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Physicochemical and protein characterization of lima bean (*Phaseolus lunatus* L) seed

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Abstract

Lima bean (*Phaseolus lunatus* L) is native to Latin America and has spread out worldwide, thus having a high level of diversity. The lima bean seed from a specific region might have different characteristics from others. This study was aimed to characterize the physicochemical and protein of the dry seed of lima bean from Indonesia based on its solubility, electrophoretic pattern, and amino acid profile. The result showed that carbohydrate (68.89±1.55%) was the major component, with starch (41.96±1.10%) as the predominant. The amylopectin was higher than amylose. Dietary fibre (27.87±0.37%) was significant and dominated by insoluble one (25.47±0.32%). The fat content (1.15±0.04%) was low and ash (3.67±0.47%) comprised of magnesium, phosphorus, potassium, calcium, and iron with the content of 184, 75, 38, 11, and 10 mg/100 g, respectively. Total phenolic compounds of this seed were 1.29±0.02 mg/g, phytic acid 11.57±0.03 mg/g, saponins 16.84±0.42 mg/g, and trypsin inhibitors 36.07±0.11 TIU/g. HCN was found significantly at 30.99±0.29 ppm. Oligosaccharides were 5.93±0.29% that comprised of raffinose 1.22±0.08% and stachyose 4.61±0.21%. Protein content was moderate (15.93±0.55%) that comprised of albumin 18.47±0.62%, globulin 56.20±2.00%, prolamin 3.14±0.20%, and glutelin 22.69±1.60%. The molecular weight of this protein was 10-141 kDa with 12 polypeptides. Globulin had 12 polypeptides of 10 to 124 kDa. Albumin had 18 to 116 kDa molecular weights, while glutelin had six polypeptides of 13 to 109 kDa. Prolamin did not have visible polypeptides. Lysine, leucine, valine, and phenylalanine were the primary essential amino acids with high lysine but low methionine and cysteine. In conclusion, lima bean seed is a source of carbohydrates and minerals, with a moderate protein content dominated by the globulin. The polypeptide profile of lima bean seed is varied depending on the protein fractions. The occurrence of anti-nutrition might hinder its utilization as a protein source. HCN as a toxicant should be removed to obtain a safe seed for consumption.

1. Introduction

Legumes are dicotyledonous seeds of plants belonging to the Leguminosae family (Hoover *et al.,* 2010), which have been consumed for thousands of years as a part of the traditional diet throughout the world. Legumes are considered an essential part of the human diet in many countries worldwide and the second-largest source of human food after cereals. Legumes are an excellent source of complex carbohydrates, protein, dietary fibre, and vitamins and minerals. The high lysine content in legume protein makes legume ideal for blending with other commodities. The nutritious seeds significantly contribute to food security, sustainable agriculture, biodiversity, and environmental change mitigation (Boukid *et al*., 2019). In recent years, legumes are recommended as health-promoting foods by health

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organizations and dieticians (Hall *et al*., 2017). The Food and Agriculture Organization of the United Nations formally declared 2016 as the "International Year of Pulses" (IYP) to raise awareness about these important crops essential for sustainable agriculture and their nutrient richness, which improves human health nutrition. It is expected that reviving legumes production by utilizing the local biodiversity will have economic, environmental, and societal impacts since consumer well -being and health are directly affected by the food they eat.

Phaseolus lunatus L., also known as lima bean or butter bean, belongs to the Fabaceae family, a vining tropical legume crop cultivated mostly in temperate and subtropical regions in Africa and Southeast Asia (Sandoval-Peraza, 2020). Lima bean is native to tropical America. The Spaniards introduced lima beans throughout the Americas and the Southeast Asia region (Lim, 2012). It adapted to highly leach infertile soils of the more humid regions (Aremu *et al*., 2016), with a high level of genetic diversity worldwide (e Lacerda *et al*., 2017). Therefore, the lima bean seed from a specific region might have different characteristics from other origins. So far, this legume is not extensively consumed (Diniyah *et al*., 2020) due to the low productivity and hard texture of dry beans. This high protein content is an advantage that might be used to provide nutritious food products and solve nutritional problems due to the lack of protein consumption.

Lima bean seeds are considered to be good sources of nutrients (Jayalaxmi *et al*., 2016), such as a valuable source of proteins with content from 14.24 to 24.92% (Ibeabuchi *et al*., 2019) and rich in essential amino acids (FAO, 2017). They provide complex carbohydrates, mainly starch and dietary fiber, vitamins (B complex), and minerals (zinc, iron, and calcium) (Campos-Vega *et al*., 2010). The most abundant proteins are storage proteins, primarily globulin and albumin (Agarwal, 2017). In addition to their good macronutrient composition, lima bean is considered beneficial for health because of the low glycemic index due to the presence of slow-release carbohydrates (Bello-Pérez *et al*., 2007). However, like other legumes, lima bean also contains antinutritional components, including saponin, phytic acid, trypsin inhibitors, phenolic compounds, and contains toxic compounds of cyanogenic glycosides (Bolade *et al*., 2017; Sandoval-Peraza *et al*., 2020). Antinutritions decrease the nutritional quality of the legumes. On the other hand, some of these compounds might have health benefits as an antioxidant, anti-cancer, and other health benefits (Campos-Vega *et al*., 2010).

Bean protein characteristics are very important to explore because legume is a source of essential nutrients. Protein fractions based on solubility will determine their uses in food processing. Meanwhile, the amino acid profile of the protein also affects its bioavailability in the body. Different environments and long adaptation to a specific region might result in different physicochemical and protein characteristics. This study aimed to characterize the physicochemical and protein of lima bean with long adaptation to Indonesia's growing environment.

2. Materials and methods

The seeds of lima bean (*Phaseolus lunatus L.*) were obtained from Malang, East Jawa Province, Indonesia. The seeds were carefully cleaned and freed from foreign materials and sundried for 3 days and then stored in polyethylene bags. All chemicals are pro-analysis grade and the standards for chromatography analysis (oligosaccharides and amino acids) were obtained from Sigma Aldrich (Singapore).

2.1 Physical characterization

Seed length, width, and thickness of dry lima bean seed were measured with a vernier caliper. The colour was measured using a Chromameter CR-100 (Minolta) in terms of L (lightness), a (redness and greenness), and b (yellowness and blueness). The weight and volume of one hundred and ten seeds of lima bean, respectively, were measured using the methods of Wani *et al*. (2017) with slight modifications.

2.2 Chemical analysis

2.2.1 Proximate and carbohydrate analysis

Proximate analysis of the dry lima bean was carried out to determine crude protein, ash, crude fat, and moisture content by AOAC (2005) methods. The total carbohydrate was determined by difference. The starch content was measured by the direct acid hydrolysis method (AOAC, 2005). The amylose content was determined by the spectrophotometric method (ISO 6647). Total (TDF), soluble, and insoluble dietary fibres were measured by the enzymatic-gravimetric method of AOAC (991.43). All the analysis was conducted in three replications. All data were calculated as dry basis (db).

2.2.2 Minerals analysis

The samples were dry-ashed according to the AOAC method (2005). The aliquot was analysed for potassium, magnesium, calcium, and iron using atomic absorption spectrophotometer (AA-6200 Shimadzu), while phosphorus was analysed colorimetrically by the AOAC method (2005) using Genesis 10S UV-Vis Spectrophotometer.

2.2.3 Anti-nutrition and HCN analysis

The colorimetric (Wade Reagent) method was applied to determine the phytic acid content described by Lai *et al*. (2013). Total phenolic content (TPC) was determined using Folin-Ciocalteu phenol reagent at an absorbance of 740 nm. TPC was expressed as mg gallic acid equivalent per gram (mg GAE/g) (Padhi *et al*., 2017). The saponin content was determined using the spectrophotometric method as described by Lai *et al*. (2013). Trypsin inhibitor activity (TIA) was measured following the procedure by Kakade *et al*. (1974). The determination of the cyanide (HCN) content of the samples was by the alkaline titration method of the AOAC (1990) (Bolade *et al*., 2017).

2.2.4 Oligosaccharides analysis

Oligosaccharides were analysed using the method of Wang *et al*. (2007) with a slight modification. Oligosaccharides were extracted by adding 20 mL of 70% ethanol, then heated in a water bath at 70°C for one hour. The extract was filtered by a 0.45 µm membrane filter and put in a vial. Sodium azide was added with a volume of 10% of the sample volume. The sample was injected into the HPLC system consisting of a degasser (model G1322A Agilent), a solvent pump (model G1310A Agilent), and a refractive index detector (model G1362A Agilent). HPLC columns for carbohydrates (ZORBAX carbohydrate analysis columns) with dimensions of 150 mm × 4.6 mm × 5 µm (Agilent), coated with 3-aminopropyl silane on the silica particles. The mobile phase was a mixture of acetonitrile: water (80:20) with a 1 mL/min flow rate. The standards of oligosaccharides used were raffinose and stachyose.

2.3 Protein characterization

2.3.1 Protein extraction

The total protein was extracted using a slight modification of Gupta *et al*. (2014). Dry lima bean seed was ground and sieved at 100 mesh. Lima bean flour (100 mg) was dissolved in 1 mL of 0.1 M Tris-HCl buffer (pH 8.0). The crude homogenate was stirred intermittently for 2 hrs and then centrifuged at 8,000×*g* for 20 mins. The supernatant was protein extract used for protein fraction and electrophoretic pattern analysis using Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS PAGE).

2.3.1 Protein fractionation

Extracted protein was fractionated based on solubility into albumin (water-soluble), globulin (salt-soluble), prolamin (alcohol-soluble), and glutelin (alkali-soluble). Proteins were separated by the Osborne method (Alghamdi *et al*., 2019) with a slight modification. Briefly, 100 mg of lima bean flour was mixed with distilled water (1 mL), and the mixture was stirred intermittently for 2 hrs and then centrifuged at 8,000×*g* for 20 mins. The supernatant was an albumin fraction. The residue was mixed with 5% NaCl and stirred for 2 hrs before being centrifuged at 8,000×*g* for 20 mins. The supernatant was a globulin fraction. The residue from the globulin fraction was mixed with 1 mL of 70% ethanol and stirred intermittently for 2 hrs. The mixture was then centrifuged at 8,000×*g* for 20 mins, and the supernatant was a prolamin fraction. The residue from the prolamin fraction was mixed with 1 mL of 0.1 N NaOH and stirred intermittently for 2 hrs before centrifugation at 8,000×*g* for 20 mins. The supernatant was a glutelin fraction. All these four fractions were analysed from their electrophoretic protein pattern by SDS-PAGE analysis. Protein concentration of protein extract and protein fractions were determined by the Biuret method using bovine serum albumin (BSA) as a standard (Wati *et al*., 2009).

2.3.3 SDS-PAGE analysis

Protein extract and protein fractions were analysed using SDS-polyacrylamide gel electrophoresis (12% separating gel and 4% stacking gel) by the method of Vasconcelos *et al*. (2010). Protein extract and its four fractions, each was 50 mL, was mixed separately with the loading buffer (125 mM M Tris-HCl buffer pH 6.8, containing 10% SDS, 50% glycerol, 1% bromophenol blue, and 200 mM b-mercaptoethanol) and heated at 100°C for 3–5 mins. After heating, each sample was loaded in the wells of the gel. The electrophoresis was performed at a constant voltage of 125 V for 30 mins. The protein was stained with Coomassie Brilliant Blue R -250 and de-stained with methanol–acetic acid solution after separation.

2.3.4 Amino acid analysis

Amino acid profile determination was analysed using a Waters Acquity Ultra-High-Performance Liquid Chromatography (UHPLC) H Class and H Class Bio Amino Acid Analysis System Guide. UHPLC method was used for the identification of 17 amino acids e.g L-serine (Ser), L-glutamic acid (Glu), L-phenylalanine (Phe), L-isoleucine (Iso), L-valine (Val), L-alanine (Ala), L-arginine (Arg), glycine (Gly), L-lysine (Lys), L-aspartic acid (Asp), L-leucine (Leu), tyrosine (Tyr), proline (Pro), threonine (Thr), histidine (His), cysteine (Cys) and methionine (Met). The method was equipped with column AccQ. Tag Ultra C18 1.7 µm (2.1×100 mm), with column temperature at 49°C, 1 µl of injection volume, flow rate of 0.5 m/min, and PDA detector at 260 nm wavelength. The gradient composition of mobile phase systems was as follows: A: Eluent A Acq. Tag

Ultra Eluent A 100%; B: Eluent B Acq.Tag Ultra Eluent B: Aquabidest 90:10; C: Aquabidest; D: Eluent B Acq.Tag Ultra Eluent B 100%.

3. Result and discussion

3.1 Physical properties

The information regarding the physical properties such as size, shape, density, and legumes seeds is crucial in designing equipment for harvesting, transporting, cleaning, separating, packaging, storing, and processing of the seeds into different foods (Wani *et al*., 2017). The physical properties of dry lima bean seeds are presented in Table 1. The length, width, and thickness of the lima bean were slightly higher than those for the same legume reported by Gupta *et al*. (2018). The weight of this seed was higher than other legumes such as *Phaseolus coccineus L.* var. purple scarlet runner, *Phaseolus vulgaris* (Corzo-Ríos *et al*., 2020), *Vicia fabae* var. broad bean (Zhong *et al*. (2018), and *Phaseolus vulgaris* L. (Wani *et al*., 2017). The bulk density was similar to the previous report for lima beans (Gupta *et al*., 2018). Lima bean seed has a purple striped pattern (Figure 1). The colour of the mature seed and the young one is different, which is green. The increasing age of the seed changes the seed coat colour.

Figure 1. Indonesian dry seed and fresh pods of lima bean (*Phaseolus lunatus* L.)

3.2 Chemical characteristics

3.2.1 Proximate composition

Legumes are an excellent source of dietary proteins that play an important role in human nutrition by complementing other foods such as wheat and other cereals. Protein content generally falls between 15-30% (Hall *et al*., 2017). Lima beans are characterized as food sources with relatively high carbohydrates, rich in proteins but low in fat (Chel-Guerrero *et al*., 2012). The proximate composition of dry lima bean is shown in Table 2. Dry lima bean seed had a protein content of 15.93±0.55% that was lower than that reported by Jayalaxmi *et al*. (2016) of 24.6%, Drago *et al*. (2016) of 24.6%, and Ibeabuchi (2019) of 16.81%. The proteins in seeds of different lima beans showed significant variability that depends on their origins. Ash content represents the mineral content of the seeds. Lima bean contains 3.67±0.47% ash that was slightly higher than fava bean (3.40%) (Millar *et al*., 2019), and the ash content of beans was in the range of 2-5% (Hall *et al*., 2017). In this study, lima bean contained a very low fat (1.15±0.04%.). Lima bean contained carbohydrates of 62.8%, protein of 20.62%, fat of 0.93%, and ash of 3.55% (USDA, 2018). However, the nutritional composition differs within a species, with variation attributable to genetic (G) and environmental components (E), as well as their interaction (GxE) (Halimi *et al*., 2019).

3.2.2 Carbohydrate composition

Carbohydrate plays an essential role in human nutrition, with starch representing a major source of calories, and dietary fibre contributing to gut health (Chibbar *et al*., 2010). The available carbohydrate of the dry lima bean seed is presented in Table 2. Starch was the major component of carbohydrates of lima bean with a concentration of 41.96±1.10%, slightly higher than reported by Bello-Perez *et al*. (2007) with a 37-38% concentration. This starch content was lower than Bambara groundnut (48.12%) (Oyeleke *et al*., 2012) and in the range of mungbean starch content of 38.4-45.5% (Skylas *et al*., 2017). In this study, amylopectin (27.97±2.47%) was higher than amylose (13.99±1.73%) in the lima bean's starch. Lima bean has higher amylopectin than amylose of 13.99%, and this amylose content is lower than other legumes (Halimi *et al*., 2019).

Dietary fibre has an essential role in intestinal health and prevention of some diseases (Philips, 2013), has slow-release carbohydrates during digestion that are valuable in some disease management (Hayat *et al*., 2013). Lima bean is a source of dietary fibre comprised of soluble (SDF) and insoluble (IDF) fractions. The concentrations of total (TDF), soluble (SDF), and insoluble dietary fibre (IDF) reported for lima beans were 27.87±0.37%, 2.40%, 25.47%, respectively, where insoluble fibre was the main fraction. These findings were different to *P. vulgaris* (27.24%, 5.78%, 21.46%,

Table 1. Physical characteristics of dry lima bean seed

Physical characteristics	Value	Physical characteristics	Value
Thickness (cm)	0.72±0.05	Yield of decorated seed (%)	81.14±0.90
Width (cm)	1.50±0.08	Colour	
Length (cm)	2.32±0.08	L (lightness)	73.70±0.98
Volume/10 seeds (mL)	9.67±0.29	+a (redness)	16.70±0.10
Weight/100 seeds (g)	146.40±1.06	+b (yellowness)	7.33±0.21
Bulk density (g/mL)	1.51±0.37		

Table 2. Chemical composition (in dry basis) and protein fractions of lima bean seed

Component	Value	Component	Value
Proximate (%)		**Minerals (mg/100 g)**	
• Moisture	11.78±0.85	• Calcium	11.04±0.16
• Protein	15.93±0.55	• Iron	10.19±0.02
• Fat	1.15±0.04	• Potassium	38.21±0.01
• Ash	3.67±0.47	• Magnesium	183.93±0.12
• Carbohydrate	68.89±1.55	• Phosphorus	74.95±0.42
Carbohydrate (%)		**Anti-nutrition**	
• Starch	41.96±1.10	• Total phenolic compounds (mg/g)	1.29±0.02
• Amylose	13.99±1.73	• Phytic acid (mg/g)	11.57±0.03
• Amylopectin	27.97±2.47	• Saponins (mg/g)	16.84±0.42
• Total dietary fiber	27.87±0.37	• Trypsin inhibitors (TIU/g)	36.07±0.11
• Soluble dietary fiber	2.40±0.07	• Oligosaccharides (%)	
• Insoluble dietary fiber	25.47±0.32	– Raffinose	1.22±0.08
• Oligosaccharides	5.93±0.29	– Stachyose	4.61±0.21
Protein profile (%)		Cyanide (HCN) (mg/kg)	30.99±0.29
• Albumin	18.47±0.62		
• Globulin	56.20±2.00		
• Prolamin	3.14±0.20		
• Glutelin	22.69±1.60		

respectively) but similar to lentils of 26.86%, 2.40±0.07%, 24.46±0.32%, respectively (Duenas *et al*. 2016). Generally, pulses have higher IDF than SDF, with IDF ranging from 10 to 28%, and SDF was lower than 10% (Mudryj *et al*., 2014).

3.2.3 Minerals

Dry lima bean seed contains an appreciable amount of minerals represented by the ash of 3.30%. Magnesium (184 mg/100 g), phosphorus (75 mg/100 g), potassium (38 mg/100 g), calcium (11 mg/100 g), and iron (10 mg/100 g) were found in dry lima bean seed (Table 1). Jayalaxmi *et al*. (2016) reported that lima beans contained minerals such as potassium, calcium, phosphorus, magnesium, sodium, iron, copper, and zinc. In comparison, mungbean had minerals which comprise magnesium (166 mg/100 g), potassium (363 mg/100 g), calcium (1.2 mg/100 g), and iron (3.4 mg/100 g) (Kumar and Pandey, 2020). Legumes are generally high in potassium, magnesium, iron, manganese, and other minerals of interest for the pulse family, such as calcium. The pulses' mineral composition varies and is affected by many factors, including genotype or cultivar and growing environment (Hall *et al*., 2017).

3.2.4 Anti-nutrition and HCN

Although lima bean has good nutrition, it also contains anti-nutrients that can affect the bioavailability and digestibility of nutritional components (Boukid *et al*., 2016) and toxicant cyanide. The anti-nutrition of dry lima bean seeds is presented in Table 1. Dry lima bean seed contains phytic acid of 11.57±0.03 mg/g, which was higher than that reported by Bolade *et al*. (2017) of 8.86 mg/g. A wide range of variation in phytic acid concentration has been reported by Shi *et al*. (2018) for common beans of 15.64 to 18.32 mg/g, pea of 9.93 to 12.27 mg/g, and soybean of 22.91 mg/g. Phytic acid is an important storage form of phosphorus in a plant and an abundant plant phosphorus constituent of the edible legumes, cereals, oilseeds, and nuts (Nissar *et al*., 2017). Phytic acid can form complexes with numerous divalent and trivalent ions such as phosphorus, zinc, calcium, and magnesium, thus reducing mineral bioavailability and nutrient deficiency (Campos-Vega *et al*., 2010).

Total phenolic compounds (TPC) in dry lima bean seed were 1.29±0.02 mg/g. As a comparison TPC for pea (*Pisum sativum*) was 1.16 to 1.38 mg/g, bean (*Phaseolus vulgaris*) was 1.59 to 4.33 mg/g, and chickpea (*Cicer arietinum*) was 1.57 to 2.87 mg/g (Padhi *et al*., 2016). The phenolic composition is affected by genetic factors, climatic conditions, and variation in seed coat color. Phenolic compounds act as radical scavengers, reducing agents, and chelators of metal ions (Zhao *et al*., 2014).

Dry lima bean seed contained saponin of 16.84±0.42 mg/g, which was higher than kidney bean of 9.40 to 11.80 mg/g (Shimelis and Rakshit, 2007), and 4 times greater than mungbean (4.31 mg/g), and was approximate to soybean of 18.56 mg/g (Lee *et al*., 2011). Saponins are sterol or triterpene glycosides and in plants are usually found as a non-polar aglycone moiety (or sapogenin) bound to sugar molecules. The amphiphilic nature of saponins makes them strong surface-active compounds. However, saponins have a bitter taste but are able to form stable foams in aqueous solutions. Saponins are widely distributed in plants, and the primary sources of saponins are legumes (Lee *et al*., 2011). These compounds are considered as an

antinutritional factor, but recent studies show that saponin has blood cholesterol-lowering properties (Kumar and Pandey, 2020).

Trypsin inhibitors are a group of proteins that can reduce the digestive enzyme biological activity, trypsin and chymotrypsin. Trypsin is a proteolytic enzyme that is important for the digestion of proteins in living organisms (Vagadia *et al*., 2017). In this study, trypsin inhibitory activity of lima bean was 36.07±0.11 TIU/g, which was higher than that reported by Bolade *et al*. (2018) of 29.3 TIU/g in the same legume and also higher than cowpea of 26.48 TIU/g (Kalpanadevi and Mohan, 2013). The level of trypsin inhibitor activity in legume seeds might be reduced by several small-scale processing methods, including water soaking, boiling, roasting, microwave cooking, autoclaving, fermentation, and micronization (Khattab and Arntfield, 2009).

Raffinose and stachyose were oligosaccharides in lima beans with 1.22±0.08% and 4.61±0.21%, respectively. Stachyose and raffinose are also present in soybean at 0.9% and 4.1%, respectively (Kumar and Pandey, 2020). There are limited data reporting oligosaccharide concentrations in lima bean seeds from other origins. Raffinose and stachyose are responsible for flatulence. However, more recent studies showed that they have prebiotic properties (Felker *et al*., 2018).

The HCN content in dry lima bean seed was 30.99±0.29 mg/kg, and this concentration was lower than the report of Bolade *et al*. (2017) of 46.1 mg/kg. Cyanogenic glucosides in lima beans are converted into HCN when the tissue is disrupted, and the release endogenous enzyme b glucosidase and linamarase hydrolyse and liberate HCN, a sugar, and a keto compound. Hydrolysis occurs rapidly when immersed seeds are cooked in water, and most of the HCN then evaporates. The processing methods (roasting, soaking, boiling, germination, cooking, and fermentation) are effective in reducing cyanide content in the raw lima bean seeds (Lim, 2012; Gänzle, 2020)

3.3 Protein characteristics

3.3.1 Protein profile based on solubility

The protein profile of dry lima bean seed based on solubility is shown in Table 1. The predominant protein fraction was a salt-soluble fraction (globulins) of 56.20±2.00%, followed by an alkali-soluble fraction (glutelins) of 22.69±1.60%, and a water-soluble fraction (albumins) of 18.47±0.62%. Prolamin was a minor alcohol-soluble fraction in the lima bean of 3.14±0.20% of the total seed proteins. This result was similar to the hyacinth with globulin of 55%, glutelin of 27%, and albumin of 18% (Subagio, 2006). In most pulses, globulin and albumin are the major seed storage proteins, such as in cowpea with globulin of 45% to 50.3%, albumin of 31.2% to 35.5%, respectively. Glutelin is the third most abundant protein, comprised of 15.1% to 20.5%, while prolamin is a minor fraction that comprises 0.5 to 1.3% in seed (Alghamdi *et al*., 2019). The legumes protein profile is affected by genotypes, the cultivars, and the growing environment (Vasconcelos *et al*., 2010).

Characterization of the individual protein fractions of seed proteins is important as the basis for the legume protein utilization in food processing (Gupta *et al*., 2014). The electrophoretic pattern of the seed protein of the lima bean and its protein fractions are shown in Figure 2. The proteins of lima beans had a wide range of molecular weights between 10-141 kDa with 12 polypeptides. The molecular weight of these polypeptides are 10, 12, 14, 20, 22, 27, 35, 48, 70, 85, 116, and 141 kDa, and five bands were the most highly intense bands with molecular weights of 14, 20, 35, 48, and 70 kDa. This electrophoretic protein pattern was similar to that found in cowpea. Gupta *et al*. (2014) reported that cowpea had 12-14 polypeptides with molecular weights of 10; 14.13; 17.78; 19.95; 22.39; 28.18; 35.48; 44.67; 56.23; 97.72; 100; 104.7; 112.2 and 141.3 kDa. Sparvoli *et al*. (1996) reported that the protein of lima bean had polypeptides with molecular weights of one band (70 kDa), doublet (54-58 kDa), triplet (32, 35, and 38.5 kDa). The doublet (21-25 kDa) polypeptides were identified as phaseolin. Soetan and Animasaun (2019) reported that the molecular weight of protein in lima bean was distributed in the range of 11 to 135 kDa. García-Mora *et al*. (2015) reported a protein profile from *Phaseolus vulgaris*. var. pinto, with bands between 10 to 97 kDa. Bands with molecular weights of 25, 45, and 50 kDa were identified as phaseolin.

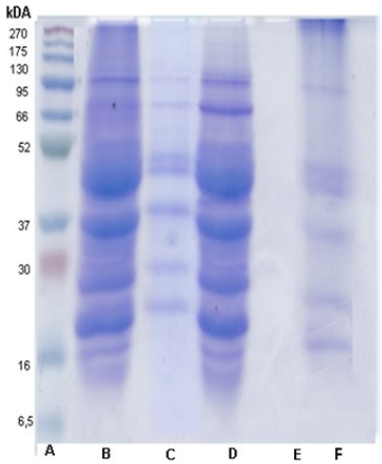

Figure 2. Protein fractions of dry lima bean seed in SDS-polyacrylamide gel electrophoresis. Line A: protein marker, B: total protein, C: albumin, D: Globulin, E: Prolamin, F: Glutelin

Globulin fraction of lima bean had 12 bands with molecular weight ranging between 10 to 124 kDa. The molecular weights of the polypeptides were 10, 13, 15, 22, 24, 27, 35, 48, 66, 90, 116, 124 kDa. Albumin had 18 to 116 kDa molecular weights, while glutelin fraction had six bands with a molecular weight of 13 to 109 kDa. According to Alghamdi *et al*. (2019), molecular weights of cowpea protein varied between 10 to 250, 15 to 110, 15 to 150, and 15 to 130 kDa for all protein, albumin, globulin, and glutelin, respectively, with some variations in number, width, and intensity of bands. Prolamin of lima bean in this study did not have visible polypeptides, and this finding was in accordance with cowpea reported by Alghamdi *et al*. (2019). Legumes have low prolamin concentration, and prolamin is mainly found in cereals (Tchiagam *et al*., 2011).

3.3.2 Amino acids composition

The protein quality of foods is assessed based on quantifying essential amino acids compared to nutritional requirements (Vaz Patto *et al*., 2015). The composition of essential and non-essential amino acids of lima beans is presented in Table 3. Lima bean contained a significant amount of essential amino acids. Lysine, leucine, valine, and phenylalanine were the major essential amino acids, and lima bean was rich in lysine. On the contrary, lima beans lacked methionine and cysteine (sulfur-containing amino acids). Methionine and cysteine are limiting amino acids of pulse proteins. Generally, the legume is an excellent ingredient combined in cereal-based flour blends to obtain a balanced and complete amino acid profile (Millar *et al*., 2019).

In this study, glutamic acid and aspartic acid were the two most abundant non-essential amino acids. Both were predominant non-essential amino acids legumes (Hall *et al*., 2107; Teka *et al*., 2020) and Bambara groundnut (Halimi *et al*., 2019). Globulins contained higher concentrations of glutamine, aspartic acid, and arginine than albumins and low concentrations of sulfur-containing amino acids (methionine, cysteine) and tryptophan (Dahl, 2012). Cysteine, methionine, and lysine contents were higher in the albumin fraction than beans' globulins (Semba *et al*., 2016).

4. Conclusion

Dry lima bean seed was a good nutrition source with a moderate concentration of protein, low in fat, and relatively high carbohydrates, which starch as a major source of carbohydrates and dietary fibre was in an appreciable amount. The globulins were the major seed protein, followed by glutelin and albumin, while prolamin was found in very low quantity. Lima bean seed was rich in essential amino acids. Like other legumes, it lacked methionine and cysteine. Dry lima bean was rich in minerals including magnesium, potassium, calcium, phosphorus, and iron, with the most abundant mineral, was magnesium. Some antinutritional compounds were found that included trypsin inhibitors, phytic acid, saponin, phenolic compounds, oligosaccharides, and a toxicant HCN.

Conflict of interest

The authors declare no conflict of interest.

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Table 3. Amino acids of dry lima bean compared to the FAO amino acids standard

Amino Acids (mg/g)	Lima Bean	*FAO Standard	Amino Acids (mg/g)	Lima Bean	*FAO Standard
Isoleucine	908	1100	Valine	983	1260
Phenylalanine	1283	1200	Tyrosine	555	740
Glutamic acid	2138	2960	Methionine	78	264
Leucine	1567	1800	Cystine	19	231
Aspartic acid	1726	2700	Histidine	624	639
Lysine	1014	1400	Threonine	1027	903
Glycine	885	883	Proline	736	950
Arginine	1355	1280	Serine	1424	1390
Alanine	755	1070			

*Source: Food And Agriculture Organization of the United Nations (2016)

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