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Characterization of nutritional and functional properties of Lima bean flour (*Phaseolus Lunatus* L.)

H T Palupi^{1,2*}, T Estiasih³, Yunianta³ and A Sutrisno³

¹Doctoral Student of Food Science, Faculty of Agricultural Technology, Universitas Brawijaya, Jalan Veteran, Malang, 65141, Indonesia

²Department of Food Science and Technology, Faculty of Agricultural, Universitas Yudharta Pasuruan, Pasuruan, 67152, Indonesia

³Department of Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Brawijaya, Jalan Veteran, Malang, 65141, Indonesia

E-mail: hapsari76@gmail.com

Abstract. Lima beans (*Phaseolus Lunatus* L.) are underutilized crops with an excellent profile. Processing to flour to enhance the starch and protein content, reducing anti-nutritional components and the same time diversifies their use as ingredients by altering their functional properties. The study aims to characterize nutritional, amino acids, anti-nutritional, pasting and functional properties of Lima bean flour from Indonesia's cultivated plant. The results showed that the Lima bean flour has a high carbohydrate concentration, moderate protein concentration, and low fat concentration. This flour has a balanced amino acid profile, rich in essential amino acids, highlighting them as a source of good quality protein for the food formulation of protein-enriched. The Lima bean flour contained 10.36 mg/g phytic acid, lower in phenolic content (0.63 mg/g) and concentration of HCN (8.83 mg/kg). The functional properties of the Lima bean flour swelling power, solubility, water absorption capacities, and oil absorption capacities were 6.88 g/g, 18.68%, 1.93 g/g, and 1.56 g/g respectively. Pasting properties of Lima bean flour exhibited peak, breakdown, final, and setback viscosity in 1172 cP, 83 cP, 2377 cP, 1288 cP respectively, and temperature pasting was high in 87°C. The study may provide useful information to consumers and food manufacturers that Lima bean flour is significant potential used to enhance the nutritional value of cereal-based foods.

1. Introduction

Legumes consumption as a source of vegetable protein for Indonesians increased in 2017 to 9.05 % compared to 2015 which was 8.56 %. To date, consumption of legumes still comes from a major commodity, soybeans, which are positioned as human food and ingredient for the food industry. Soybeans for domestic use are mostly imported. Soybean production in Indonesia in 2018 was 982.60 thousand tons, while direct consumption was around 1.99 million tons, so domestic soybean production was not able to meet current needs [1-2]. To reduce its dependence on soybean imports Indonesia, develop local commodities to substitute soybeans. Among the miscellaneous variety of legume species cultivated in Indonesia, one of which is Lima beans (*Phaseolus lunatus* L.) has been recognized as promising potential legumes [3-4]

Lima bean is an edible legume in the Leguminosae family that is considered as the best source of



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dietary proteins and essential nutrients derived from plants [5]. It is known by various names in Indonesia, including *kacang kara*, *kratok* and *kekara* [3]. This plant is well adapted in tropical environments, can be easily planted in tropical areas, and has been used for food consumed [4-5]. Lima bean seeds are considered high-quality sources of nutrients due to a valuable source of proteins ranging from 14.24 % to 24.92 %, with a balanced amino acids profile, and abundant in essential amino acids [3],[6-7]. They have a high content of complex carbohydrates (55–64 %), mainly starch and dietary fiber. The slow-release nature of the carbohydrate constituents of the most edible Lima bean is a particularly appealing feature [8-10]. However, Lima bean seeds contain anti-nutritional factors for example phytins, tannins, trypsin inhibitors and cyanogenic glycosides, which act metabolic functions in humans who consume these foods regularly [3],[11]. These effects can be positive, negative, or both [12]. Antinutritional factors are those that harm diet quality. On the other hand, these same compounds may have health benefits that exhibit antioxidant, anti-cancer, and other specific properties [13]. This legume is among that underutilized leguminous plant [4] and minor grain legume and has diverse varieties worldwide [14]. According to the USDA, Lima bean is well-known as potentially underutilized legumes due to their hardness, exceptional nutritional profiles, and desirable protein content, particularly in their seeds [15-16]. Figure 1 depicts the plant and the dry seed of Lima bean, its dehulled seed and its flour.



Figure 1. The plant of Lima bean (1), the dry seed of Lima bean (2), dehulled seed (3) and Lima bean flour (4).

Different research into legume-based flours and the potential applications have been interested growing due to flour that can conveniently be used for the food formulations and demonstrated to be a way of improving the availability and consumption of the legume seeds [17-20]. Legumes flour contains a high concentration of high-quality protein, resistant starch, and dietary fiber. They are incorporated into the diet alongside cereals as complementary protein sources, whereas legumes are high in lysine but low in methionine and cysteine [18]. The successful utilization of flour from different legumes is dependent on pasting and functional properties [20], which enhance their efficiency and performance in the food products. The functional properties of legume flours are mainly influenced by carbohydrates, proteins and other components to various extents [17],[21]. Legume flours could be applied in the preparation of ready-to-eat breakfast cereals, snacks, bread, frying batters, and others [17]. A study of the characterization of Lima bean flours is necessary because flours from different sources will have a differential on nutritional profile and functional properties, considering the wide variety of Lima bean subspecies. The study aims to characterize the nutritional, amino acids, anti-nutritional, pasting, and functional properties of Lima bean flour.

2. Material and methods

2.1. Materials

The Lima bean seed (*Phaseolus lunatus* L.) was obtained from a local farmer in Malang regency, East Java, Indonesia. Material for analysis are hydrobath, gallic acid, Folin-Ciocalteu, NaOH, HCl, Iodin, acetic acids and glucose standard. All reagents analyses used were of analytical grade.

2.2. Equipment

The equipment used in this study were a digital scale, 100 mesh sieve, cabinet dryer, and disc mill grinder. Equipment used for analysis included glassware, thermometer, spectrophotometer (UNICO UV Vis-2100), centrifuged (PLC-012E), shaker, color reader, water bath, LCMS (the Waters Xevo TQD), Rapid Visco Analyzer (RVA).

2.3 Lima bean flour preparation

The dehulled Lima bean flour was prepared according to the method of [22]. After cleaning and removing of any broken seeds, dirt, and other extraneous materials, 1 kg portions of Lima bean were washed and soaked in 2 L of potable water at room temperature ($30 \pm 2^\circ\text{C}$) for 8 h. The soaked seeds were drained and dehulled manually by rubbing in between palms to remove the hulls. The dehulled seeds were rinsed, spread on the trays and dried in a cabinet dryer at 40°C for 12 h with occasional stirring. The dried beans were grinded so that the flour would pass through a 100-mesh stainless steel sieve.

2.4 Flour analysis

2.4.1 Proximate and carbohydrate analysis. Proximate analysis of the Lima bean flour was carried out to determine crude protein, ash, crude fat, and moisture content by AOAC (2005) methods [23]. The total carbohydrate was determined by difference ($100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ protein} + \% \text{ fat})$). The starch content was measured by the direct acid hydrolysis method [23]. The apparent amylose content of flour was determined by the method of William et al. (1970) with slight modification [17]. Total Dietary Fiber (TDF), Soluble Dietary Fiber (SDF), and Insoluble Dietary Fiber (IDF) were measured by the enzymatic-gravimetric method of AOAC (991.43) [23]. All the analysis was conducted in three replications.

2.4.2 Amino acid analysis. LC-MS/MS was used to determine the amino acid profile. This procedure was investigated as described by Chang et al. (1989) in [6]. In the presence of 1 % phenol (v/v), the sample was hydrolyzed with 6 N HCl at 110°C for 24 hours. The sample solution was neutralized by adding 6 N NaOH to the sample solution and diluting it with distilled water. A 0.22- μm syringe filter was used to filter the solution. The LS-MS/MS injected the sample derivatives (the Water Xevo TQD, Waters, USA). Solvent A: 0.1 % pentadecafluorooctanoat acid in water/ CH_3CN with 0.1 % formic acid (99.5 %:0.5 %) and Solvent B: 0.1 % pentadecafluorooctanoat acid in water/ CH_3CN with 0.1 % formic acid (99.5 %:0.5 %) were used (10 %:90 %).

2.4.3 Antinutritions and HCN analysis. The total phenolics content (TPC) was determined using a method modified slightly from that described by Lai et al. [24]. Based on a standard prepared with various concentrations of gallic acid standard the results were expressed in mg gallic acid/g extract. Lai et al. [24] used the colorimetric (Wade Reagent) method to determine the phytic acid content. The results were calculated and expressed as mg sodium phytate/g extract using the sodium phytate calibration curve. The alkaline titration method [11] was used to determine the cyanide (HCN) content of the samples.

2.4.4 Pasting property analysis. A Rapid Visco Analyzer (RVA) was used to determine the pasting properties of a flour suspension. The starch slurry (9 %, w/w, db) was loaded into the RVA instrument and equilibrated at 50°C for 1 min before being heated to 95°C in 3.5 min and held at 95°C for 3 min then cooled to 50°C in 3.5 min and held at 50°C for 2 min. A rotation speed of 160 rpm was maintained throughout the analysis, except for rapid stirring at 960 rpm to disperse the sample for the first 10 sec. Parameters that has been measured were pasting temperature, peak, through, final, breakdown, and setback viscosity.

2.4.5 Functional properties. The solubility, swelling power, water absorption capacity, and oil absorption capacity of Lima bean flour were investigated. Swelling power and solubility were determined using a slightly modified method [25]. 1 g (S) flour was placed in a previously weighed centrifuge tube, dispersed in 50 mL of distilled water, and heated in a water bath at 90°C for 30 min. The tube was then cooled and centrifuged for 15 min at 3000 g. The obtained supernatant was decanted carefully, and the wet mass of the sedimented retained was weighed and recorded (B). The supernatant was transferred to a pre-weighed petri dish and dried at 110° C until its constant weight (A). The solubility was expressed as a percentage of dried solid weight based on the dry sample weight. The swelling power was calculated as the weight of wet sediment divided by the weight of the initial dry sample.

$$\text{Solubility \%} = \frac{A}{S} \quad (1)$$

$$\text{Swelling power (g/g)} = B \times \frac{100}{S (100 - \% \text{solubility})} \quad (2)$$

A= Weight of dried supernatant

S= Weight of sample

B= Weight of wet sediment

Water absorption capacity (WAC) and oil absorption capacity (OAC) were determined using the method described by [17] with slight modifications. A centrifuge tube weighed with 10 mL of distilled water or oil and 1 g of flour. The mixture was vortexed for 10 sec every 5 min for 30 min, followed by 30 min of centrifugation at 3000 rpm. The supernatant was decanted after centrifugation, and the centrifuge tubes and precipitate were re-weighed. WAC and OAC were calculated as grams of water/oil absorbed per gram of sample.

3. Results and discussion

3.1. Proximate and carbohydrate analysis

Lima bean have a high carbohydrates concentration, moderate concentration of protein and low fat concentration (Data is not shown). The proximate composition of this study is similar to previously reported values by Du et al. [8] where Lima bean flour contained moisture, protein, fat, ash and starch content were 10.2, 23.92, 1.15, 4.30, and 44.55% respectively. Lima bean flour can be used as a substitute for high-protein foods to help people overcome protein deficiency [19]. Total ash content generally represents the mineral content of legumes. The high ash content of pulse flour means they have the potential to increase mineral intake in the diet when combined in food products with low ash flour such as wheat [18],[20]. Starch is the main content of carbohydrate component in Lima bean flour. The high starch content indicates that these flour have the potential to be developed as a food crop.

Table 1. The carbohydrates composition of Lima bean flour.

Parameter	Lima bean flour (% wb)
Carbohydrates	71.69 ±0.34
Total dietary fiber (TDF)	13.34 ±2.68
Soluble dietary fiber (SDF)	2.31 ±0.85
Insoluble dietary fiber (IDF)	11.03 ±1.83

Values are expressed as means±standard deviation (n = 3)

Lima bean flour exhibited 13.34 % total dietary fiber. This value was similarly reported by Chinma et al. [20] in 13.3% for raw Bambara groundnut flour. Dietary fiber can be further divided into SDF and IDF fractions. Lima bean flour exhibited a greater amounts concentration of IDF (11.03 %) than SDF (2.31 %). For all legumes, this pattern has been established [28]. Consumption of food rich in

dietary fiber was recommended within diets for management due to protective effects against chronic diseases such as diabetes, cardiovascular diseases, cancer and hypercholesterolemia [20],[28].

3.2. Amino acids

Legumes are best known for being a good source of proteins with a well-balanced amino acid profile and a high concentration of essential amino acids [31]. Table 2 shows the amino acid composition of Lima bean flour. The amino acid content in this study was in close range with those previously reported for Bambara groundnut flour [20]. The essential amino acids phenylalanine, leucine, and lysine had the highest concentrations. The most abundant non-essential amino acids in Lima bean flour were glutamic acid (2.14 mg/100 g) and aspartic acid (1.88 mg/100 g), respectively. In Lima bean flour, sulfur-containing amino acids (methionine and cysteine) are considered limiting essential amino acids. Methionine and cysteine were higher in quality than wheat flour, but lysine, phenylalanine, arginine, and aspartic acid were lower [18]. The amino acid content of Lima bean flours is similar to that of most other legume flours [18],[20]. Boukid et al. [32] proposed that legume flour be used in wheat-based flour blends to achieve a balanced and complete amino acid profile.

Table 2. Amino acids composition (g/100g) of Lima bean flour.

Amino acids	Value (g/100 g)	Amino acids	Value (g/100 g)
Essential amino acids		Non-Essential amino acids	
Lysine	0.80	Valine	0.81
Thryptophan	0.02	Cysteine	0.08
Phenylalanine	0.91	Aspartic acid	1.89
Methionine	0.08	Glutamic acid	2.14
Threonine	0.84	Serine	1.13
Leucine	1.42	Glycine	0.74
Isoleucine	0.77	Arginine	0.54
Histidine	0.09	Alanine	0.85
		Proline	0.87
		Tyrosine	0.21

3.3 Antinutrient

Lima bean flour had a total phenolic content (TPC) of 0.63 mg/g. The content was lower than fava bean, green pea, and yellow pea flour [18]. Tannins and phenolic acids are two types of total phenols that can affect the overall digestibility of pulse flour. Lima bean flour may have a higher protein bioavailability due to its lower TPC. Lima bean flour had a phytic acid content of 10.36 mg/g. These results had a higher phytic acid content than Bambara groundnut [20] but were lower than Ratnawati et al [27] value in soybean flour. While phytic acid may have some beneficial effects [17], high consumption can be harmful to health due to their ability to produce chemical complexes with minerals, particularly iron and zinc, reducing their absorption through the diet [18]. Moreover, Lima bean flour was at a low concentration of HCN (8.83 mg/kg). The flour values in the study were safe for direct human consumption because the levels of cyanide in the flour do not exceed the WHO safe limit level of 10 mg/kg in flour [33].

Table 3. The antinutritional factor of Lima bean flour.

Anti-nutrient	Value
TPC (mg/g)	0.63 ± 0.20
Phytic acid (mg/g)	10.36 ± 0.56
HCN (mg/kg)	8.83 ± 1.26

Values are expressed as means±standard deviation (n = 3)

3.4 Pasting and functional properties

Table 4 shows the Lima bean's pasting properties. Peak viscosity, breakdown viscosity, final viscosity, setback viscosity, and pasting temperature were tested as pasting properties. The variation in the viscosity of starch over water during heating and cooling treatments in a controlled conditions is referred to as pasting properties. Pasting properties are influenced by starch properties and interactions with other components [29]. Peak and trough viscosity were discovered in 1172 and 1089 respectively. The peak viscosity was showed higher than mungbean (909 cP) and red kidney bean (827 cP) [27]. The peak viscosity refers to the range of starch granules' ability to freely swell upon heating, resulting in a rise in paste viscosity before amylose dissolution [21]. The peak viscosity indicated how high or low the sample's viscosity could go, which was determined by the structure of the starch granules, the proportion of amylose and amylopectin in the granules, and the possible combinations with other ingredients [34]. A decrease in viscosity was observed after trough viscosity, known as breakdown viscosity. Lima bean flour was observed 83 cP in breakdown viscosity and it similar was reported for red kidney bean flour (85 cP) [27]. The lower breakdown viscosity exhibited Lima bean flour is indication of its good paste stability and strong shearing resistance [17].

The final and setback viscosities of Lima bean flours were 2377 cP and 1288 cP. This value is considered high when compared to red kidney bean flour (1606 cP and 863 cP) and mungbean flour (1387 cP and 518 cP) [27]. The final viscosity, also known as cold paste viscosity revealed the quality and stability of cooked starch. The high of final viscosities could be attributed to its high amylose content [21]. Setback viscosity has a result of temperature fall leading to amylopectin molecules reassociating and amylose leaching. It act as an index of starch retrogradation and is associated with amylose content. This parameter is a useful index in the prediction of flour products storage life [30] The Lima bean flour had a high temperature pasting at 87⁰ C, exhibited lower than in a previous investigation by Du et al. [8] in 83⁰ C. The high pasting temperature of Lima bean flour indicates that its starch is highly resistant to swelling and rupture. High viscosity is desirable and advantageous for industrial application where the goal is the thickening power and retrogradation must be controlled.

Flours have functional properties that increase their use as a food raw material. The functional properties of flour are physicochemical characteristics that can affect the behaviour of flour in the food system during the process. They are strongly influenced by the flour composition and reflect interactions between flour components [29]. Characteristics of flour in beans commonly observed are swelling, solubility, WAC and OAC. Table 4 shows the swelling power and solubility of Lima bean flour. As shown in the data, the flour had a swelling power of 6.8 g/g. and markedly lower than mung bean (10.52 g/g) and red kidney bean (10.09 g/g) [27], which is close to the results of Bambara groundnut flour (7.13 g/g) [20]. The degree of swelling and the amount soluble swelling is determined on the starch species. It has been proposed that Bonding forces within starch granules are thought to affect swelling power [22]. Meanwhile, Du et al [8] stated swelling power is related to the hydrophilicity and gelation capacity of biomacromolecules, such as starch and protein, in flour. The amorphous region of starch can a lot of swelling by absorb much water, causing starch swelling. However, the various components of legume flours may cause different interactions with water. The solubility of the Lima bean flour was high 18.68%. This value was above those of Bambara groundnut (12.85 %) [22] and similar values of solubility were reported by Ratnawati et al. [27] for of mung bean (18.80 %). The solubility may be due to the presence of soluble molecule components (e.g. soluble sugars and amino acids) because of the disruption of starch granule [21],[27].

Lima bean flour had a WAC of 1.56 g/g. The findings are consistent with the reported by Du et al. [8] Lima bean, mung bean, and red kidney bean flour have WAC in the range of 1.17–1.67 g/g. WAC influences food preparations' functional and sensory properties. Using flours with a higher WAC may aid in the preservation of a soft texture [8]. It also indicates that the higher proportion of hydrophilic residues in carbohydrate and protein constituents of legumes flours [35]. Lima bean flour had a high OAC content of 1.93 g/g. The OAC revealed that protein molecules have a higher proportion of hydrophobic than hydrophilic groups on their surfaces. OAC was impacted by a large number of available non-polar side chains in its protein molecules [8, 35].

Table 4. Pasting and functional properties of Lima bean flour.

Parameters	Value	Parameters	Value
Pasting properties		Functional properties	
Peak viscosity (cP)	1172	Swelling power (g/g)	6.88 ±0.06
Through viscosity (cP)	1089	Solubility (%)	18.68 ±0.11
Breakdown viscosity (cP)	83	WAC (g/g)	1.56 ±0.55
Final viscosity (cP)	2377	OAC (g/g)	1.93 ±1.20
Setback viscosity (cP)	1288		
Pasting temperature (°C)	87		

Values are expressed as means±standard deviation (n = 3), except pasting properties

4. Conclusions

The Lima bean flour was found to be a good and valuable source of proteins, starch, and dietary fiber. Besides, they contained balanced levels of essential amino acids which suggest the potential use in food formulation. Likewise, Lima bean flour was observed to have a lower content of total phenolic content so that it can increase protein digestibility. Lima bean flour has high peak viscosity and low breakdown viscosity exhibited good paste stability and strong shearing resistance. The Lima bean flour had a high temperature pasting at 87°C, exhibited high resistance to swelling and rupture. This source of starch has functional characteristics that make it exciting and appealing for various industrial applications. This has opened avenues that Lima bean flours to be used in food systems. However, future studies are still needed to processing flour to major components (proteins concentrate and starch), this strategy can be adopted to add value and increase consumption of Lima bean herein.

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