al*. (1998) examined isozyme diversity and Random Fragment Length Polymorphism in sixty accessions of lima bean followed by cluster analysis. Their study revealed two distinct groups each representing the two gene pools, as well as an

intermediate group that was representative of transitional group. A cluster analysis of eleven cowpea varieties from Turkey revealed eight groups (Vural and Karasu, 2007). Kalaimagal *et al.* (2008) have also applied the principal component technique in pigeon pea. An assessment of forty-nine genotypes of pigeon pea on twelve characters using principal component analysis and cluster analysis revealed eight clusters.

Variability in proximate composition of fifteen *Canavalia ensiformis* varieties and further classification into groups by means of cluster analysis and principal component analysis provided a guide to selection of genotypes for interspecific hybridization to maximize genetic gains in proximate composition (Dada *et al*., 2013). There is dearth of information on the proximate composition, seed storage proteins and functional properties of lima beans cultivated in Ghana. Assignment of Ghana lima bean accessions into heterotic groups for improving protein content, and functional properties has hitherto not been carried out.

The main objective of this research therefore was to characterize lima bean accessions in Ghana for their proximate composition, storage protein content, and functionalities of the flour and on the basis of these parameters, assign the accessions into clusters for purpose of interspecific hybridization. Furthermore, the research aims to identify the traits that contribute most to the variation in the data by means of principal component analysis. The specific objectives of the study include:

1. To evaluate the proximate composition of ten lima bean genotypes collected in Ghana.
2. To determine the mineral composition of the accessions specifically potassium, calcium, iron and phosphorus.

3. To isolate and quantify the storage proteins, albumins, globulins, glutelins and prolamins in lima bean flour.
4. To evaluate the functionality and physicochemical properties of the lima bean flour
5. To classify the ten lima accessions into groups by means of cluster analysis and principal components analysis.

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomy of lima beans

Lima bean (*Phaseolus lunatus* L.) belongs to the family Leguminosae. It originated over a wide area in the Americas from where it was domesticated. Its complex taxonomy has given rise to many controversies due to its occurrence in many forms. On the basis of morphology of pod and seed, authors classify lima bean into type and named *P. lunatus*. The genepool of *P. lunatus* comprises the wild populations and the landraces of lima bean, which can be grouped into two main races: the Andean and the Mesoamerican.

Each race is characterized by distinctive morphological characters (Debouck *et al*., 1987; Maquet, 1995), ecological adaptation, seed storage proteins (Debouck *et al.,* 1989; Lioi, 1994; Gutierrez Salgado *et al*., 1995; Maquet, 1995; Lioi *et al*., 1999), allozymes (Maquet *et al*., 1997; Lioi *et al*., 1998) and molecular markers (Nienhuis *et al*., 1995; Fofana *et al*., 1997; Lioi *et al*. 1998; Caicedo *et al*. 1999). Escaped forms and weedy forms (natural hybrids between the wild form and a landrace) are observed throughout Latin America. Currently, no natural interspecific hybrids involving *P. lunatus* have been reported.

The secondary genepool of the lima bean probably consists of the South American species (P. *augusti,* P. *bolivianus,* and P. *pachyrrhizoides*). These species differ very little (Caicedo *et al*., 1999; Delgado *et al*., 1999) and may in fact constitute a single species with geographic variants. The tertiary genepool of P. *lunatus* includes the following species from the U.S.A. and/or Mexico with varying levels of relationship and compatibility: P. *jaliscanus Piper*, P. *juquilensis Delgado,* P. *maculatus Scheele,* P. *marechalii Delgado,* P. *polystachyus Britt,* Stern and Pogg, P. *ritensis Jones*, P. *salicifolius Piper,* P. *sonorensis Standley,* and P. *xolocotzii Delgado* (Debouck, 1999;

Delgado *et al*., 1999; Delgado, 2000). Two variants of P. *polystachyus* are usually recognized: P. *sinuatus Nutt* and P. *smilacifolius Pollard*.

2.2 Geographic Distribution

The wild form of P. *lunatus* is found only in the Americas, along the Pacific slopes of the mountains of Mexico and Guatemala, and further south in Mesoamerica while the cultivated form is widely distributed all over the tropical regions at temperatures of 16°C to 27°C and at elevation of up to 2,500 m.a.s.l in temperate regions. However, large-seeded wild populations are best adapted to drier conditions and are tolerant of temperatures below 15°C of the world and in some temperate regions (Baudoin, 2006).
The small-seeded wild form of P. *lunatus* extends at low altitudes from Sinaloa, Mexico (23-26°N) to Salta, Argentina (25°S) and through the Caribbean islands (Maquet and Baudoin, 1997).

In Ecuador and northern Peru, deciduous forests give way to steppes due to low rainfall. This could suggest that small-seeded landraces are also distributed throughout South America (Maquet and Baudoin, 1997) at lower altitudes (an average of 700 m.a.s.l) while large seeded landraces at 1880 m.a.s.l (Maquet, 1995). In the eastern part, they are particularly common in northeastern Brazil. The frequency of large-seeded landraces increases in central and southern Brazil (Erickson, 1982).

Consequently, a hybrid zone has developed, characterized by landraces with intermediate seed sizes. Landraces are cultivated in the Andean region from Venezuela to Argentina. Lima bean landraces are thus adapted to ecological systems ranging from the dry Peruvian coast to the tropical humid Amazonian region of Ecuador and Peru, to the

temperate high altitudes of 2800 m.a.s.l. (Baudoin, 2006). Weedy types resulting from gene flow between wild and cultivated forms have been observed where these grow simultaneously without interbreeding (Maquet, 1995). For example, hybridization between the small-seeded wild form and the cultigen is known from Mexico, Guatemala, Costa Rica, the Bahamas, Cuba, Puerto Rico and Jamaica (Correll and Correll, 1982; Liogier and Martorell, 1982; Maquet, 1991; Esquivel *et al*., 1993). The weedy type is also present in the Andean region and particularly in Ecuador and Peru (Debouck, 1990). It is distributed from 1800 to 2000 m.a.s.l in Peru and even up to 2400 m.a.s.l in Ecuador.

2.3 Domestication

Two separate domestication events have been demonstrated, from two different wild forms, with different distribution ranges and distinct ecologies (Gutierrez Salgado *et al*., 1995; Maquet, 1995; Fofana *et al*., 1997). Among the large-seeded wild type, one domestication event occurred within the southern Andes of Ecuador and the northwestern Andes of Peru. In contrast, the range of the small-seeded wild lima bean is huge, and a precise location of the domestication of the small-seeded cultivated lima beans is still to be determined. Domestication events were characterized by selection for larger pods and seeds reduction in hard seededness, dormancy and antinutritional factors, including antitrypsin factors and cyanide glycosides and low levels of linamarin (Baudoin, 1991).

Farmers have selected for white-seeded cultivars. This mutation appears from time to time in wild populations, for example in Yucatan, Mexico (Debouck, 1999). The original viny habit with profuse branching, observed in the wild form was selected against to obtain annual landraces with determinate growth habit. In addition to these changes in plant habit, some selection has been undertaken for photoperiod insensitivity. Most of the

evolutionary changes in beans are due to mutations of just a few genes; the increase in seed size is a noteworthy exception (Koinange *et al*., 1996).

2.3.1 Genetic Diversity

Domestication leads to an overall reduction in genetic diversity due to a founder effect. Using seed-protein markers, Maquet *et al*. (1990), Gutierrez *et al*. (1995) and Maquet (1995) showed reduced genetic diversity among the small-seeded Mesoamerican landraces, and among large-seeded Andean landraces. Generally, seed storage proteins have been used as markers in the following four main areas: analysis of genetic diversity within and among populations, plant domestication in relation to genetic resources conservation and breeding, genome relationships especially in polyploid series, and as a tool in plant breeding (Gepts, 1990).

Seed storage protein satisfies the requirements of genetic markers for the following reasons: they are highly polymorphic, their polymorphism is genetically determined and the molecular sources of their polymorphism are known; their control of qualitative genetic variations is simple and involves limited number of loci of nuclear genome; and they show that most protein variants are unique and can therefore, be used as evolutionary markers. Finally, homologies have been established between seed storage proteins of different taxa (Gepts, 1990).

In the international collection of P. *lunatus* held by the Centro Internacional de Agricultura Tropical (CIAT) in Cali, Colombia, gene diversity is mainly distributed among rather than within accessions (Maquet *et al*., 1997). Lima bean germplasm is characterized by a high inbreeding coefficient. In spite of this, and a low rate of gene

flow, the intrapopulation gene diversity estimated from the CIAT collection is significantly different from zero and higher than that of other selfing species, such as P. *acutifolius* (Schinkel and Gepts, 1989) and P. *vulgaris* (Koenig and Gepts, 1989). Because of the wide distribution of their wild ancestors and multiple domestication events, cultivated common bean and lima bean probably have a wider genetic base than the other cultigens in the genus (P. *acutifolius,* P. *coccineus* and P. *polyanthus)* (Debouck, 1999).

2.4 Lima Bean Production in Ghana

Lima beans are the second most economically important species of *Phaseolus* and one of the 12 primary grain legumes (Broughton *et al*., 2003). In Ghana lima bean production is fourth in importance after cowpea, groundnut and bambara groundnut while in Nigeria, it is placed second in importance to cowpea (Asante *et al*., 2008). In tropical Africa, the crop is interplanted with crops such as maize, sorghum, sweet potato, coffee, cotton and yam. The crop is hardy and may be advantageous in adverse conditions where other leguminous vegetables do not grow well (FAOSTAT, 2012). Lima bean grows better in areas where temperatures are ranging from 16 to 27°C and where annual rainfall is about 900-1500 mm. Once well established, it can withstand rainfall as low as 500-600 mm. Perennial forms of lima bean are considered drought resistant (Baudoin, 2006). Lima bean is tolerant of a wide range of soils but prefers well-drained soils with pH above 6. Some cultivars do well in acid soils with pH as low as 4.4 (Baudoin, 2006).

Lima bean is borne in a dehiscent pod of size of about 5-12 cm long, and contains 2-4 seeds per pod (Baudoin, 2006). Seeds are very variable in size, shape and colour. Cultivar groups are distinguished on the basis of seed differences (Baudoin, 2006). Lioi (1994)

and Esquivel *et al*. (1990) identified five different morphotypes of lima based on seed weight and seed length as follows: Big Lima (100–110 g, 25 mm long and 14 mm wide), Sieva (30–45.3 g, 12 mm long and 8mm wide), Potato (35.5 g, 9 mm long and 8 mm wide), Potato-Sieva (36.3 g, 11 mm long and 8 mm wide) and Sieva-Big (77.5 g, 17 mm long and 11 mm wide).

Lima bean is a minor grain legume in spite of its diversity and yield potential. In Africa, about 120,000 to 200,000 ha are devoted to lima bean cultivation in the sub-humid and humid areas of Sierra Leone, Liberia, Côte d'Ivoire, Ghana, Nigeria and DR Congo (Nwokolo, 1996). Currently, explicit global production statistics are lacking as lima beans are aggregated with other grain legumes (FAOSTAT, 2012). However, world lima bean production is not likely to exceed 200,000 mt/year compared to about 5 million mt for cowpea for instance.

In 1995, the U.S.A. was the most important producer with 70,000 mt/year, followed by Madagascar (8000 mt) and Peru (5000-5500 mt). About 50,000–100,000 mt came from Africa during the same period (Baudoin, 2006). Lima bean sprouts, leaves, young pods and green immature seeds are eaten as vegetable. The dry seeds are eaten boiled, fried, ground into powder and baked, and used in soups and stews. The vines, leaves and empty pods left after harvest serve as fodder, and can be made into hay or silage. Lima bean may be used for manure or as cover crop (Baudoin, 2006).

2.5 Importance of Lima Beans

Legumes are a significant food source worldwide and particularly so in the developing world, where they function as a protein source in the absence of animal protein (Redden

1998; Siddhuraju *et al*., 2002). Millions of people in the tropics depend on several legume species as their principal source of dietary protein (Okafor *et al*., 2002). Isoelectric precipitation of lima bean protein from the meal yields approximately 72% protein making it a promising protein functionality ingredient in food systems, such as bakery products, seasonings and sausages (Chel-Guerrero *et al*., 2002; Betancur-Ancona *et al*., 2004).

The oil from lima beans is a good source of potassium, iron, copper and manganese (Redden, 1998). Its low sodium content is recommended for reduction in blood pressure. The soluble fiber of lima bean contributes to cholesterol-lowering effect (Ensminger and Ensminger, 1996; McIntosh and Miller, 2001; Singh *et al.,* 2002) through its binding with the bile acids and subsequent elimination from the body. The cholesterol-lowering effect is enhanced by its rich content of beta-sitosterol (Ensminger and Esminger, 1996; Gardner *et al.,* 2005). When consumed as food, the insoluble fiber of lima bean increases stool bulk and prevents digestive disorders such as irritable bowel syndrome and diverticulosis (Han *et al.*, 2002; Bazzano *et al.*, 2003).

Lima is a good source of the trace element, molybdenum, which is an integral component of sulfite oxidase, the enzyme required for detoxification of sulfites used food preservative (Bogden, 2000; U.S.D.A., Research Service, 2013). A hundred gram (100g) of lima bean supplies 99% of folic acid needed by the body (U.S.D.A., Research Service, 2013). Like other grain legumes, lima bean meal contains 21- 26% protein, 55- 64% carbohydrate contents, a low fat content of 1.0 – 2.3%, fiber levels of 3.2 – 6.8% (Holland *et al*., 1991; Oshodi and Aletor, 1993), including high levels of minerals such as potassium, Zinc, Calcium and iron, and low levels of sodium and phosphorus. They are

rich in niacin, thiamine, and riboflavin (Sathe *et al*., 1984). The essential amino acid composition per 100 g raw lima beans is: tryptophan 180 mg, lysine 1440 mg, methionine 280 mg, phenylalanine 1160 mg, threonine 800 mg, valine 980 mg, leucine 1560 mg and isoleucine 950 mg (Paul and Southgate, 1980). As in other pulses, the main limiting amino acids are methionine and cysteine.

2.6 Nutritional and Anti-Nutritional Composition

The composition of the edible portion of lima on a dry weight basis is water 11.6 g, energy 1214 kJ (290 kcal), protein contains 21- 26 %, a low fat content of 1.0-2.3%, , carbohydrate content of 55-64%), and a dietary fibre of about 19%. Other nutritional factors in lima bean include 85 mg Ca, 190 mg, 320 mg P, 5.9 mg Fe, 2.8 mg Zn, trace of carotene, 0.45 mg thiamin, 0.13 mg riboflavin, 2.5 mg niacin, 0.51 mg vitamin B6 and trace of ascorbic acid (Sathe *et al*., 1984; Holland *et al.,* 1991). The proximate composition (Table 2.1) of wild and cultivated lima beans is similar (Kathirvel and Kumudha, 2011).

The Mineral content of wild and cultivated lima beans (*Phaseolus lunatus L*.) germplasm as studied has been represented in the Table 2.2. There were significant differences in the mineral constituents between the two germplasms. The observed different concentrations of the mineral elements were as a result of the concentration levels in the soil, translocation rates of the elements by the cultivars from the soil, and detection limits of the characterization techniques. This could also be due to differences in their ability to take up mineral elements from the soil (Purves *et al.*, 1992).

Table 2.1 Proximate composition of wild and cultivated seeds of *Phaseolus lunatus* [1, 2] (g/100g)

Components	**Wild germplasm**	**Cultivated germplasm**
Moisture	11.78 ± 0.05^{a}	12.07 ± 0.04^{b}
Crude protein	22.84 ± 0.07^{c}	20.05 ± 0.06^{a}
Crude lipid	2.63 ± 0.09^{b}	2.55 ± 0.06^{a}
Crude fibre	5.48 ± 0.07^{c}	4.54 ± 0.04^{a}
Ash	3.42 ± 0.03^{b}	2.91 ± 0.03^{a}
Nitrogen Free Extracts (NFE)	65.63	69.95
Calorific value (kJ 100g-1DM)	1576.6	1599.14

[1, 2] Mean values in the same row sharing different superscript are significantly different ($P < 0.05$)

Source: Kathirvel and Kumudha (2011)

Amino acids serve as substrates for protein synthesis and play other roles as tissue repairs, hormone synthesis, precursors of heme as well as synthesis of enzymes that catalyze biochemical reactions in the cells (Maduka *et al.,* 2004). Like all leguminous seeds, *Phaseolus* seeds are deficient in sulphur-containing amino acids and, to a lesser extent, in tryptophan, but they contain relatively large amounts of lysine (Mahe *et al.*, 1994) with the wild and cultivated forms having similar amino acid profiles. Compared to soya bean protein, the lysine content in pulses tends to be higher and the content of sulphur containing amino acids (methionine and cystine), and tryptophan tends to be lower (Norton *et al.*, 1985; Jansman, 1996).

Table 2.2 Mineral compositions of wild and cultivated seeds of *Phaseolus lunatus*[1] expressed in mg/100g.

Component	**Wild germplasm**	**Cultivated germplasm**
Sodium	37.84 ± 0.06^{d}	30.84 ± 0.07^{a}
Potassium	1892.05 ± 0.64^{d}	1698.68 ± 0.52^{a}
Calcium	352.56 ± 0.71^{a}	720.88 ± 0.26^{d}
Magnesium	225.64 ± 0.33^{d}	155.74 ± 0.52^{b}
Phosphorus	247.91 ± 0.51^{c}	177.81 ± 0.37^{b}
Iron	2.09 ± 0.03^{b}	2.25 ± 0.05^{c}
Copper	1.58 ± 0.03^{b}	1.12 ± 0.07^{a}
Zinc	0.54 ± 0.06^{b}	0.19 ± 0.04^{a}
Manganese	4.75 ± 0.04^{bc}	5.16 ± 0.05^{c}

[1]Mean values in the same row sharing different superscript are significantly different ($P < 0.05$)

Source: Kathirvel and Kumudha (2011)

2.7 Legume Proteins

Generally, grain legume seeds have high protein contents ranging from 20% to 40% of their dry matter, most of which is located in the cotyledon (Otoul, 1976; Norton *et al.*, 1985; Baudoin, 1991). The major storage proteins in legume seeds are the globulins that usually account for about 70% of the total protein. The remaining 30 % is shared between Glutelins (10–20%) and albumins (10–20%). The principal storage globulins in most legumes are legumin and vicilin, the latter predominating in common bean (Jansman, 1996). Difference in amino acid composition is also observed among the protein fractions of the seeds.

Table 2.3 Amino acid composition of Lima bean (*Phaseolus lunatus*) and soybean (Glycine max) (g/100g protein)

Amino Acids	**Lima bean (*Phaseolus lunatus*)**	**soybean (*Glycine max*)**
Lysine	6.54±0.01	7.27±0.01
Histidine	2.27±0.01	2.61±0.01
Arginine	6.99±0.01	7.94±0.01
Aspartic acid	10.61±0.01	12.26±0.01
Threonine	4.01±0.01	3.90±0.01
Serine	3.06±0.01	3.56±0.01
Glutamic acid	15.87±0.01	16.83±0.01
Proline	3.21±0.01	3.56±0.01
Glycine	4.92±0.00	5.31±0.01
Alanine	3.05±0.01	3.54±0.01
Cystine	1.54±0.01	1.43±0.01
Valine	5.41±0.01	4.92±0.01
Methionine	1.58±0.01	1.72±0.01
Isoleucine	4.51±0.01	4.90±0.01
leucine	7.04±0.01	8.62±0.01
Tyrosine	3.46±0.01	3.45±0.00
Phenylalanine	4.96±0.01	5.72±0.01

Values are mean of five replicates. Source**:** Kizito (2010)

Globulins are relatively poor in sulphur-amino acids. Albumins are richer in sulphur-amino acids and lysine than globulins. The protein quality of a food or feed depends on its amino acid composition and digestibility (Hahn *et al*., 1981).

2.7.1 Lima Bean Protein Fractions

2.7.1.1 Lima bean globulins

Osborne (1988) classified seed storage proteins according to their solubility in diverse solvents. The albumins dissolve in water, globulins dissolve in saline solutions, prolamins dissolve in alcohol and glutelins dissolve in weak acid or base. The major storage proteins in legume seeds are the globulins (Gepts, 1990) which account for about 70% of the total protein. In contrast, globulin content of wild and cultivated lima beans total proteins has been found to be 54.63 and 55.16% respectively (Kathirvel and Kumudha, 2011). On the basis of sedimentation coefficient, the principal storage globulins in most legumes are 7S vicilin-type globulins and the 11S legumin-type globulins (Shewry *et al.*, 1995; Jansman, 1996).

Like other legumes including peas, soybean, broad bean and french bean, which have been studied extensively, the globulins of lima have been characterized as 7S heterotrimer globulins devoid of disulfide linkages having relatively low molecular weight of 72 kDa and higher thermal stability than typical vicilins, while the 11S legumin is a heterodimer of 336 kDa linked by disulfide linkages (Chel-Guerrero *et al.*, 2007). Research on the functional properties of legume seed storage proteins has largely focused on purified 11S and 7S globulins. The protein quality of a food or feed depends on its amino acid composition and digestibility (Hahn *et al*., 1981).

Globulins of legumes can be separated on polyacrylamide gel electrophoresis into two classes of proteins, namely, the 7/8S vicilins and 11/12S legumins. Purified 7S and 11S reserve proteins from faba bean protein possess excellent physicochemical properties (Kimura *et al.,* 2008) which arise from increased hydrophobic interactions that promote protein-protein interactions thereby decreasing solubility. Soybean contains mostly 11S proteins, the properties of which have been extensively researched, leading to its broad use as a food ingredient (Chavan *et al*., 2001). Basic research has shown that vicilins (7S) proteins are generally more surface active than legumin (11S) proteins and also exhibit better emulsifying and foaming properties (O'Kane *et al*., 2005).

2.7.1.2 Albumins

Albumins fraction is a diverse group of proteins usually soluble in water with a sedimentation coefficient of approximately 2S, of which some are rich in cysteine. They have been reported to be basic proteins with molecular weights ranging from about 10 to 18 kDa (Kortt and Caldwell, 1990; Anisimova *et al.,* 1995; Raymond *et al*., 1995; Popineau *et al*., 1998). 2S albumins, defined on the basis of their sedimentation coefficient (Youle and Huang, 1981) are a major group of seed storage proteins widely distributed in both mono- and dicotyledonous plants. As storage proteins, they are deposited in protein bodies of developing seeds and are used by the plant as a source of nutrients during subsequent germination and seedling growth (Agizzio *et al.*, 2003).

Recent findings have demonstrated that 2S albumins can also play a protective role in plants as defensive weapons against fungal attack (Agizzio *et al.,* 2003). In addition to their physiological role in plants, these small globular proteins are becoming of increasing interest in nutritional and clinical studies. The amino acid composition of 2S

albumin proteins from many plant species has revealed their high content of sulphur-containing amino acids (Youle and Huang, 1981). Typically, 2S albumins show high levels of cysteine residues, ranging from 6 to 13 mol %.

2.7.1.3 Glutelins

Glutelins are major storage proteins that aggregate in protein bodies in the endosperm of maize (Prat *et al*., 1985; Leite *et al*., 1991). They comprise the second largest protein fraction in maize endosperm (Prat *et al*., 1985) (with prolamins being the largest), and show sequence similarities to other cereal storage proteins, such as gliadins, glutenins, hordeins, etc. Glutelins have a well-defined structure, including an N-terminal region containing varying numbers of repeats of the sequence PPPHVL (Leite *et al.,* 1991); a Gln rich region that can be separated into two domains; and a Cys rich C-terminal domain that shows some regions of internal similarity.

Glutelin is extractable with dilute alkali or detergent containing reducing agent and makes up of 30 to 35% of the total protein (Landry and Moureaux, 1970). The two major subunits of glutelin have molecular weight near 40 and 20 kDa (Juliano and Boulter, 1976; Luthe, 1983; Yamagata, 1982), and rice glutelin is synthesized as a precursor polypeptide that is post translationally cleaved to form two smaller subunits (Luthe, 1983, Yamagata, 1982). Like oat globulin (Zhao *et al.*, 1983), rice glutelin appears to be preferentially synthesized on membrane bound polysomes (Luthe, 1983, Yamagata, 1982) and it is packaged in protein bodies (Yamagata, 1982).

The major storage proteins of rice endosperm are the glutelins, which may constitute up to 75% of the grain protein (Tecson *et al.,* 1971; Villareal and Juliano, 1978). In contrast,

the alcohol-water soluble prolamins, which are the predominant storage proteins in most of the other major cereals, compose less than 5 to 10% of the total rice grain protein. Glutelins are synthesized at about 4 to 6 DAF (days after flowering.), whereas prolamins accumulation is first detected several days later (Luthe, 1983). These proteins are deposited exclusively into two different protein bodies, which can be distinguished, by morphological characters and size (Tanaka *et al.,* 1980).

2.7.1.4 Prolamins

Osborne (1924) first identified prolamins as proteins extracted in aqueous alcohol, after removal of the water and salt soluble proteins. Later, large amounts of Prolamins were extracted when a reducing agent was added to the aqueous alcoholic solvent. Landry and Moureaux (1970) incorporated the use of reducing agents into their much used fractionation schemes for maize zeins. The Landry-Moureaux method divides Prolamins into two major classes: those extractable in aqueous alcohol alone and those extractable in aqueous alcohol and a reducing agent. Prolamins are classified into four groups termed alpha-zeins, beta-zeins, gamma-zeins and delta-zeins.

The alpha-zeins are the major components in maize accounting for about 70% of the total prolamins. SDS-PAGE shows two broad bands of mass about 19,000 (Z19) and 22,000 (Z22) but isoelectric focusing shows that each of these comprises a number of components (Hagen and Rubenstein, 1981; Wilson, and Larkins, 1984). Gamma-zeins are the second most abundant group of proteins in maize, with two bands of Mr about 27,000 and 16,000. They differ from all other zeins in being soluble in water as reduced subunits. All gamma-prolamins also contain ten conserved cysteine residues and are rich in proline, glutamine and non-polar amino acids (Leite *et al.,* 1991).

The beta-zeins of maize have molecular masses of about 15,000 by SDS-PAGE with true masses of about 17,500. They comprise about 160 amino acids with 18 residues of methionine and 7 residues of cysteine (Pedersen *et al.,* 1986). The delta-zeins of maize comprise two minor components of Mr about 10,000 and 18,000 with true masses of about 14,400 and 21,100 (Coleman and Larkins, 1999). Both are methionine-rich, with the Mr 18,000 component having apparently been derived from the Mr 10,000 protein by duplication of part of the methionine-rich central region.

2.8 Isolation and Purification of Lima Bean Proteins

A wide variety of extraction and fractionation tools for proteins are available based on their physicochemical and structural characteristics such as solubility, hydrophobicity, molecular weight, isoelectric point and so on. Generally, different technologies such as cell disruption, precipitation, and enrichment systems are needed to obtain the protein fraction of interest. Removal of interfering compounds (mainly lipids, nucleic acids, phenolic compounds, carbohydrates, proteolytic and oxidative enzymes, and pigments) is crucial (Uebersax *et al*., 1991).

These procedures need to be optimized to minimize proteins modifications and proteolysis as well as to be compatible with subsequent analysis. The growing body of research on the health benefits associated with consumption of bean proteins has led to a corresponding growth of interests in developing bean protein-based functional food products. High protein ingredients intended for incorporation as food components or for experimental purposes can be produced in several ways. This includes flour fractions, concentrates and isolates processed by alkali, salt and acid extraction with subsequent purification by chromatographic techniques or ultrafiltration (Uebersax *et al.,* 1991).

2.9 Functional Characteristics

There is increasing demand for promising plant sources of functional ingredients to improve food nutritional quality and control costs (Sanchez-Vioque *et al.,* 1999). Functional characteristics of food material refer to intrinsic physicochemical properties that influence the behavior of proteins in food systems during processing and storage. They include hydration properties such as solubility, wettability, swelling, water binding, thickening, and gelling. In addition are surface properties such as emulsion, foaming, protein-lipid interactions, film formation, lipid and flavor binding. Finally, there are structural/rheological properties comprising elasticity, grittiness, cohesiveness, chewiness, aggregation, gelation, stickiness, viscosity, texturization, fiber formation, dough-forming ability, extrudability, and adhesion (Onimawo and Akubor, 2005).

The functionality of proteins is closely associated their physical and chemical properties, such as molecular weight, amino acid composition and sequence, structure, surface electrostatic charge, and effective hydrophobicity. Some food ingredients, including water, salts, proteins, sugars, and fats, as well as processing methods (Damodaran, 1990), also affect it. Kaur and Singh (2005) investigated the functionality of chickpea and revealed that in addition to proteins, the complex carbohydrates of legumes, such as starch, fibers, pectins and mucilages, contribute to their functionality.

2.9.1 Water Absorption Capacity

Water absorption capacity is the amount of water that can be held per unit weight of the protein material (Sikorski, 2002). It is an index of the maximum amount of water that a food product would absorb and retain (Mosha and Lorri, 1987; Marero *et al.,* 1988). Water absorption index is related to the hydrophilicity and gelation capacity of

biomacromolecules such as starch and protein in flour (Kaur and Singh, 2005). As reported by Hoover and Sosulski (1986), water absorption capacity is influenced by the degree of intermolecular arising from hydrogen and covalent bonding between starch polymers. It has been reported that water binding capacity of proteins is a function of several parameters including size, shape, steric factors, conformational characteristics, hydrophilic–hydrophobic balance of amino acids in the protein molecules as well as lipids, carbohydrates and tannins associated with proteins (Chavan *et al.,* 2001).

The water absorption characteristics of flour are represented by its ability to associate with water under conditions where water is limited such as doughs and pastes (Singh, 2001). Water absorption index and water solubility are important functional properties that have been widely studied in food because they can affect product yield, consistency, body, retention of water and hence the applicability (Aziz *et al.,* 2011; Waramboi *et al.,* 2011). Water absorption index generally reflects changes in the molecules such as protein denaturation and starch fragmentation, which results in retention of water.

Water binding capacity's data are useful for assessing the technological suitability of bean protein materials in food applications. High water binding capacity is attributed to lose structure of starch polymers whiles low values indicates the compactness of the structure (Nwokocha and Peter, 2011). In the preparation of baked products, extruded snacks and mash, water-binding capacity is an important parameter to be considered. Water binding capacity is very essential in the development of ready to eat foods and so foods with high water binding capacity will assure product cohesiveness (Houson and Ayenor, 2002). It is a useful indication of whether flour or isolate can be incorporated into aqueous food formulations especially those involving dough handling (Okerie and Bello, 1988; Giami,

1993). Water absorption capacities ranging from 149.1 % to 471.5 % are considered critical in viscous foods such as soups, and gravies (Aletor *et al*., 2002).

2.9.2 Oil Absorption Capacity

Oil absorption capacity is the amount of oil, which can be retained per unit weight of the protein material (Sikorski, 2002). The oil absorbing mechanism involves capillary interaction that allows the absorbed oil to be retained. Hydrophobic proteins play the main role in oil absorption. The Oil absorption capacities of different legume flours are influenced by particle sizes, starch and protein contents, protein types (Sathe *et al.,* 1982), and non-polar amino acid side chain ratios on the protein molecule surface (Chau *et al.*, 1997).

More hydrophobic proteins show superior binding of lipids indicating that non-polar amino acid side chains bind the paraffin chains of fats (Kinsella, 1976). Based on this suggestion, legume flour that shows higher oil absorption capacity likely contains a higher amount of available non-polar side chains in its protein molecules. Oil Absorption Capacity is important because oil acts as flavour retainer and increases the mouth feel of foods (Aremu *et al.*, 2007).

2.9.3 Emulsion Capacity

The emulsion activity reflects the ability of a protein to aid in the formation of an emulsion and is related to the protein's ability to absorb at the interfacial area of oil and water in an emulsion. Emulsion stability normally reflects the ability of a proteins to impart strength to an emulsion for resistance to stress and changes and is therefore related to the consistency of the interfacial area over a defined period (Singh *et al.,* 2010).

Emulsion capacity is governed by the hydrophilicity and hydrophobicity of proteins as they create electrostatic repulsion on oil surface (Moure *et al*., 2006). Unfolding of proteins at oil and water interfaces plays a significant role in formation and stability of emulsions.

Other factors such as adsorption kinetics, interfacial load, decrease of interfacial tension, rheology of the interfacial film and its surface hydrophobicity also affect emulsion properties. This could be attributed to protein denaturation during isolation (Damodaran, 1997). The ability of proteins to aid the formation and stabilization of emulsions is important in many applications including mayonnaise, milks, comminuted meats and salad dressings (Adeyeye *et al*., 1994). Proteins with high oil and water binding are desirable for use in meats, sausages, breads and cakes, while proteins with high emulsifying capacity are good for sausages, bologna, soups and salad dressing. Emulsion stability is important in food emulsions as it indicates the capacity of emulsion droplets to remain dispersed without separation by creaming, coalescing and flocculation (Damodaran, 1997).

2.9.4 Foam Capacity

Foaming is as result of proteins forming an interface that keeps air bubbles in suspension and prevents their collapse. As protein concentration is increased, the formation of interfacial protein membranes at the air–water interface is increased, and this enhances encapsulation of air bubbles. The formation of foams where water molecules surround air droplets represents the non-polar phase and is related to soft texture of food products. Some proteins and peptides in dispersion are capable of reducing the surface tension at the water-air interface that leads to foaming. Normally, foaming properties of proteins are

measured by three indices, namely, foam expansion, foam capacity and foam stability (Boye *et al.,* 2010).

Many factors affect foaming properties of proteins, which include the source of protein, methods and thermal parameters of processing, such as temperature, pH, protein concentration, mixing time and method of foaming (Zayas, 1997). Foam stability requires the specific properties of protein films such as formation of cohesive, viscous, elastic, continuous, air-impermeable film around each gas bubble. Film thickness, mechanical strength, protein-protein interactions and environmental factors such as pH and temperature have very strong influence on foam stability (Sai-Ut *et al.,* 2009). Foam formation of proteins is a very important characteristic that is widely employed in beverages, mousses, meringue cakes and whipped toppings. The proteins and their hydrolysates are good foaming agents as they easily diffuse into the air-water interface forming cohesive, adhesive and elastic films by partial unfolding themselves (Sreerama *et al.,* 2012).

2.9.5 Swelling power

Swelling power is defined as the maximum increase in volume and weight which protein undergoes when allowed to swell freely in water (Balogopalan *et al*., 1988). It is a measure of hydration capacity, because the determination is a weight measure of swollen starch and protein granules and their occluded water. The swelling capacity is a function of the process conditions, nature of the material and the type of treatment. Biopolymers such as starch and proteins contribute to the development of these characteristics (Gujska *et al.,* 1994; Hoover and Manuel, 1995). The extent of swelling in the presence of water depends on the temperature, availability of water, species of starch, and extent of starch

damage due to thermal and mechanical processes and other carbohydrates and protein such as pectins, hemicelluloses and cellulose (Hoover and Manuel, 1995; Gujska *et al.*, 1994).

High swelling power is reported to constitute better thickening as well as a bulking agent. Swelling is reported to be related to the water absorption index of the starch-based flour during heating (Iwuoha and Nwakanma, 1998). Food eating quality is often connected with retention of water in the swollen protein and starch granules. For incorporation into foods, new protein additives should possess certain water binding and swelling capacity, which vary for different protein materials. Swelling is an important protein functional property because most foods are water-swollen systems. There is a possible relationship between cooking quality and swelling volumes. The higher the swelling volume the better the cooking quality (Moorthy and Ramanujan, 2001).

2.9.6 Bulk Density

Bulk density is defined as the weight of the sample per unit volume of the sample (g/mL). It is essentially a measure of the degree of coarseness of the sample. The consistency of energy density of the food and the frequency of feeding are also important in determining the extent to which an individual will meet his or her energy and nutrient requirements (Omueti *et al.,* 2009). The bulk density is influenced by particle size and the density of the flour and is important in determining the packaging requirement and material handling (Karuna *et al.,* 1996). Plaami (1997) reported that bulk density is influenced by the structure of the starch polymers and loose structure of the starch polymers could result in low bulk density. It also depends on interrelated factors including intensity of attractive inter particle forces, particle size and number of contact points.

Increase in bulk density is desirable in that it offers greater packaging advantage, as a greater quantity may be packed within a constant volume (Fagbemi, 1999). The bulk density of legume flour plays an important role in weanling food formulation, that is, reducing the bulk density of the flour is probably helpful to the formulation of weanling foods (Milán-Carrillo *et al.,* 2000). It gives an indication of the relative volume of packaging material required. Generally, higher bulk density is desirable for the greater ease of dispersibility and reduction of paste thickness, which is an important factor in convalescent child feeding (Padmashree *et al*., 1987).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Lima Bean Accessions

Ten lima bean landrace populations collected from eight locations at four regions in Ghana and kept by the Crops Research Institute (CRI) of the Center for Scientific and Industrial Research, Fumesua, were evaluated in current study. Hitherto, the accessions had been named by description of their collection site and colour as CR01, CR02, CR03, CR04, CR05, CR06, CR07, CR08, CR09, and CR10. Each population was regenerated in the previous year by planting on two rows with 12 plants per row on the research fields of Crops Research Institute.

Table 3.1 Origin of lima bean seeds grown in eight locations in four regions of Ghana.

ID	**Origin**	**Region**	**Gene pool**	**Collection site**		
				Altitude (m.a.s.l)	**Longitude**	**Latitude**
CR01	Suhum black & white	Eastern	Andean	186	-0.45	6.03
CR02	Kumawu Bodomase white	Ashanti	Andean	440	-1.28	6.90
CR03	Bososo cream	Eastern	Andean	460	-0.38	6.39
CR04	Nkurakan black	Eastern	Andean	172	-0.26	6.09
CR05	Nsawam cream & black	Eastern	Andean	61	-0.35	5.81
CR06	Kolenu mottled brown	Volta	Andean	173	0.47	7.15
CR07	Kolenu red	Volta	Andean	173	0.47	7.15
CR08	Kolenu small black & white	Volta	Meso-American	173	0.47	7.15
CR09	Techiman cream & brown	Brong Ahafo	Andean	398	-1.94	7.59
CR10	Mampong white	Ashanti	Andean	395	-1.40	7.06

After harvest, the accessions were given designations to reflect the collector and rank of collection. Table 3.1 lists the origin, gene pool, and collection sites of the accessions. At maturity, all pods harvested per population were shelled, bulked and partitioned into triplicates. Two kilograms of each replicate were uniformly sun-dried for 48 h for characterization and flour preparation.

3.2 Characterization of Lima Bean Accessions

For each genotype and replicate, seeds were picked at random and evaluated for hundred seed weight, seed size, and color. One hundred seed weight is a measure of the compactness of kernel with respect to starch, protein and other macro components (Osborne and Mendel, 1914). Following cleaning of beans to remove dust, dirt, debris and broken seeds, the mass of one hundred seeds of each accession was determined on an electronic balance (OHAUS AS260D, New Jersey, USA) was measured. Fifteen seeds were similarly evaluated for their size characteristics including length, width and thickness by means of a Vernier calliper.

3.3 Flour Preparation

Sun-dried seeds were milled into flour using a Laboratory Scale Hammer Mill (Schutte-Buffalo Hammer mill, Buffalo NY, U.S.A.) and sieved through a 0.2 mm screen. Flour samples were kept in plastic bottles and stored in a cool and dry environment until required for chemical analyses. The chemical evaluations encompassed proximate analyses, storage protein content, mineral composition and functional properties.

3.4 Proximate Analysis

Moisture, ash, fiber, oil, protein, and carbohydrate content of flour were determined in triplicate and mean values reported.

3.4.1 Moisture Content Determination

Moisture content was determined by the method of A.O.A.C. No. 945.38 (A.O.A.C., 2005). Two grams of flour were weighed into a dried and weighed crucible and dried in a hot air oven (MIDO/3/SS/F Model D3S, Genlab Widens, England) at 105°C for 6 h. The sample was removed, cooled to room temperature in a desiccator and weighed. The sample was returned to the oven, dried, and reweighed until constant mass was obtained. The difference in mass between the wet and dried sample was calculated and expressed as a percentage of the mass of the original sample (Appendix 1).

3.4.2 Ash Content Determination

Ash content was determined by the method of A.O.A.C. No. 936.07 (A.O.A.C., 2005). Two grams of flour were transferred into a dried and weighed pre-ignited and pre-weighed porcelain crucible and combusted in a muffle furnace (Gallenkamp, Model OV160, Leicestershire, United Kingdom) at 600 °C for 2 h at which time the sample had turned white and was free of carbon. The crucibles containing ash were cooled to room temperature in a desiccator and re-weighed. Loss in mass was calculated as percentage ash content (Appendix 2).

3.4.3 Determination of Crude Fat Content

Crude fat content of lima bean flour was determined following the method of A.O.A.C. No. 2003.05 (A.O.A.C., 2005). Two grams of sample were transferred into a 22 × 80 mm

paper thimble and capped with glass wool, dropped into a thimble holder and attached to a pre-weighed 500 ml round bottom flask containing 200 ml petroleum ether (60 ^{o}C) and assembled on a semi-continuous soxhlet extractor. Contents of the thimble were refluxed for 16 h after which the petroleum ether was recovered on a steam water bath. The flask and its contents were heated for 30 min in an oven at 103 ^{o}C to remove residual petroleum ether, cooled in a desiccator and weighed. Increase in mass of flask was recorded as crude fat from which percentage crude fat was calculated (Appendix 3).

3.4.4 Determination of Crude Protein

Protein content was measured following the Kjeldahl nitrogen determination of A.O.A.C. No. 2001.11 (A.O.A.C., 2005). Two grams of the defatted sample in a Kjeldahl flask was added 25 ml of 98% H_2SO_4 with catalyst (3.5 g potassium sulphate :0.105 g copper sulphate: 0.105 g titanium oxide) and digested till the colour of the solution turned clear. The solution was transferred into a volumetric flask and the volume made up to the 100-ml mark with distilled water. Ten milliliters of the solution were distilled and titrated against 0.1 M hydrochloric acid against a blank and titre values recorded. Percentage nitrogen was calculated (Appendix 4) and converted to percent crude protein by multiplying by a factor of 6.25.

3.4.5 Crude Fibre Determination

Crude fibre was determined following the method of A.O.A.C. 920.86 (A.O.A.C. 2005). Two grams of defatted flour was transferred into 750 ml Erlenmeyer flask, 200 ml of boiling 1.25 % H_2SO_4 was added and refluxed for 45 min. The mixture was screened through cheesecloth and residue washed with large volume of boiling water until filtrate was no longer acidic. The reflux was repeated with 1.25 % sodium hydroxide, screened

and washed to remove all alkali as before. The residue was transferred to a previously weighed porcelain crucible (M1), dried for 1 h at 100°C in a hot air oven (MIDO/3/SS/F Model D3S, Genlab Widens, England), cooled in a desiccator, and re-weighed (M2). The crucible was ignited in the muffle furnace (Gallenkamp, Model OV160, Leicestershire, United Kingdom) at 600 ^{o}C for 30 min and re-weighed after cooling in a desiccator (M3). Difference in mass of the crucible following ignition was recorded as crude fibre and expressed as percentage of the original mass of flour (Appendix 5).

3.4.6 Carbohydrate Content Determination

Carbohydrate content was calculated by the difference between 100 and the sum of all other proximate parameters (moisture + ash + protein + fibre + fat).

3.4.7 Energy Content

Gross energy in joules per 100 g dry matter was calculated based on the formula:

Gross energy = (Crude protein ×16.7) + (Crude lipid×37.7) + (Carbohydrate ×16.7) (Ekanayake *et al.*, 1999).

3.5 Determination of minerals

Mineral content was determined using the Official Methods of analysis by A.O.A.C.968.08 (A.O.A.C.2005). One (1) gram of flour samples were ashed at 550^{o}C followed by boiling with 10 ml of 20 % hydrochloric acid in a beaker and then filtered and diluted to the 100-ml mark with deionized water into a standard flask. The minerals were determined from the resulting solution. Potassium was determined by means of the standard flame emission photometer using potassium chloride as the standard (A.O.A.C., 2005). Phosphorus was determined colorimetrically using the Spectronic 20 (Gallenkamp,

UK) with potassium dihydrogen phosphate as standard (Kirk and Sawyer, 1991**)**. Calcium and iron were determined using Atomic Absorption Spectrophotometer (AAS Model 210VGP, East Norwalk, USA). All values were expressed in mg/100g.

3.6 Determination of Functional Properties of Lima Bean Flour

3.6.1 Water Absorption Capacity

Water absorption capacity of lima bean flour was determined according to the method of Sathe *et al*. (1982) with slight modifications. Two grams of the flour samples were mixed with 20 ml distilled water in a blender at high speed for 30 sec. Samples were allowed to stand at room temperature (30±2°C) for 30 min then centrifuged at 10,000 r. p. m. for 30 min. The volume of supernatant in a graduated cylinder was recorded. Water absorption capacity was expressed in gram of bound water per gram of sample on a dry matter basis. Means of triplicate determinations were reported.

3.6.2 Oil Absorption Capacity

Oil absorption capacity of the flour was determined according to the method of Sathe *et al.* (1982) with slight modifications. Two grams of flour samples were mixed with 20 ml refined vegetable oil in a blender at high speed for 30 sec. Samples were then allowed to stand at room temperature (30±2°C) for 30 min then centrifuged at 10,000 r. p. m. for 30 min. The volume of supernatant in a graduated cylinder was recorded. Density of oil was taken to be 0.93 g/ml. Oil absorption capacity was expressed in gram of oil bound per gram of sample on a dry matter basis. Means of triplicate determinations were reported.

3.6.3 Foaming Capacity

Foaming capacity was determined using the method described by Sathe *et al*. (1982) with slight modifications. One gram of flour sample was added to 40 ml distilled water and blended for 5 min at 100 r. p. m. or top speed in a blender. The contents of the blender were transferred into a measuring cylinder and allowed to stand for 1 min. The volume of the layer of foam in the cylinder was recorded. Triplicate measurements were taken for each sample and mean values recorded.

3.6.4 Emulsion Capacity

Emulsifying properties were determined according to the method reported by Kaur and Singh (2005). Flour samples (3.5 g) were mixed with 50 ml distilled water and homogenized at 100 r. p. m. using a blender at high speed for 30 s. Peanut oil (25 ml) was added and the mixture was homogenized again for 30 s. this step was repeated with 25 ml. and the mixture was homogenized for 90 s. The emulsion was divided evenly into two 50-ml graduated centrifuge tubes and centrifuged at 1,100 × g for 5 min. The heights of emulsion and water layers were measured separately. Emulsion capacity was calculated as:

$$\text{Emulsion capacity} = \frac{\text{Volume of emulsion height}}{\text{Volume of water height}} \times 100$$

3.6.5 Swelling Index

The method of Abbey and Ibeh (1998) was employed. To one gram of flour was transferred into a 10 ml graduated measuring cylinder and tapped several times to remove air spaces. To the flour was carefully added 5 ml distilled water without agitation and the volume occupied by the sample was recorded. The sample was then allowed to stand

undisturbed for 30 min and change in volume after swelling was recorded. Swelling index was calculated as:

$$\text{Swelling index} = \frac{\text{Volume occupied by sample after swelling}}{\text{Volume occupied by sample before swelling}}$$

3.6.6 Bulk Density

Bulk density was determined according to the method described by Okaka and Potter (1977) with slight modifications. Ten gram flour sample was transferred into a 50 ml graduated cylinder and tapped 10-20 times to remove free air spaces. The volume occupied by the flour was recorded. Bulk density was calculated as mass per unit volume of sample. Measurements were made in triplicate.

3.7 Sequential Isolation of Lima Bean Storage Proteins

The storage proteins of lima bean were isolated and quantified by the sequential isolation methods of Landry *et al.* (1999) and Gorinstein *et al*. (1991). It involved extraction of albumin, globulin, prolamin and glutelins. Forty grams of each flour sample were defatted in a Soxhlet extractor using petroleum ether for 2 h. The defatted samples were stored at 4^{o}C until required for use.

3.7.1 Extraction of water-soluble nitrogen-containing substances

Total water-soluble nitrogen-containing substances were first extracted by stirring 20 g of defatted flour with 200 ml distilled water at 4^{o}C for 90 min. The mixture was centrifuged at 10,000-×g for 30 min at room temperature in a Wagtech-C2 series, U.K centrifuge. After each centrifugation, the flour residue was kept. This operation was repeated twice with 150 ml distilled water and all the supernatants were bulked (200 ml + 150 ml + 150 ml = 500 ml) as water-soluble nitrogen-containing substances composed of both protein

nitrogen and non-protein nitrogen substances. The nitrogen content of the extract was determined by the Kjeldahl method as described previously.

3.7.2 Extraction and quantification of albumins

The non-protein nitrogen-containing substances were separated from the total nitrogen-containing substances by precipitation of the water extract with trichloroacetic acid (TCA) to a final concentration of 10 % (w/v) (Landry *et al*., 1999). In this method, 5 g of TCA was added to 50 ml of the water extract and mixed thoroughly by stirring for 5 min. The mixture was kept overnight at 4^{o}C and centrifuged at 10,000 × g at 4^{o}C. The supernatant was harvested and analyzed for total nitrogen by Kjeldahl method. The nitrogen measured in the supernatant was considered as non-albumin nitrogen-containing substances and the difference from the total nitrogen content of the water extract was considered as albumin.

3.7.3 Extraction and quantification of globulins

To the bulk meal residue from the water-soluble extract was added 180 ml of 0.5 M NaCl. The mixture was stirred for 90 min at 4^{o}C and centrifuged at 10,000 × g at 4^{o}C for 30 min. The residue was extracted two more times with 160 ml of the NaCl solution and the supernatants bulked. The supernatant was analyzed for total nitrogen by Kjeldahl method and the meal residue kept for extraction of prolamins. Globulin was separated from non-globulin nitrogen-containing substances by precipitation with trichloroacetic acid (TCA) to a final concentration of 10 % (w/v) (Landry *et al*., 1999). In this method, 5 g of TCA was added to 50 ml of the salt-soluble extract and mixed thoroughly by stirring for 5 min. The mixture was kept overnight at 4^{o}C and centrifuged at 10,000 × g at 4^{o}C. The supernatant was harvested and analyzed for total nitrogen by Kjeldahl method. The

nitrogen content measured in the supernatant was considered as non-globulin nitrogen-containing substances and the difference from the total nitrogen content of the salt-soluble extract was considered as globulin content.

3.7.4 Extractability of alcohol-soluble proteins (prolamins)

Prolamins were isolated from 1.0 g lima bean meal residue from globulin extraction was added 10 ml 70 % ethanol containing 1% sodium acetate (solid; solvent ratio of 1:10 v/w) with constant stirring at 25^{o}C for 1 h at room temperature. In this method, 1 g of TCA was added to 10 ml of the water extract and mixed thoroughly by stirring for 5 min. The mixture was centrifuged at 10,000 × g for 30 min at 4^{o}C. The supernatant was transferred into a beaker and the residue extracted two more times with the same solvent. Total nitrogen content of the bulked supernatant was determined by Kjeldahl method. The bulked supernatant was treated with TCA up to 10% for 30 min at 4^{o}C and centrifuged as before. Total nitrogen content of the supernatant obtained after TCA treatment was determined. Prolamin content was expressed as percentage of the total nitrogen in each sample (Gorinstein *et al*., 1991).

3.7.5 Extraction of glutelin

To the residue was added 10 ml of borate buffer at pH 10 containing 0.05 % 2-ME (0.5% NaOAc, 0.05 % 2-ME) and extracted for 3 times, 90 min each at room temperature. The mixture was centrifuged at 10,000 × g for 30 min. The total nitrogen content of the bulked supernatant was determined.

3.8 Statistical Analysis

3.8.1 Descriptive statistics of traits and accessions of lima bean

Means, standard deviation, range, standard error of the mean, and coefficient of variation were calculated for the triplicate measurements. Analysis of variance was computed and differences in means were located by the Duncan's Multiple Range test (Steel *et al.*, 2004). Pearson correlation coefficients among the traits were computed. Data analyses were carried out using PROC MEANS and PROC GLM of SAS 9.3 (SAS Institute, Cary, NC, U.S.A.).

3.8.2 Assessment of relationships between lima bean accessions

3.8.2.1. Distance measurements

The relationships among traits and accessions were evaluated by means of distance measurements and cluster analysis. A 10×18 data matrix of seed size, proximate composition, storage proteins and functional properties was created. The data was standardized to remove bias arising from differences in units of measurements from the means. The YBAR and STD options in NTSYS-pc. The Euclidean distance coefficients, which represent the level of dissimilarity among all pairs of genotypes, were computed using formula 1.

$$d = \sqrt{\sum_{i=1}^{n} (X_i - Y_i)^2} \quad \text{.....1}$$

where d is the distance measure, X_i is the $_i$ th allele frequency from population 1 and Y_i is the ith allele frequency from population 2. A distance matrix of dissimilarity coefficients was generated.

3.8.2.2 Cluster analysis

This was performed on the Euclidean distance matrix to identify groups with similar morpho-agronomic and phenotypic characters using the descriptors. This method identifies groups that are homogeneous as possible and heterogeneous among groups (Franco *et al.,* 2001) or associations between the descriptors. The hierarchical method of grouping used was the Unweighted Pair Group Method with Arithmetic Average (UPGMA) of Sequential Agglomerative Hierarchical Nesting (SAHN) based on Euclidean distance coefficients (matrix). Dendrograms were generated from the cluster analysis. Computations were performed using NTSYSpc 2.21c package (Rohlf, 2009).

3.8.2.3. Principal components analysis

Principal component analysis (PCA) was performed on the accession by trait correlation (that is, the standardized variance-covariance) matrix in order to depict non-hierarchical relationships among the genotypes and determine the traits that are most effective in discriminating between accessions. Analysis to reveal the proportion of variance, which was contributed by each principal component, and the traits that were most important for that component were determined by calculation of eigen values and eigenvectors from the correlation matrix. Relationships between traits and accessions were further investigated by generation of 2-dimensional principal components plot. All calculations and plots were carried out using NTSYS-pc 2.1 software (Rohlf, 2009).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Seed characteristics

Ten lima bean accessions originating from eight locations in four regions of Ghana were evaluated in current study. Fifteen seeds of each genotype were examined for seed size, colour, and hundred seed weight. Table 4.1 shows the physical characteristics of the ten lima bean seeds. Seeds showed wide variations in size, shape and color. With regard to seed color, some seeds showed primary and secondary colours with cream being dominant. Seed size was measured in length, width and thickness. Seed length ranged from 10.2 mm for CR08, of the Meso-American gene pool to 23.0 mm for CR04, an Andean type. The overall mean seed length was 20.2 mm.

Seed width ranged from 7.7 to 15.5 mm with a mean value of 12.9 mm. The highest and lowest widths were also found in CR04 and CR08. Values of seed thickness ranged from a minimum of 5.7 mm for CR08 to a maximum of 7.6 mm for CR09 with a mean of 6.6 mm. The 100-seed weight ranged from 35.3 g for CR08 to 138.1 g for CR01 with a mean of 114.5 g (Table 4.1). Lioi (1994) and Esquivel *et al.* (1990) identified five morphotypes of lima bean on the basis of hundred-seed weight, seed length, and seed width.

These types include Big lima (100–110 g, 25 mm long, 14 mm wide), Sieva (30 - 45.3 g, 12 mm long, 8 wide), Potato (35.5 g, 9 mm long, 8 mm wide), Potato-Sieva (36.3 g, 11 mm long, 8 mm wide) and Sieva-Big (77.5 g, 17 mm long, 11 mm wide).

Table 4.1 Physical characteristics of the ten lima bean accessions grown in Ghana.

ID	**Mean seed size (mm)**			**100- seed weight (g)**
	Length (mm)	Width (mm)	Thickness (mm)	
CR01	20.7±0.32^{cd}	13.5±0.39^{bc}	7.1±0.45^{ab}	138.1±0.35^{a}
CR02	20.8±0.61^{bcd}	13.0±0.35^{cd}	6.6±0.12^{bc}	128.0±1.05^{c}
CR03	21.1±1.00^{bcd}	13.1±0.86^{cd}	6.5±0.27^{c}	125.4±0.78^{d}
CR04	22.8±0.25^{a}	15.2±0.34^{a}	6.6±0.08^{bc}	119.2±1.21^{f}
CR05	21.6±0.54^{bc}	13.9±0.65^{b}	6.3±0.04^{c}	122.3±0.58^{e}
CR06	21.6±0.77^{bc}	13.4±0.58^{bc}	6.4±0.06^{c}	130.6±0.97^{b}
CR07	21.8±0.62^{ab}	13.1±0.22^{cd}	6.6±0.35^{c}	117.1±0.76^{g}
CR08	10.3±0.25^{e}	7.9±0.26^{e}	6.1±0.51^{c}	35.3±1.15^{i}
CR09	20.7±0.96^{cd}	12.5±0.27^{d}	7.2±0.33^{a}	118.5±1.19^{gf}
CR10	20.2±0.32^{d}	13.4±0.19^{bc}	6.4±0.44^{c}	109.9±1.29^{h}
Min	10.2	7.7	5.7	34.2
Max	23.0	15.5	7.6	138.5
Mean	20.2	12.9	6.6	114.5
SD	3.4	1.8	0.4	27.91
CV (%)	17.1	14.3	6.5	24.39

Based on these categories nine accessions, CR01 to CR07, CR09 and CR10 belonged to the South American large seeded type (Harlan, 1971; Heiser, 1965; Kaplan, 1965; Vavilov, 1949) classified as big lima while one accession, CR08 could be considered as the Meso-American Sieva or Potato morphotype. The use of seed morphotype for classification must be used with caution because environmental effects can influence seed weight, length and width.

Fig. 4.1 shows images of lima beans used in current study, depicting wide variations in shapes, sizes and colors.

Fig. 4.1 Lima bean accessions showing range of sizes, shapes and colors.

From top left to right shape, color: CR01- flat, cream with black stripes; CR02 - flat, white; CR03 - flat, cream; CR04 – flat, black; CR05– flat, cream with black stripes; CR06– flat cream with reddish brown stripes; CR07– tan, flat with white stripes; CR08– small, grey with black spots rounded; CR09– flat, cream with reddish brown stripes; CR10 – flat, white.

4.2 Phenotypic Variability

Pattern of variation among the accessions was different for the various proximate components. The highest coefficient of variation was 26.46% for fat content while the lowest value of 0.73 % was recorded for carbohydrate content whereas the largest variation occurred in fibre, ash and fat contents. The coefficients of variation for these three variables were 8.42 %, 10.92 %, and 26.46 %, respectively (Table 4.2). The large variability in fat content signifies the possibility to improve on the fat content of lima via breeding. A low coefficient of variation of less than 2 % was observed for protein and carbohydrate, which suggests that little progress, can be made through selection. The variation in chemical composition among the different kinds of accessions may have arisen from differences in their genetics, origin and environment of cultivation (Kaur *et al*., 2007).

4.3 Proximate Composition

Proximate composition of the ten lima bean accessions was determined. Accessions showed a wide variation in fiber, protein, and carbohydrate content while ash and fat contents were similar among the accessions (Table 4.3). Generally, all the accessions recorded lower moisture values of 9.81–13.90% compared to 14.09% reported by Kizito (2010) for improved lima bean cultivars, but corroborated with the 4% to 13% moisture content reported by Rahma *et al.* (2000). Low moisture content of food enhances the storage life of food commodities (Alozie *et al*., 2009; Temple *et al*., 1996).

Fiber content varied from 3.37 % for CR08 to 9.11 % for CR04 with a mean of 4.81 % (Table 4.3). Crude fibre content of wild and cultivated lima beans cultivated in Pakistan was found to be 5.48% and 4.54%, respectively (Kathirvel and Kumudha, 2011). Kizito (2010) and Moses *et al*. (2012) reported crude fiber content of some Nigerian lima accessions to be 2.61 % and 5.23 %, respectively. Those of lima bean originating from various geographical locations demonstrate similarity to the Ghana accessions.

In general, the crude fiber content of lima exhibited similarity to that of soybean (4.21 %). The significance of fibre in human nutrition is its role in lowering blood cholesterol level and is beneficial in reducing the risk of diabetes, hypertension, cancer (Singh *et al*., 2002: Koulshon *et al*., 2005; Han *et al*., 2002; Brown *et al*., 1999; Gadner, 2005; Anderson, 2005: Messina, 1999; Oboh and Omofoma, 2008). All accessions exhibited low crude fat content ranging from 0.94 % to 1.66 % lower than values of jack bean (3.4–4.7%) (Vadivel and Janardhanan, 2001), chickpea (3.1–5.5%) (Kaur *et al.,* 2005) and soybeans (18.56–21.66%) (Redondo-Cuenca *et al*., 2006). The ash content of lima was within the range of 4.04 % for

CR07 to 5.98 % in CR09 (Table 4.3) higher than the values of 2.91 to 3.42 % reported by Kumudha and Kathirvel (2011) for both wild and cultivated lima.

Table 4.2 Means, standard deviations, range, coefficient of variation and mean squares of proximate composition of ten lima bean accessions

Variable (%)	**Mean**	**SD[1]**	**SE[2]**	**Min.**	**Max.**	**CV[3] (%)**	**Mean Square**
Moisture	12.13	1.40	0.25	9.41	14.68	5.52	5.28***
Fat	1.24	0.37	0.07	0.56	1.72	26.46	0.20
Ash	4.87	0.69	0.13	3.29	6.70	10.92	0.92
Fiber	4.81	1.78	0.33	2.93	9.63	8.42	9.87***
Protein	22.49	1.08	0.20	20.50	24.56	1.87	3.40***
Carbohydrate	53.88	3.12	0.57	47.02	58.12	0.73	31.03***
Calorific Val (kJ/100g)	1,322.07	53.67	9.80	1,205.76	1,407.27	1.22	8,702.75***

[1]Standard deviation; [2]Standard error; [3]Coefficient of variation; ***P<0.0001.

The carbohydrate content of lima accessions in current study was characterized by wide variation (P<0.001) ranging from 47.6 to 58.0 % with a standard deviation of 3.12 and a coefficient of variability of 0.73 %. Accessions CR07 and CR04 had the lowest and highest values, respectively (Table 4.3). Moses *et al*. (2012) reported relatively lower variability in carbohydrate content of lima bean accessions grown in Nigeria. A narrower range of carbohydrate content of 56.4-57.1% was obtained for some varieties. Mean carbohydrate content of the lima accessions was greater than that reported for soybeans (46.80%) by Kizito (2010).

Lima beans contained high crude protein content of 20.7 % to 24.0 % similar to that of cowpea (*Vigna unguiculata*) (24.7%) (Appiah *et al*., 2011) and green pea (24.9%) but lower than soybean protein content (37.1%) (Iqbal *et al*., 2006; Ensminger *et al*., 1990).

Table 4.3 Proximate composition (% dry matter) of ten accessions of Lima bean flour [1, 2].

Accession	Moisture	Fat	Ash	Fibre	Protein	Carbohydrate	Calorific value
CR01	13.9±0.7^{a}	1.3±0.3^{ab}	5.3±0.7^{ab}	4.2±0.0^{c}	24.0±0.8^{a}	50.6±0.4^{f}	1,296.5±18.8^{de}
CR02	12.6±0.9^{bcd}	0.9±0.3^{b}	4.9±0.6^{bcd}	4.4±0.4^{c}	23.9±0.2^{a}	52.5±0.1^{e}	1311.9±14.8^{cd}
CR03	12.5±0.6^{bcd}	1.3±0.4^{ab}	4.5±0.0^{bcd}	3.8±0.4^{cd}	22.9±0.3^{b}	54.8±0.2^{c}	1346.6±19.9^{b}
CR04	13.7±0.8^{ab}	1.0±0.3^{b}	4.9±0.6^{bcd}	9.1±0.7^{a}	22.9±0.3^{b}	47.6±0.9^{g}	1212.7±6.0^{f}
CR05	12.1±0.5^{cd}	0.9±0.3^{b}	5.2±0.6^{abc}	7.1±0.2^{b}	21.2±0.5^{ef}	52.8±0.3^{e}	1271.6±12.4^{e}
CR06	11.7±0.6^{d}	1.1±0.6^{ab}	4.5±0.1^{bcd}	3.9±0.3^{cd}	20.7±0.3^{f}	57.4±0.1^{ab}	1346.7±19.7^{b}
CR07	9.8±0.5^{e}	1.7±0.0^{a}	4.0±0.7^{d}	4.2±0.3^{c}	21.9±0.4^{de}	58.0±0.1^{a}	1396.8±9.4^{a}
CR08	13.2±0.6^{abc}	1.5±0.3^{a}	4.9±0.6^{bcd}	3.4±0.7^{d}	22.4±0.5^{bcd}	53.8±0.2^{d}	1329.4±22.5^{bc}
CR09	11.7±0.5^{d}	1.1±0.0^{ab}	5.9±0.6^{a}	4.1±0.1^{c}	22.2±0.4^{cd}	54.6±0.6^{c}	1323.5±17.5^{bcd}
CR10	10.2±0.8^{e}	1.5±0.3^{ab}	4.4±0.0^{cd}	4.1±0.1^{cd}	22.8±0.2^{bc}	56.8±0.2^{b}	1385.2±12.8^{a}

[1, 2] Mean values in the same column having different superscripts are significantly different ($P<0.05$).

CR01 had the highest protein content (24.0%). The large variability in protein content of lima (Table 4.2) indicates the possibility of raising the current protein content to a higher level through breeding protocols. Lima bean with higher protein content can serve as inexpensive protein source for both humans and livestock. Energy content of lima varied from 1,212.7 J/100g for CR04 to 1,396.8 J/100g for CR07 with a mean of 1,322.1 J/100g (Table 4.3). According to Health Canada Food Guide, on average about 8 kJ per day is required by adult male and female engaged in sedentary and active lifestyles. High-energy content of the samples is attributable to the high levels of carbohydrate and crude protein. In most food crops, genetic variability in protein content is considered an important factor for improvement of protein quality by selection and breeding (Singh and Eggum, 1984).

4.4 Variability in Mineral Content

The lima accessions demonstrated ample variation in mineral content. The largest variation was observed for calcium and phosphorus contents with values of coefficients of variation being 16.02% and 10.61%, respectively compared to values of 0.50 for potassium and 5.19 for iron (Table 4.4). Potassium content varied from a minimum value of 833.33 mg/100g for CR08 to a maximum value of 936.67 mg/100g for CR03 (Table 4.5). Calcium content ranged from 143.33 to 396.67 mg/100g; iron content from 2.27 to 6.87 mg/100g, and phosphorus composition ranged from 85 to 460 mg/100g (Table 4.5). The overall mean for the ten accessions were 893.00, 212.00 and 207.50 mg/100g for potassium, calcium, and phosphorus, respectively. The high coefficient of variability indicates possibility of improvement in mineral content of lima via selection (Price *et al*. 2002; Asch, *et al*. 2005; Nassir and Adewusi 2012).

Table 4.4 Means, standard deviations, range, coefficient of variation and mean squares of mineral composition of ten lima bean accessions.

Variable	**Mean**	**SD[1]**	**SE[2]**	**Min.**	**Max.**	**CV[3]**	**Mean Square**
Potassium	893.00	29.26	5.34	830.00	940.00	0.50	27,14.44***
Calcium	212.00	77.48	14.15	130.00	450.00	16.02	16,779.26***
Iron	4.99	1.42	0.26	2.00	7.20	5.19	6.31***
Phosphorus	207.50	121.17	27.10	70.00	480.00	10.61	30,458.33***

[1]Standard deviation; [2]Standard error; [3]Coefficient of variation; ***P<0.001.

Among the ten lima accessions, CR03 demonstrated the highest contents of potassium (936.67 mg/100g), iron (6.87 mg/100g) and phosphorus (460.00 mg/100g) (Table 4.5). CR01 had the highest calcium content of 396.67 mg/100g. Potassium and phosphorus

Table 4.5 Mineral content of lima bean flour[1] (mg/100g)

Accession	Potassium	Calcium	Iron	Phosphorus
CR01	883.33±5.7^{e}	396.67±75.7^{a}	3.90±0.2^{f}	115±21.2^{d}
CR02	913.33±5.7^{bc}	143.33±11.6^{e}	3.60±0.4^{f}	115±21.2^{d}
CR03	936.67±5.7^{a}	176.67±55.1^{cde}	6.87±0.4^{a}	460±28.3^{a}
CR04	886.67±5.7^{e}	200.00±36.1^{cde}	6.40±0.2^{b}	115±21.2^{d}
CR05	890.00±0.0^{de}	233.33±20.8^{bc}	5.17±0.3^{d}	385±21.2^{b}
CR06	896.67±5.7^{d}	260.00±17.3^{b}	5.90±0.2^{c}	215±21.2^{c}
CR07	860.00±0.0^{f}	153.33±5.7^{e}	6.33±0.2^{bc}	85±21.2^{d}
CR08	833.33±5.7^{g}	220.00±17.3^{bcd}	4.53±0.2^{e}	185±21.2^{c}
CR09	910.00±0.0^{c}	173.33±5.7^{de}	2.27±0.3^{g}	185±21.2^{c}
CR10	920.00±0.0^{b}	163.33±15.3^{de}	4.93±0.2^{de}	215±21.2^{c}

[1]Values in the same column having different superscripts are significantly different (P<0.05).

were the most abundant minerals in all the accessions as was also reported by Kathirvel and Kumudha (2011) for wild and cultivated Indian types. In their report, the mineral contents were 247.91 and 177.81 mg/100g for phosphorus, 2.09 and 2.25 mg/100g for iron, 1,892.05 and 1698.68 mg/100g for potassium, and finally, 352.56 and 720.88 mg/100g for calcium in wild and cultivated lima, respectively. This observation indicates that the legume flours would serve as good sources of minerals such as calcium and phosphorous, which are considered essential for bone and teeth development in children.

4.5 Storage Proteins of Lima Bean

The study revealed a large variability in storage protein content among the accessions. Glutelins were the most variable (CV of 23.25 % and a significant mean square) while globulins were the least variable (CV of 13.96 %) (Table 4.6). Typically, the major storage

proteins in legume seeds are the globulins, which usually account for about 70 % of the total protein while glutelins (10–20%) and albumins (10–20%) make up the remainder.

Table 4.6 Descriptive analysis of protein profile of Lima bean accessions (%).

Variable	**Mean**	**SD[1]**	**SE[2]**	**Min**	**Max**	**CV[3]**	**Mean Square**
Albumins	37.40	17.52	3.20	5.49	66.46	18.67	880.58***
Glutelins	14.07	8.73	1.59	2.59	37.50	23.25	221.87***
Prolamins	11.68	5.44	0.99	2.53	20.62	23.14	79.30***
Globulins	36.86	12.93	2.36	17.91	70.33	13.96	479.74***

[1]Standard deviation; [2]Standard error; [3]Coefficient of variation; ***P<0.001.

Prolamins are usually in limiting concentration in legumes (Jansman, 1996). Globulins had the highest content of 19.27- 61.88%, followed by albumins (10.53 - 57.67%), glutelins (6.68-35.77 %) with the least value of 3.13-16.94% for prolamins (Table 4.7). Prolamins are rather predominant in cereal crops. The results observed for globulins, albumins, glutelins and prolamins in all the accessions were similar to those reported by Kathirvel and Kumudha (2011) being 54.63 % and 55.16 % for globulins, 28.55 % and 28.87 % for albumins, 11.96 %and 9.34 % for glutelins, and 4.86 % and 6.63% for prolamins, in wild and cultivated lima, respectively. CR03 demonstrated the highest globulin (61.88%) and prolamin contents (16.94%) (Table 4.7).

CR07 was the highest in terms of albumins content (57.67%). Finally, CR10 had the most glutelin content of 35.77%. Typically, globulins represent between 50% and 75% of the total protein found in the seeds of common beans (*Phaseolus vulgaris*) (Muller and Gottschalk, 1983; Ali *et al*., 2012).

Table 4.7 Storage protein Fractions of lima bean seed flour (%)

	Seed Protein (100%)			
Accession	**Albumins**	**Glutelins**	**Prolamins**	**Globulins**
CR01	37.99±7.0^{c}	6.68±2.2^{c}	14.29±0.1^{ab}	41.04±4.1^{c}
CR02	55.94±1.3^{ab}	17.46±2.6^{bc}	7.34±2.7^{cd}	19.27±1.4^{e}
CR03	10.53±4.1^{e}	10.65±0.3^{de}	16.94±3.7^{a}	61.88±8.4^{a}
CR04	23.74±2.3^{d}	12.85±2.1^{cd}	13.42±4.7^{ab}	49.99±9.0^{b}
CR05	39.41±12.7^{c}	8.15±2.9^{de}	14.17±3.6^{ab}	38.28±6.2^{cd}
CR06	38.35±7.9^{c}	12.94±0.9^{cd}	10.74±3.6^{bc}	37.97±5.2^{cd}
CR07	57.67±9.5^{a}	8.35±5.6^{de}	3.13±0.6^{d}	30.85±3.2^{d}
CR08	44.44±4.5^{bc}	18.75±1.3^{b}	4.08±1.3^{d}	32.73±1.1^{cd}
CR09	53.98±8.5^{ab}	9.11±6.8^{de}	16.05±0.5^{a}	20.86±2.3^{e}
CR10	11.92±0.6^{de}	35.77±1.7^{a}	16.62±1.6^{a}	35.69±0.7^{cd}

[1]Values in the same column having different superscripts are significantly different (P<0.05).

While globulins constituted the highest storage protein fraction in only four of the accessions (CR01, CR03, CR04 and CR10), the occurrence of higher albumin than globulin content in six of the accessions, namely CR02 (55.97%), CR05 (39.41%), CR06 (38.35 %), CR07 (57.67%), CR08 (44.44%), and CR09 (53.98%) was unexpected as legumes contain more globulins than albumins. This discrepancy may have been arisen from leaching of the globulins into the albumins during extraction as was also reported by Ragab *et al*. (2004) in which cowpea demonstrated higher albumin than globulin content. In most food crops, genetic variability in protein content is a desirable attribute as it indicates the potential to improve upon the content by means of selection and breeding (Singh and Eggum, 1984).

4.6 Means, Standard Deviations, Range, Coefficient of Variation and Mean Squares of Functional Properties of Lima Bean Flour

Overall means of the ten lima accessions in terms of water and oil binding capacity, foam capacity and emulsion capacity were 1.54 g/g, 1.29 g/g, 19.58 % and 66.79 %, respectively (Table 4.8). The standard errors were within the range of 0.01 to 0.37 signifying high precision in the results. Water absorption capacity, emulsion capacity and bulk density demonstrated highest variation among the six functional properties as demonstrated by the highly significant mean squares. The wide variability in these functional properties is an indication of availability of opportunity for genetic improvement in these traits. Nassir and Adewusi (2012), Asch *et al*. (2005) and Price *et al*. (2002) corroborated this observation. Oil absorption capacity, foam capacity, and swelling index did not vary among the accessions (Table 4.8)

4.6.1 Bulk Density of Lima Storage Proteins

Bulk densities of the ten lima accessions are shown in Table 4.9. Significant differences were observed between CR01, CR08 and CR04. The bulk densities for the seed flours varied from 1.43 to 1.63 g/ml. Accessions with highest bulk density were CR03, CR05, CR06, CR07 and CR10. Values of bulk density for lima flour (1.4 to 1.7 g/ml) were higher than those of ten other seed legumes including lima (0.54-0.82 g/ml) (Du *et al*., 2013), cowpea (1.35 g/ml) (Odedeji and Oyekele, 2011) and lower than that of soybean (1.85 g/ml) (Ali *et al*., 2012).

Table 4.8 Means, standard deviations, range, coefficient of variation and mean square of functional properties of lima bean flour.

Functional Property	**Mean**	**SD[1]**	**SE[2]**	**Min**	**Max**	**CV[3]**	**Mean Square**
Water Absorption Capacity (g/g)	1.54	0.45	0.08	1.00	2.50	14.99	0.54***
Oil binding Capacity (g/g)	1.29	0.35	0.06	0.70	1.86	26.99	0.13
Foam Capacity (%)	19.58	2.05	0.37	16.51	23.21	9.66	5.58
Emulsion Capacity (%)	66.79	11.17	2.04	47.06	80.00	3.42	390.52***
Swelling Index (ml)	1.19	0.09	0.02	1.07	1.45	7.31	0.01
Bulk Density (g/ml)	1.55	0.08	0.01	1.40	1.70	2.36	0.02***

[1]Standard deviation; [2]Standard error; [3]Coefficient of variation; ***P<0.001.

Bulk density is an important parameter that determines the packaging requirement of a product. It signifies the behaviour of a product in dry mixes and may vary with fineness of particles (Butt and Batool, 2010).

4.6.2 Water Absorption Capacity (WAC)

The WACs of the legume flours ranged from a least value of 1.00 g/g for CR02 and CR07 to 2.33 g/g for CR09 (Table 4.10) and were higher than the values of 1.12-1.89 g/g reported by Du *et al*. (2013) for ten different seed legumes including lima beans. They were also greater than the 1.60 and 1.94 g/g for some cowpea varieties in Nigeria (Chinma *et al*., 2008) as well as 1.68 g/g of soybean flour (Edema *et al*., 2005). Water absorption capacity of flour plays an important role in food preparation because it influences texture and sensory properties.

Table 4.9 Functional properties of lima bean flour cultivated in Ghana

Accessions	Water Absorption Capacity (g/g)	Oil Absorption Capacity (g/g)	Foam Capacity (%)	Emulsion Capacity (%)	Swelling Index (ml)	Bulk Density (g/ml)
CR01	1.67±0.3^{bcd}	1.01±0.1^{a}	18.02±1.3^{bc}	75.53±2.0^{a}	1.20±0.0^{ab}	1.50±0.0^{c}
CR02	1.00±0.0^{f}	1.09±0.3^{a}	21.20±1.0^{ab}	78.08±2.2^{a}	1.21±0.0^{ab}	1.47±0.1^{cd}
CR03	1.17±0.3^{ef}	1.01±0.4^{a}	21.21±2.3^{ab}	77.38±4.2^{a}	1.14±0.1^{b}	1.58±0.0^{ab}
CR04	1.83±0.3^{bc}	1.40±0.5^{a}	21.31±1.5^{a}	74.61±1.7^{a}	1.18±0.0^{b}	1.43±0.0^{d}
CR05	1.93±0.4^{b}	1.55±0.3^{a}	19.34±2.0^{abc}	76.33±2.4^{a}	1.22±0.0^{ab}	1.63±0.1^{a}
CR06	1.42±0.1^{de}	1.24±0.3^{a}	19.84±2.5^{abc}	62.22±1.9^{c}	1.13±0.1^{b}	1.60±0.0^{ab}
CRO7	1.00±0.0^{f}	1.40±0.5^{a}	19.63±2.4^{abc}	54.75±2.6^{d}	1.17±0.0^{b}	1.63±0.1^{a}
CR08	1.52±0.3^{cde}	1.24±0.3^{a}	19.49±2.2^{abc}	49.02±1.7^{e}	1.33±0.2^{a}	1.57±0.1^{b}
CR09	2.33±0.1^{a}	1.55±0.4^{a}	17.72±1.7^{c}	68.03±0.3^{b}	1.22±0.2^{ab}	1.48±0.0^{cd}
CR10	1.50±0.0^{cde}	1.40±0.5^{a}	18.02±1.3^{bc}	51.95±1.9^{de}	1.11±0.0^{b}	1.60±0.0^{ab}

[1]Values in the same column having different superscripts are significantly different (P<0.05)

The legume flours containing several water-loving components, such as polysaccharides and hydrophilic proteins generally have high water absorption capacity and impart soft texture to cereal-based foods (Wall, 1979; Kaur and Singh, 2005). In the current study, water absorption capacity had a low but significant and negative correlation (r=-0.38, P<0.05) (Table 4.12) with carbohydrate content. An R^2 of 0.14 interprets that 14 % of the variation in water absorption capacity is explained by the carbohydrate content. The protein quality of legume flours also affects their WAC (Kaur and Singh, 2005).

4.6.3 Oil Absorption Capacity (OAC)

The OACs of the legume flours ranged from 1.01 g/g for CR03 and CR01 to 1.55 g/g for CR05 and CR09 with no statistical difference (Table 4.10). The current study demonstrates a higher OAC for lima bean flour than that of cowpea (0.39 to 0.53 g/g) (Chinma *et al*., 2008), black bean (1.38g/g) and red kidney bean (1.20 g/g) (Du *et al*., 2013).

Oil absorbing mechanism involves a capillary action that allows retention of absorbed oil in foods. It is influenced by particle size, starch and protein contents, as well as nature of the protein (Sathe *et al*., 1982). Hydrophobic proteins have higher preference for binding to lipids (Kinsella, 1976; Chau *et al*., 1997). Based on this explanation, legume flours that show higher oil absorption capacity are likely to contain higher proportion of hydrophobic amino acid residues. Legume flours with high OAC are used in foods to impart desired texture and mouthfeel.

4.6.4 Emulsion Activity

Emulsion activity reflects the ability of a protein to aid in the formation of an emulsion and is related to a protein's ability to absorb at the interfacial area of oil and water in an emulsion. Lima bean flours demonstrated high emulsion capacities (49.02-78.08 %) compared to 23.30 % for cowpea (Odedeji and Oyekele, 2011). The highest values were exhibited by CR02 (78.08%), CR03 (77.38%), CR05 (76.33%), CR01 (75.53%) and CR04 (74.61%) with no significant differences among them.

These values were similar to 81.70 % emulsion capacity of soybean (Ali *et al*., 2012). Accession CR08 exhibited the lowest emulsion activity (49.02%) (Table 4.9). According to James and Norman (1979), differences in protein, starch, fat, and sterol contents of legume flours give rise to variation in emulsion activities. Flours having high emulsion activity may act as excellent binders of fat and water, and are good adhesive agents. As such, they may find uses in processed meat products, both coarse and fine emulsions including batters and sausages.

4.6.5 Foam Capacity

Foam capacity generally depends on the interfacial film formed by proteins, which maintain air bubbles in suspension and slows down the rate of coalescence. Foaming properties are influenced by protein and carbohydrate composition of the flour (Sreerama, *et al*., 2012). The lima bean flours demonstrated both low values and little variation in foam capacity. Values ranged from a minimum of 17.72 % in CR09 to 21.31% in CR04 (Table 4.10) being about three times lower than that of cowpea (57%) (Odedeji and Oyekele, 2011) and soybean (69.48%) (Ali *et al*., 2012).

The low foam capacity of lima beans may be attributed to the globular nature of storage proteins. In general, globular proteins are spherical and resist denaturation at air-water interface, as such, they are not flexible to orient with hydrophobic and hydrophilic regions of food leading to low foaming capacity (Grahams and Phillips, 1976). Proteins, which exhibit low foam capacity, may not be suitable for food products that require a high percentage of porosity such as ice cream and cakes.

4.6.6 Swelling Index

The swelling index of the lima bean flours ranged from 1.11 ml to 1.33 ml (Table 4.10) about half the values reported for cowpea (2.65 to 2.68 ml) (Appiah *et al*., 2011) and less than a quarter of the swelling index of soybean (5.89 ml) (Edema *et al*., 2005) . The highest values of swelling power were found in CR08, CR09 (1.22 ml), CR05 (1.22 ml), and CR02 (1.21 ml) with no significant differences among them.

CR10 registered the lowest value of 1.11ml. Swelling power of flour is its ability to increase in volume when mixed with water. The extent of swelling depends on temperature,

availability of water-loving species such as starch, extent of starch damage due to thermal and mechanical processes and presence of other carbohydrates such as pectins, hemicelluloses, and celluloses (Kinsella, 1979; Iwuoha and Nwakanma, 1998).

4.7 Correlation Analysis of Seed Size, Proximate Components, Storage Proteins and Functional Properties of Lima Bean Flour

Correlation analysis was carried out on seed size, three proximate components, the storage proteins and functional properties of lima bean flour. In general, the correlation coefficients among the four groups of traits were mostly weak and nonsignificant except between bean size, hundred seed weight, and storage protein content (Table 4.10). Bean size exhibited contrasting associations with proximate components, storage protein, and functional properties.

Table 4.10 Pearson correlation coefficients of seed size, proximate composition, storage proteins and functional properties

	WD	TN	HS	PT	FA	CH	SP	AL	GL	PR	GT	WB	OB	FC	EC	SW	BD
LG	**0.94****	**0.38***	**0.92****	**-0.51****	0.22	0.07	**-0.51****	-0.34	-0.20	0.23	**0.50****	0.04	0.10	0.10	**0.56****	**-0.48****	-0.08
WD		0.31	**0.87****	**-0.39***	0.08	-0.11	**-0.57****	**-0.46****	-0.11	0.31	**0.56****	0.09	0.08	0.13	**0.56****	**-0.40***	-0.14
TN			**0.43***	0.02	0.08	-0.12	-0.06	0.11	-0.26	0.21	-0.07	**0.37***	0.23	-0.33	0.32	0.05	**-0.39***
HS				**-0.40***	0.24	0.07	**-0.52****	-0.31	-0.26	0.23	**0.50****	-0.02	-0.07	0.04	**0.65****	-0.45	-0.13
PT					0.10	-0.49	0.07	-0.11	0.13	0.08	0.03	-0.05	**-0.42***	0.03	-0.10	0.31	**-0.42***
FA						0.35	-0.21	-0.03	0.14	-0.14	0.01	**-0.37***	-0.21	0.03	-0.10	-0.17	0.07
CH							-0.20	0.10	0.23	-0.21	-0.21	**-0.38***	0.03	-0.15	**-0.49****	-0.24	**0.62****
SP								**0.78****	-0.33	-0.30	**-0.71****	0.32	0.23	-0.09	-0.08	**0.51****	-0.12
AL									**-0.45****	**-0.60****	**-0.80****	0.13	0.14	-0.11	-0.24	0.35	0.00
GL										0.08	-0.09	-0.21	0.05	-0.06	**-0.45****	-0.16	0.08
PR											0.34	**0.49****	0.17	-0.17	0.26	-0.06	-0.08
GT												-0.24	-0.30	0.26	**0.53****	-0.34	-0.02
WB													**0.39***	-0.35	0.15	0.09	-0.16
OB														-0.27	-0.09	0.09	0.23
FC															0.23	-0.12	-0.18
EC																-0.12	**-0.39***
SW																	-0.19

LG = seed length; WD = seed width; TN = seed thickness; HS = hundred seed weight; PT = protein; FA = fat; CH = carbohydrate; SP = storage protein; AL = albumin; GL = globulin; PR = prolamin; GT = glutelin; WB = water binding capacity; OB = oil binding capacity; FC = foaming capacity; EC = emulsion capacity; SW = swelling power; BD = bulk density; *P < 0.05; **P < 0.01.

A strong and positive correlation between seed size and hundred seed weight was expected.

Seed size was contributed mostly by length and width with minimal contribution from the thickness. Positive correlation coefficients of r = 0.92, r = 0.87 and r = 0.43, respectively give R^2 values of 0.84, 0.76 and 0.18 which indicate that at least 84 %, 76 %, and 18% of variation in hundred seed weight is explained by length, width and thickness. Larger seeds were associated with low protein (r = -0.50), low storage protein (r = -0.57), low albumin (r=-0.40) unlike with glutelin content which had a positive correlation with seed size (r = 0. 56).

Again seed size in terms of length and width showed equal and positive correlation with emulsion capacity (r= 0.56) but about equal and negative correlation with swelling index (r = -0.48). In contrast, seed thickness was associated with different traits, a positive and significant correlation with water binding capacity (r = 0.37) and a negative significant correlation with bulk density (r = -0.39). It appears that different sets of proteins with varying amino acid composition govern seed size and functionality of lima bean.

The negative and significant correlation between fat and water binding capacity (r = -0.37) and between protein and oil binding capacity (r = -0.42) were expected as lima contains relatively higher proportions of water-soluble storage proteins. Hydrophilic components is the basis for high water binding capacity, hence the negative correlation in both instances.

Similarly, abundant and predominant hydrophilic proteins would contribute to a negative association between protein and oil binding components. The negative correlation between carbohydrate and water binding capacity was unexpected as carbohydrates are hydrophilic by nature. Dietary fiber makes up most of the carbohydrates in lima bean. The insoluble fraction of the dietary fiber constitutes about 75% of the total fiber while the remaining 25 % is soluble (gums, pectin, mucilage, and beta-glucan) (Anderson, 1990). Insoluble dietary fiber, made up of cellulose, lignin and hemicellulose is insoluble in water which partly explains the

negative correlation (r = -0.38, P = 0.03) between carbohydrate and water binding capacity in lima. An R^2 value of 0.14 indicates that 14 % of the variation in water binding capacity is explained by the carbohydrate content.

A negative and significant correlation (r =-0.45, P=0.01) between globulin and emulsion capacity indicates that as globulin content increases emulsion capacity decreases. An R^2 value of 0.2 means 20% of variation in emulsion capacity is explained by globulin content. In contrast, high glutelin content was associated with high emulsion capacity, judged from the positive and significant correlation (r=0.53, P=0.0027) (Table 4.10). An R^2 value of 0.28 indicates that 30% of variation in emulsion capacity is explained by glutelin content. Water binding capacity was shown to be correlated with prolamin content (r=0.49, P=0.0056).

A negative but significant correlation existed between globulin and albumin content (r = -0.45, P=0.01) (Table 4.12) indicating that as globulin content increases there is a simultaneous decrease in albumin content. An R^2 value of 0.20 signifies that 20 % of the variation in globulin content is explained by albumin content. This study also indicated a significant negative correlation between glutelins and albumins (r = -0.80, P=<0.01). This result suggested that there is a compensatory effect in the modulation of storage proteins, whereby increase in one storage protein leads to a decrease in another. Finally, high carbohydrate content was associated with high bulk density (r = 0.62, P = 0.01).

4.8 Principal Components Analysis of Seed Size, Proximate, Storage Proteins and Functional Properties

A principal component analysis (PCA) was applied to the data to explain the variance-covariance structure and expose the relationship between variables as well as accessions and to reduce the original number to a few uncorrelated variables in terms of their contribution to

the total variance. Results of the principal component analysis are shown in Table 4.11. The first six principal components accounted for 95% of the total variance (Table 4.11). In the first PC, which explained 32.44 % of the total variance, the most important traits were seed size and hundred-kernel weight, the storage protein albumins, glutelins, and swelling power.

Table 4.11 Results of the principal component (PC) analysis for the first six PCs from the correlation matrix of seed size, proximate, storage proteins and functional properties of 10 lima bean accessions grown in Ghana

Trait	PC1	PC2	PC3	PC4	PC5	PC6
LG	**0.890**	0.109	0.333	0.236	0.060	0.132
WD	**0.900**	0.178	0.226	0.074	0.098	0.218
TN	0.296	0.559	0.295	0.263	**-0.644**	-0.065
HS	**0.888**	0.140	0.251	0.298	-0.109	-0.042
PT	-0.293	0.222	**-0.654**	-0.247	-0.546	-0.061
FA	0.306	**-0.602**	0.041	0.380	-0.567	0.067
CH	0.013	**-0.783**	0.499	0.103	-0.091	-0.181
SP	**-0.803**	0.391	0.141	0.276	0.142	0.039
AL	**-0.692**	0.133	0.341	**0.607**	-0.048	-0.030
GL	-0.058	-0.512	-0.085	-0.571	-0.234	0.531
PR	0.423	0.390	0.117	**-0.736**	-0.060	-0.273
GT	**0.806**	0.010	-0.452	-0.135	0.247	-0.209
WB	-0.126	**0.776**	0.447	-0.420	0.036	-0.004
OB	-0.202	0.178	**0.760**	-0.227	0.325	0.331
FC	0.250	-0.119	**-0.625**	0.349	0.552	0.238
EC	0.589	0.583	-0.245	0.308	0.150	-0.088
SW	**-0.863**	0.376	-0.208	0.188	0.082	-0.057
BD	-0.080	**-0.676**	0.350	-0.151	0.334	-0.467
Eigen value	5.84	3.57	2.75	2.27	1.76	0.91
Total variance (%)	32.44	19.83	15.28	12.61	9.78	5.06
Cumulative Variance (%)	32.44	52.27	67.55	80.16	89.94	95.00

*Values >0.6 are presented in bold face and represents traits important for definition of principal component. LG = seed length; WD = seed width; TN = seed thickness; HS = hundred seed weight; PT = protein; FA = fat; CH = carbohydrate; SP = storage protein; AL = albumin; GL = globulin; PR = prolamin; GT = glutelin; WB = water binding capacity; OB = oil binding capacity; FC = foaming capacity; EC = emulsion capacity; SW = swelling power; BD = bulk density; *P<0.05; **P<0.01.

The second PC (19.83 %,), the predominant traits were fat, carbohydrate, water binding capacity, and bulk density. The third PC explained 15.28 % of the total variance and was represented by traits related to protein and the functional properties, oil binding capacity and

foam capacity. Albumins and prolamins were the quantitative traits that were evident in PC 4 (Table 4.11). Only seed thickness exhibited variation in PC 5. To determine which PCs were significant among the six, the Noel *et al*. (1986) test for statistical significance of PCs given by formula $\delta\lambda = \lambda(2/N)^{\frac{1}{2}}$ was applied, where $\delta\lambda$ is the error associated with a PC which is equivalent to the physical distance between two adjacent PCs, that is, $\lambda_1 - \lambda_2$ and λ_1 is the magnitude of eigenvalue of a PC1 and λ_2 is magnitude of eigen value of a PC2, N= sample size.

Application of this test revealed that PC3 and PC4 were both not significant and exhibited a mixed mode phenomenon arising from leakage of one PC into another and rendering them identical. Upon this basis PC4 was rejected, likewise PC6 in which none of the traits showed variation. A plot of the first two PCs, which cumulatively accounted for 52.27 % of the total variance revealed five major correlation groupings in which each of the storage proteins was identified with a separate group. Glutelins correlated with seed size, albumins were grouped with protein, oil binding capacity and swelling power, prolamins with emulsion capacity, globulins with fat, carbohydrate, foam capacity and bulk density, while water binding capacity showed little relationship with the other groups (Fig. 4.2A).

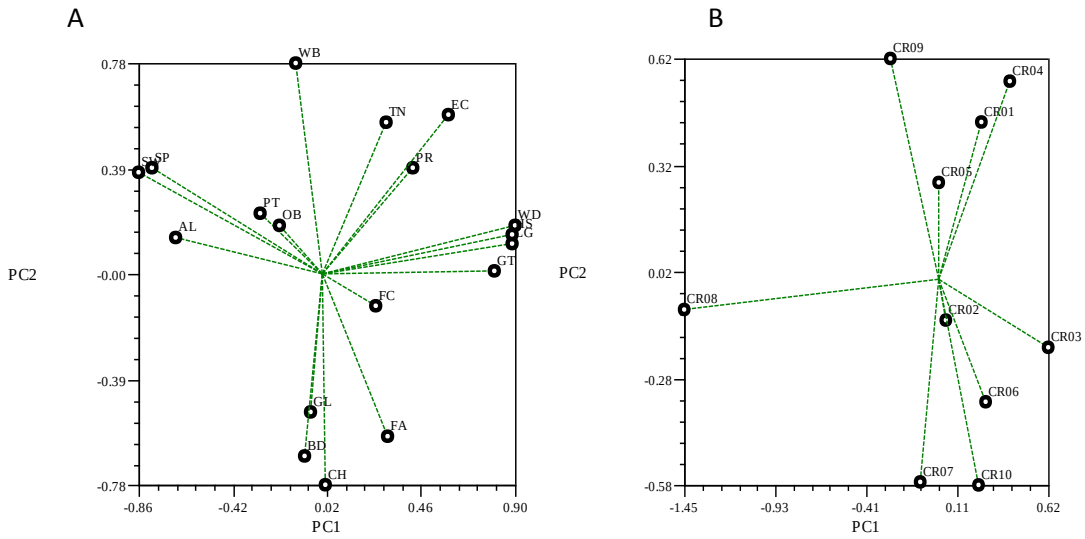

Fig. 4.2. 2D plots of 10 lima bean accessions grown in Ghana on the first two principal components obtained from the analysis of 18 quantitative traits. A) Principal components showing groups of traits; B) principal components showing groups of accessions. Fig. 4.2B shows a plot of PC1 and PC2 on the 10 lima accessions.

Principal component 1 separated the accessions on the basis of seed size, the water-soluble proteins albumin and glutelin and water-related properties, such as swelling power. The second PC separated accessions on the basis of the proximate composition variables, fat and carbohydrate, the water-related property, water binding capacity, and bulk density. It is worthy of note that CR08 was isolated from the other accessions.

4.9 Cluster Analysis of Seed Size, Proximate, Storage Proteins and Functional Properties

The level of genetic dissimilarity among lima bean accessions was estimated based on Euclidean distance coefficients generated from the 18 traits. The Euclidean distance matrix, which represents an estimate of the genetic distance between pairs of accessions based on morphological differences, is shown in Appendix 7. A cluster analysis was performed on the Euclidean matrix to classify and order the genetic variability, identify and analyze the genetic

associations among the accessions. Fig. 4.3 shows the dendrogram generated from the distance matrix. The dissimilarity coefficients ranged from -0.21 for CR07 to 0.40 for CR05. Insertion of a reference line at 0.10 distance units classified the genotypes into five distinct clusters. Clusters III and V had the highest membership of 30 % each of the total accessions. Cluster II had two members (20%) while clusters I and IV were each represented by a single genotype (10%). Clusters I and V had the highest inter-cluster distance. Clusters I and IV were the closest in terms of dissimilarity coefficient.

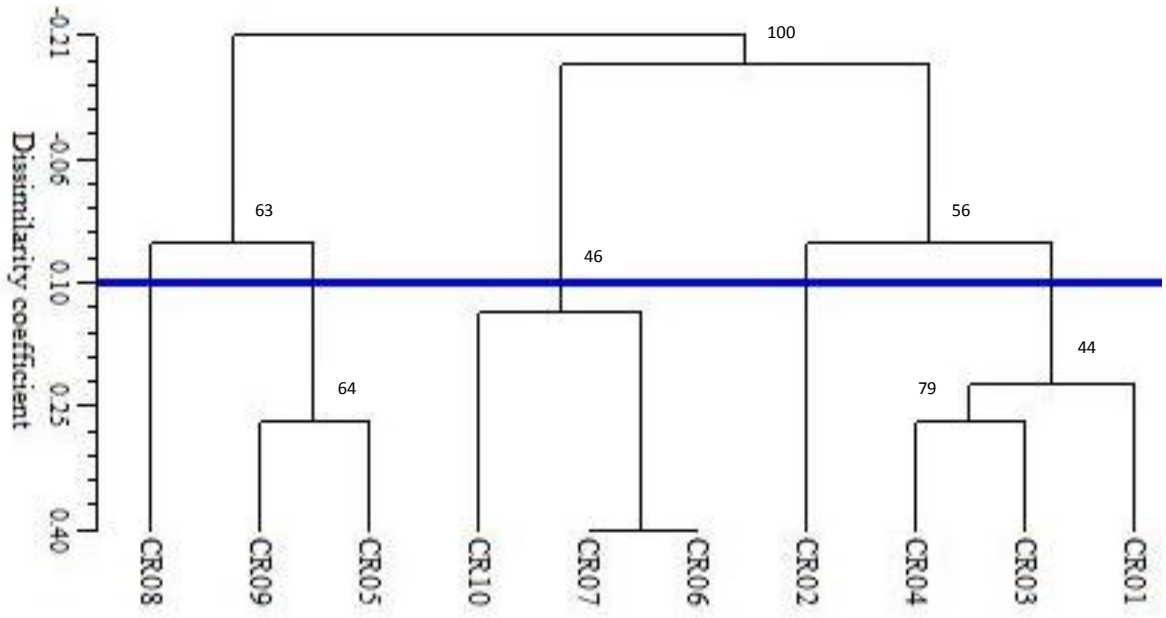

Fig. 4.3 A dendrogram generated from UPGMA clustering of 10 lima beans grown in Ghana based on measurement of 18 traits. Numbers at the nodes are bootstrap values.

The variability that existed among the clusters suggests the possibility of making selection for introgression of superior accessions with outstanding performance for various quantitative traits. Crosses between individuals from different clusters may result in heterosis (Stuber, 1994).

4.9.1. Clusters and their variances from the overall mean

The intra-cluster variability of the ten accessions within five clusters is presented in Table 4.12.

Cluster I genotypes

The first cluster constituted a single member, genotype CR08. Mean values for seed size, hundred seed weight, fat and carbohydrate content were the least among all the ten genotypes. In contrast, the crude protein and storage protein contents exceeded the overall mean values. Cluster I had the highest albumin and lowest glutelin content. The storage protein was chiefly contributed by albumin which exceeded the overall mean by about 50 % and was least represented by glutelin which was about 47 % less than the mean. All traits demonstrated lower standard deviation than the overall standard deviation values except thickness (Table 4.12).

Cluster II genotypes

Genotypes of cluster II were CR05 and CR09. Mean values for seed size, hundred seed weight, albumin and prolamin content were higher than the overall mean, whereas for traits such as crude protein, fat, carbohydrate, globulin and glutelin values were lower than the total mean with globulin and foam capacity being the least of all the accessions (Table 4.12).

Cluster III

The third cluster had three members consisting of CR06, CR07, and CR10 genotypes. Mean values of seed thickness, storage proteins, albumins, globulins, prolamins, glutelins, water binding capacity, foam capacity, and emulsion capacity were lower than the overall mean, with least storage protein and swelling power occurring in this cluster. The highest

carbohydrate and globulin contents of all the accessions were in this group. With regard to functional properties, this cluster exhibited the highest bulk density.

Cluster IV

Genotype CR02 was the only member of cluster IV. It recorded the largest mean values of hundred seed weight, fat, foam and emulsion capacities, but the least mean values of prolamin, water binding oil binding and bulk density.

Table 4.12 Cluster means and their differences from the overall mean

	Mean and standard deviation (in parenthesis) of clusters (I to V) and their variance from the overall mean										
Trait	Overall	I	Variance	II	Variance	III	Variance	IV	Variance	V	Variance
LG	20.16 (3.4)	10.33 (0.3)	-9.83	21.15 (0.9)	0.99	21.19 (0.9)	1.03	20.84 (0.6)	0.68	21.51 (1.1)	**1.35**
WD	12.92 (1.8)	7.99 (0.3)	-4.93	13.24 (0.9)	0.32	13.33 (0.4)	0.40	13.03 (0.4)	0.10	13.91 (1.1)	**0.99**
TN	6.58 (0.40)	6.07 (0.5)	-0.50	6.76 (0.6)	**0.18**	6.47 (0.3)	-0.11	6.60 (0.1)	0.02	6.73 (0.4)	0.15
HS	114.46 (27.9)	35.27 (1.2)	-79.19	120.40 (2.3)	5.94	119.24 (9.1)	4.79	128.03 (1.1)	**13.58**	127.58 (8.4)	13.12
PT	22.49 (0.37)	23.96 (0.2)	1.47	21.70 (0.7)	-0.79	21.78 (1.0)	-0.71	22.42 (0.5)	-0.07	23.27 (0.7)	0.77
FA	1.24 (1.08)	0.94 (0.3)	-0.30	1.03 (0.2)	-0.21	1.42 (0.4)	0.18	1.51 (0.3)	**0.28**	1.20 (0.4)	-0.04
CH	53.88 (3.12)	52.47 (0.1)	-1.41	53.67 (1.1)	-0.21	57.42 (0.5)	**3.54**	53.77 (0.2)	-0.11	50.99 (3.2)	-2.89
SP	5.69 (1.62)	8.21 (0.6)	**2.52**	7.30 (1.0)	1.61	4.55 (1.1)	-1.14	6.41 (0.2)	0.72	4.68 (0.6)	-1.01
AL	37.40 (17.5)	55.94 (1.3)	**18.54**	46.69 (12.5)	9.30	35.98 (20.8)	-1.42	44.44 (4.5)	7.04	24.09 (12.7)	-13.31
GL	14.07 (8.7)	17.46 (2.6)	3.39	8.63 (4.7)	-5.44	19.02 (13.1)	**4.95**	18.75 (1.3)	4.68	10.06 (3.1)	-4.01
PR	11.68 (5.4)	7.34 (2.7)	-4.34	15.11 (2.5)	**3.43**	10.16 (6.2)	-1.52	4.08 (1.3)	-7.60	14.88 (3.4)	3.21
GT	36.86 (12.9)	19.27 (1.4)	-17.59	29.57 (10.4)	-7.29	34.84 (4.4)	-2.02	32.73 (2.1)	-4.12	50.97 (11.2)	**14.11**
WB	1.54 (0.5)	1.52 (0.3)	-0.02	2.13 (0.4)	**0.60**	1.31 (0.2)	-0.23	1.00 (0.0)	-0.54	1.56 (0.4)	0.02
OB	1.29 (0.4)	1.24 (0.3)	-0.05	1.55 (0.3)	**0.26**	1.35 (0.4)	0.06	1.09 (0.3)	-0.20	1.14 (0.4)	-0.15
FC	19.58 (2.1)	19.49 (2.2)	-0.09	18.53 (1.9)	-1.05	19.16 (2.1)	-0.41	21.20 (1.0)	**1.62**	20.18 (2.2)	0.60
EC	66.79 (11.2)	49.02 (1.7)	-17.77	72.18 (4.8)	5.39	56.31 (5.0)	-10.48	78.08 (2.2)	**11.29**	75.84 (2.8)	9.05
SW	1.19 (0.1)	1.33 (0.2)	**0.14**	1.22 (0.10)	0.03	1.14 (0.1)	-0.05	1.21 (0.0)	0.02	1.17 (0.0)	-0.02
BD	1.55 (0.08)	1.57 (0.0)	0.02	1.56 (0.1)	0.01	1.61 (0.0)	**0.06**	1.47 (0.1)	-0.08	1.50 (0.1)	-0.05

LG = seed length; WD = seed width; TN = seed thickness; HS = hundred seed weight; PT = protein; FA = fat; CH = carbohydrate; SP = storage protein; AL = albumin; GL = globulin; PR = prolamin; GT = glutelin; WB = water binding capacity; OB = oil binding capacity; FC = foaming capacity; EC = emulsion capacity; SW = swelling power; BD = bulk density. The least and greatest variances are underlined and in bold, respectively.

Cluster V

Cluster V constituted three members, viz., CR04, CR03 and CR01. Seed size was the highest among all accessions with their mean values exceeding that of the overall mean. In addition, it demonstrated the greatest glutelin content whereas carbohydrate and albumin content were the least.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

Pattern of variation among the accessions was different for the various proximate components. The highest coefficient of variation was 26.46% for fat content while the lowest of 0.73 % was observed for carbohydrate content. The large variability in fat content signifies the possibility to improve fat content of lima via breeding.

The proximate analysis revealed CR01 as the accession with the highest protein content (24.0%) while CR06 (20.7 %) was the lowest. Lima bean with higher protein content can serve as inexpensive source of food for both humans and livestock. A low crude fat content ranging from 0.94% to 1.66% was obtained in the accessions studied. Generally, all the accessions recorded lower moisture values of 9.81–13.90%. Low moisture content of food enhances the storage life of food commodities.

Mean mineral contents were 893 mg/100 g for potassium, 212 mg/100 g for calcium, 4.9 mg/100 g for iron, and 207.5 mg/100 g for phosphorus. Potassium and phosphorus was the most abundant mineral in lima beans. With regard to storage proteins, globulins were present at 19.27-61.88 % and albumins at 10.53-57.67 %. Glutelins and prolamins occurred at least concentrations of 6.68-35.77 % and 3.13-16.94 %, respectively.

Lima bean flour possessed high water and oil binding capacities of 1.00-2.33 g/g and 1.01-1.55 g/g, respectively. However, emulsion and foaming capacities were low in comparison to soybean and cowpea. Correlation coefficients among the four groups of

traits were mostly weak and nonsignificant except between bean size, hundred seed weight, and storage protein content.

UPGMA cluster analysis based on seed size, proximate, storage proteins and functional properties clustered the ten accessions into five main groups. Principal component analysis revealed that the first six principal components accounted for 95 % of the variation in the data. Size and weight, protein, fat and carbohydrate, albumin, prolamin, glutelin and the functional properties, water and oil binding capacities, swelling capacity and bulk density were the most important traits that contributed to the variance.

5.2 Recommendation

The successful nutritional characterization of lima bean accessions and their classification has made it possible for the exploitation of these legumes in Ghana. It is therefore recommended that increased efforts would be made to encourage the cultivation of lima beans as well as its consumption. The differences observed among the accessions available in Ghana would be of immediate importance for broadening the lima bean genepool and may be used in hybridization and breeding programmes.

Finally, the greatest impediment to utilizing underutilized legumes as a food and feed is the presence of certain antinutritional factors, which might not be only toxic, but also can be lethal in extreme situations. These constituents when present in high quantities, results in loss of appetite, reduction in dry matter intake and has effect on protein digestibility. It is therefore imperative to do further experiment to screen and quantify the levels of antinutrients in the lima bean accessions. In addition, to evaluate various modern and

traditional foods processing methodologies that can be applied to either reduce or eliminate antinutritional compounds in lima bean accessions.

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APPENDICES

Appendix 1: Moisture Content Determination

$$\% \text{ Moisture content} = \frac{(M2\text{-}M1)\ X\ 100}{M2\text{-}M1}$$

where

M1 = Initial Mass of empty dish

M2 = Mass of dish + undried sample

M3 = Mass of dish + dried sample

Appendix 2: Ash Content Determination

$$\% \text{ Ash} = \frac{\text{Mass of ash X } 100}{\text{Mass of sample}}$$

Appendix 3: Fat Content Determination

$$\% \text{ Fat} = \frac{W2\text{-}W1}{W3} \times 100$$

where

W1 = Mass of the empty extraction flask

W2 = Mass of the flask and oil extracted

W3 = Mass of the sample

Appendix 4: Crude Protein determination

$$\% \text{ Total nitrogen} = \frac{100\ (Va - Vb) \times 0.1 \times 0.01401 \times 100\%}{\text{weight of sample} \times 10}$$

Where;

Va = titre value of the blank

Vb = titre value of the sample

Appendix 5: Crude Fibre Determination

$$\% \text{ Crude fiber} = \frac{M1\text{-}M2}{M3} \times 100$$

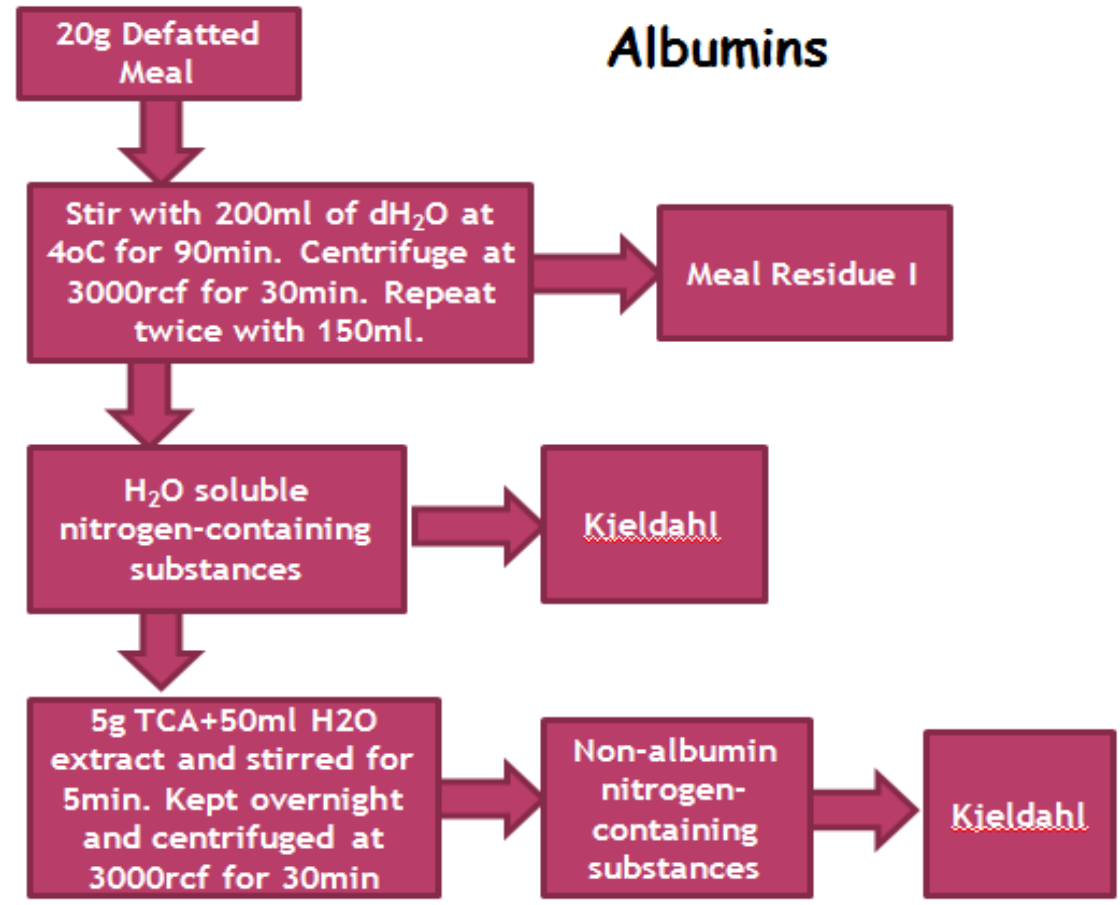
M1 = Mass of sample before incineration

M2 = Mass of sample after incineration

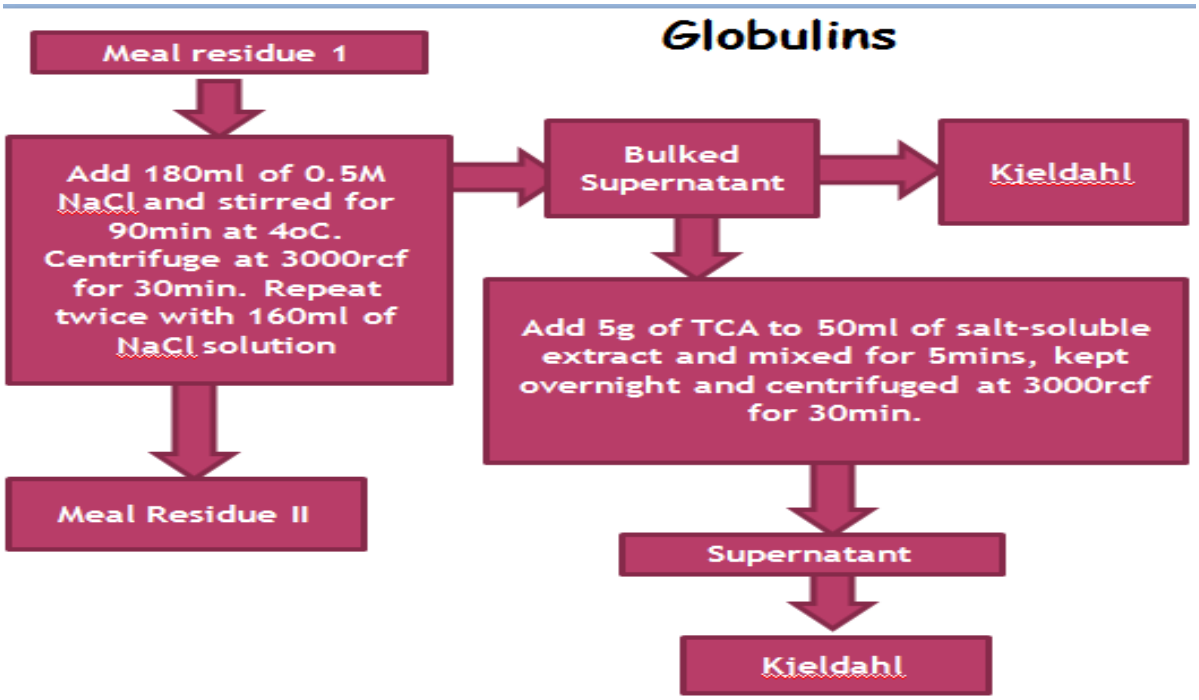
M3 = Mass of original sample.

Appendix 6: Schematic representation of storage protein determination

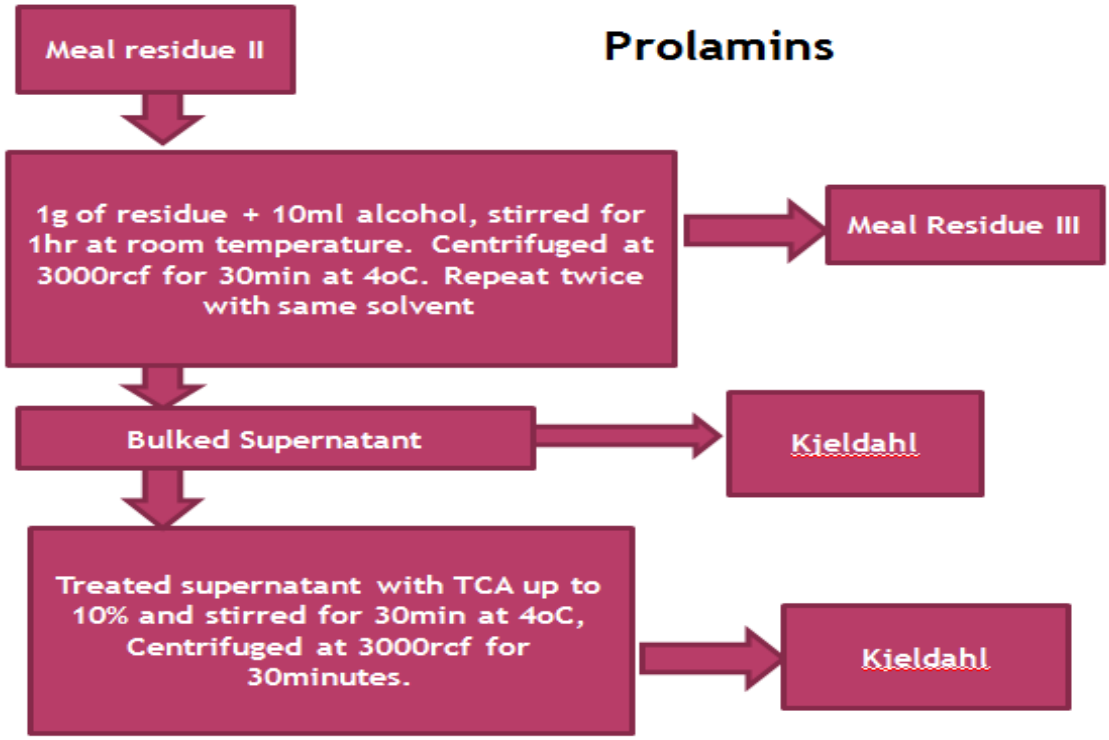
2.2 Extraction of albumin



2.3 Extractability of globulins



2.4 Extraction of Prolamins



Appendix 7: Euclidean distance matrix of the 18 variables measured on ten accessions of lima beans

	CR01	CR02	CR03	CR04	CR05	CR06	CR07	CR08	CR09
CR01									
CR02	4.67								
CR03	4.68	4.884							
CR04	4.481	5	4.661						
CR05	5.414	5.493	5.465	4.547					
CR06	5.318	4.531	4.233	5.22	3.831				
CR07	5.944	4.274	5.972	6.839	5.278	3.702			
CR08	8.157	7.38	8.925	8.577	7.491	8.063	7.557		
CR09	4.672	6.043	7.295	5.727	4.371	5.583	5.982	7.481	
CR10	6.119	6.244	5.389	6.395	6.052	4.547	5.673	8.365	6.62