Moses et al., 1:7
http://dx.doi.org/10.4172/scientificreports.349

Open Access Scientific Reports

Research Article Open Access

The Proximate Composition and Functional Properties of Full-Fat Flour, and Protein Isolate of Lima Bean (*Phaseolus Lunatus*)

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Abstract

The proximate composition and functional properties of full fat flour, defatted fat flour and protein isolate from lima bean (*Pheseolus lunatus*) were determined through laboratory analysis. The protein isolated was found to have 86.4% of protein while the full fat and defatted flours have 26.13% and 6.83% protein respectively. Also the protein isolate contain more ash and moisture 6.00 and 5.99%, than the free fat flour which had 5.45% ash and 5.24% moisture content. The defatted flour contained 5.53% ash and 5.81% moisture, both of which were slightly higher than that of the full fat flour. Both the full fat flour and the defatted flour contained more carbohydrate, fiber and fat than the protein isolate which was found to be fibre and fat free. The protein isolate was also found to exhibit better functional properties with higher water and oil absorption capacities than the flour samples. The full fat flour had the highest foaming capacity (7.33%) while the defatted flour had the higher emulsifying capacity (89.17%). The protein isolate had the least emulsifying capacity of 36.62% but recorded maximum swelling index of 2.8 as against 1.32 and 1.16 for full fat flour and defatted flour respectively. It was therefore observed that the lima bean protein isolate has good potentials as an indigenous ingredient in food products.

Introduction

Protein malnutrition is one of the major nutritional problems in the developing world. The specific maladies like kwashiorkor and marasmus are prevalent in the children owing to protein deficiency, whereas in adults, results in poor health and reduced work capacity. Bridging the gap between increased food consumption and production is among the most challenging tasks round the globe especially in developing countries [1]. The existing problems of food security and malnutrition coupled with escalating population, uncertain crop yield and high cost of animal based food supplies have urged to identify and incorporate unconventional protein sources to enrich the traditional formulation [2].

Lima bean (*Phaseolus lunatus*) like many other legumes is a rich source of plant protein which compares favorably with other legumes one of which are leading protein source in many parts of the world. Notwithstanding the similarities between nutritional qualities of lima bean with other legumes, not much information is available on its protein isolate. Against this background, this project was designed to produce protein isolate, defatted and full fat flour form lima bean and determine its quality and functional properties as a means of assessing its acceptability and application in food system.

Generally there are two sources of protein i.e. animal and plant; provision of adequate animal proteins is difficult due to high cost and changing consumer's attitudes towards animal based proteins. Consumers are more conscious in the food selection owing to growing awareness about nutritional dependent ailment. An alternative for improving protein intake of the people is to supplement for diet with plant proteins. For that reason, consumption of plant protein isolate with special reference to legumes is beneficial [3]. Legumes are inexpensive source of proteins with high nutritional profile and after cereal important food source for humans [4]. Protein content in legumes ranged from 17 – 40%, contrasting with that of cereals 7 – 13% and comparable with meat 18 – 25% [5]. Being a cheap source of protein for low income group of population, legumes are commonly used as a substitute for meat and play a significant role in alleviating the protein –energy malnutrition. In addition, they are also a good source of complex carbohydrates. Dietary fiber and contain significant amounts of vitamins and minerals. Protein isolates obtained from the legumes through isoelectric precipitation have high percentage of protein contents, which make them potential protein sources for food industries applications and this potential usefulness will depend on their functional properties. Functional properties are the physical and chemical characteristics of the specific protein influencing its behavior in food system during processing, storage, cooking and consumption. The examples of functional properties include bulk density, protein solubility, water and oil absorption capacity, emulsifying and foaming properties [6].

The factors that affect functional behavior of proteins in foods are their size, shape, amino acid composition and sequence, net charge, hydrophobic acid structure, molecular rigidity in response to external environment (humidity, temperature, salt concentration) or interaction with other food constituents [7]. Proteins form legumes have gained immense importance in modern food design due to their nutritional value and favourable functional properties.

Materials and Methods

Source of materials and reagents used

Mature Lima bean seeds (*Phaseolus lunatus*) were obtained from the central laboratory service unit of National Root Crops Research Institute, Umudike, Abia States. All chemicals used were of analytical grade.

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Received September 21, 2012; **Published** September 28, 2012

Citation: Moses O, Olawuni I, Iwouno JO (2012) The Proximate Composition and Functional Properties of Full-Fat Flour, and Protein Isolate of Lima Bean *(Phaseolus Lunatus).* 1:349. doi:10.4172/scientificreports.349

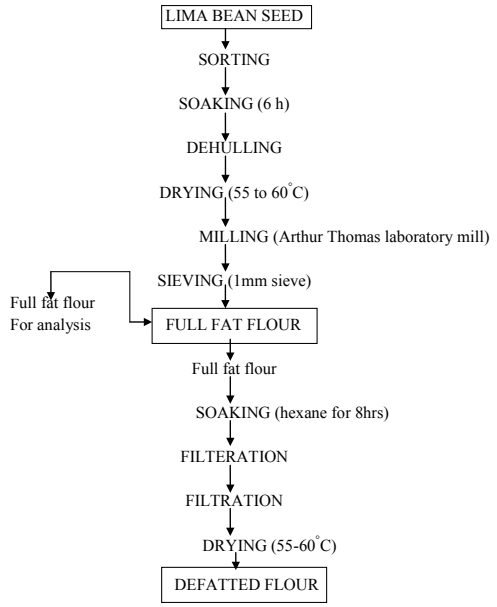

Figure 1: Production of full fat and defatted lima bean flour. Source: Hassan et al. (2010).

Production of defatted flour form lima bean

Defatted and full-fat lima bean flour was produced according to the method of Hassan et al. [8]. This is shown in figure 1. Lima bean seeds were sorted removing diseased ones and other extraneous materials. The "healthy" seeds were soaked in water for six hours to facilitate the removal of the hulls. Dehulling was done manually and the dehulled seed cotyledon were spread on laboratory tray and dried in Carbolite moisture extractor oven at 55 to 60°C for 24 hours. The dry cotyledons were ground into powdered from in an Arthur Thomal casoratry machine in which the grind sample was sieved through a 1 mm test sieve. Part of this ground sample was set apart for analysis as raw flour sample while another part was defatted by soaking in normal hexane for 8 hours after which it was removed by filtration and air-dried at room temperature. The defatted sample was set aside for protein isolate production and analysis.

Production of protein isolate

Protein isolates were produced according to the method described by Makri et al. [9] as shown in figure 2. Plate 1 shows the dehulled, undehulled lima bean. Legume flours were defatted by slurring the sample in an organic solvent using soxhlet apparatus. After extraction, solvent was recovered through rotating evaporator. The defatted flour was dispersed in distilled water (1/10); pH was adjusted to 9.5 with the aid of 1 N NaOH and shaken for 40 min using mechanical shaker. Following centrifugation at 400 rpm for 20 min supernatant was collected. The residue was collected and dispersed in distilled water (1/10) and stirred. Following centrifugation at 4000 rpm for 20 min, the respective supernatant was collected and combined with the supernatant collected from the first centrifugation and the pH was adjusted to 4.5, the precipitated protein was recovered by centrifugation at 4000 rpm for 20 min, neutralized and freeze dried.

Proximate analysis

Associations of Official Analytical Chemist (A.O.A.C) [10], procedure were used to determine the proximate composition of the different vegetable sample. Triplicate determinations were made with the average reported.

Moisture Content Determination

Two grams of each of the sample was weighed into dried weighed crucibles. The sample was put into a moisture extraction oven at 105°C and heated for 3h. The dried sample was put into desiccators, allowed to cool and reweighed. This process was repeated until a constant weight is obtained. The difference between the weight was calculated a percentage of the original sample.

$$\text{Percentage moisture} = \frac{W2 - W3 \times 100}{W2 - W1}$$

Where

W1 = Initial weight of empty dish

W2 = Weight of dish + undried sample

W3 = Weight of dish + dried sample.

Ash Content Determination

Two grams of each of the samples was weighed into crucible, heated in a moisture extraction oven for 3 h at 100°C before being transferred into a muffle furnace until it turned white and free of carbon. The sample was then removed from the furnace, cooled in desiccators to a room temperature and reweighed immediately. The weight of the residue was then calculated as ash content expressed in percentage.

$$\text{Percentage ash} = \frac{\text{Weight of ash} \times 100}{\text{Weight of sample}}$$

Crude Protein Determination

The micro kjeldahl method described by A.O.A.C [10] was used. Two grams, each of the samples was mixed with 10 ml of concentrated H_2SO_4 in a heating tube. One tablet of selenium catalyst was added to the tube and mixture heated inside a fume cupboard. The digest was transferred into a 100 ml volumetric flask and made up with distilled water. Ten milliliter portion of the digest was mixed with equal volume of 45% NaOH solution and poured into a kjeldahl distillation apparatus. The mixture was distilled and the distillate collected into 4% boric acid

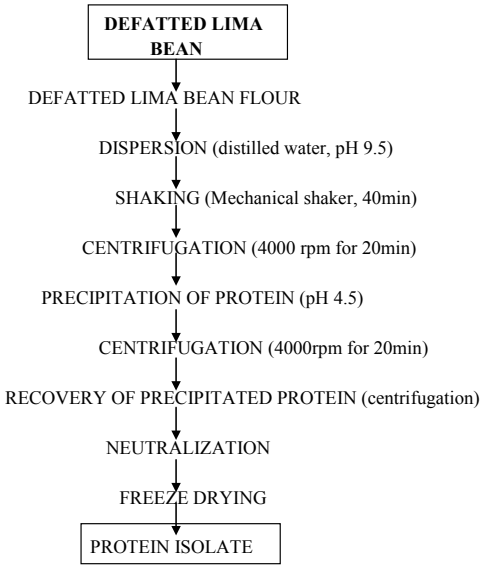

Figure 2: Production of protein isolate.

solution containing 3 drops of zua zaga indicator. A total of 50 ml distillate was collected and titrated as well. The sample was duplicated and the average value taken. The nitrogen content was calculated and multiplied with 6.25 to obtain the crude protein content.

$$\text{This is given as Percentage Nitrogen} = \frac{(100 \times N \times 14 \times v_f)\, T}{100 \times v_a}$$

Where;

W = Weight of the ample

N = Normality of the titrate (0.1N)

v_f = Total volume of the digest = 100ml

T = Titre value

v_a = Aliquot volume distilled.

Fat Content Determination

Two grams sample was loosely wrapped with a filter paper and put into the thimble which is fitted to a clean round bottom flask, which has been cleaned, dried and weighed. The flask contained 120 ml of petroleum ether. The sample was heated with a heating mantle and allowed to reflux for 5 h. The heating was then stopped and the thimbles with the spent samples kept and later weighed. The difference in weight was received as mass of fat and is expressed percentage of the sample. The percentage oil content is calculated;

$$\text{Percentage fat} = \frac{W2 - W1 \times 100}{W3}$$

Where

W_1 = Weight of the empty extraction flask

W_2 = Weight of the flask and oil extracted

W_3 = Weight of the sample

Crude Fibre Determination

Two grams sample was put into 200 ml of 1.25% of H_2SO_4 and boiled for 30 minutes. The solution and content then poured into bucheur funnel equipped with muslin cloth and secured with elastic band. This was allowed to filter and residue washed with hot water to free it from acid. The residue was then put into 200 ml boiling 1.25% NaOH and boiled for 30 min, then filtered. It was then washed twice with alcohol; the material obtained was washed thrice with petroleum ester. The residue obtained was put in a clean dry crucible and dried in the moisture extraction oven to a constant weight. The dried crucible was removed, cooled and weighed. Then difference of weight (i.e. loss in ignition) is recorded as crucible fibre and expressed in percentage of the original weight.

$$\text{Where Percentage crude fibre} = \frac{W_1 - W_2 \times 100}{W_t}$$

W_1 = Weight of sample before incineration

W_2 = Weight of sample after incineration

W_3 = Weight of original sample.

Carbohydrate Content Determination

The nitrogen free method described by A.O.A.C [10] was used. The carbohydrate is calculated as weight by difference between 100 and the summation of other proximate parameters as Nitrogen Free Extract (NFE).

Percentage carbohydrate (NFE) = 100 – (M + P + F_1 + F_2)

Where

M = moisture

P = protein

F_1 = fat

A = ash

F_2 = fibre

Functional Properties of the Flour

The following functional properties of the flour were evaluated;

Water and oil absorption capacity

Water and oil absorption properties of the composite flour were determined with the following methods of Sathe et al. [11]. Briefly, the flour samples (2 g) were mixed with 20 ml distilled water for water absorption and 20 ml of oil for the absorption in a blender at high speed for 30 (s). Samples were then allowed to stand at 30°C for 30 min then centrifuged at 10,000 rpm for 30 min. The volume of supernatant in a graduated cylinder was noted. Density of water was taken to be 1 g/ml and that of oil determined to be 0.93 g/ml. Means of triplicate determinations were reported.

Foaming capacity

Foaming capacity was studied according to the methods described by Desphance. The flour sample (0.5 g) was blended for 30 min in distilled water (40 ml) at top seed in a blender. The blender was rinsed with 10 ml distilled water and then gently added to the graduated cylinder. Foam volume in the cylinder. Foam volume in the cylinder was recorded per sample after 30 min standing. Triplicate measurement was taken for each sample and mean values recorded.

Emulsion capacity

A flour sample (2 g) and distilled water (100 ml) were blended for 30 (s) in a blender at high speed (ca.100 rpm). After complete dispersion, peanut oil was added from a burette in streams of about 5 ml. Blending continued until there appears separation into two layers. Emulsification determinations were then carried out at 30°C and expressed as grams of oil emulsified by 1 g flour. Triplicate measurements were made and average results taken.

Swelling index

The method of Abbey and Ibeh [12] was employed. One gram of the flour samples were weighed into 10 ml graduation measuring cylinder. Five milliliters of distilled water was carefully added and the volume occupied by the sample was recorded. The sample was allowed to stand undistributed in water for 1 h and the volume occupied after swelling.

Composition		Samples		
(%)	Full Fat	Defatted	Protein Isolate	LSD
Moisture	5.24 ± 0.02^c	5.813 ± 0.0115^b	5.993 ± 0.0416^a	0.0549
Protein	26.13 ± 0.1966^c	26.833 ± 0.202^b	86.46 ± 0.3453^a	0.5142
Ash	5.453 ± 0.0115^c	5.53 ± 0.0115^b	5.9967 ± 0.0115^a	0.0231
Fat	0.873 ± 0.0115^a	0.093 ± 0.0231^b	-	0.0298
Crude Fiber	5.23 ± 0.0115^b	5.31 ± 0.0231^a	-	0.0298
Carbohydrate	57.08 ± 0.1916^a	56.43 ± 0.206^a	1.6+0.399^c	1.123

Table 1: Mean values of the proximate composition of the full fatted, defatted flour and protein isolate from lima bean.

Property	Samples			
(%)	Full fat	Defatted	Protein Isolate	LSD
WAC	1.8667 ± 0.057^{c}	2.766 ± 0.057^{b}	4.23 ± 0.115^{a}	0.1673
OAC	2.75 ± 0.9526^{c}	3.26 ± 0.052^{b}	3.69 ± 0.052^{a}	1.101
EMC	82.4 ± 0.2879^{c}	89.18 ± 0.4497^{b}	36.62 ± 0.3646^{a}	0.7458
SI	1.3167 ± 0.0231^{b}	1.16 ± 0.0436^{c}	2.8+0.0854^{a}	0.1138
FC	7.33 ± 0.5774^{b}	12.33 ± 1.1547^{a}	3.667+1.1547^{c}	1.9979

Values are means of triplicate determinations
Means with different superscript along the row differs significantly ($p<0.05$).
WAC – Water absorption capacity (g/ml)
OAC – Oil absorption capacity (g/ml)
EMC – Emulsifying capacity (%)
SI – Swelling power (%)
FC – Foaming capacity (g/ml)

Table 2: Mean values of the functional properties of the full fat defatted flour and protein isolate from lima bean.

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

Statistical analysis

Determinations were done in three replicates. The Least Significant Difference test (LSD) was used to test differences between means.

Results and Discussion

Proximate composition

The result of the proximate composition of the defatted flour, full fat and protein isolate from lima bean is shown in Table 1.

Proximate composition is important in determining the quality of raw material and often the basis for establishing the nutritional value and overall acceptance of the consumers. The values for moisture contents in full fat flour (5.24 ± 0.02%) and defatted flour (5.81 ± 0.02%) were statistically equal at 5% level. There was no moisture content in the protein isolate. Findings of present study are collaborated with the research investigation by Rahma et al. [13] who reported values of 4% to 13% moisture for lima beans. This low moisture content was desirable because if the moisture content of the flour is more than 14%, there is danger of bacterial action and mould growth and such flour was kept badly and develops hydrolytic rancidity.

Protein isolates exhibit maximum protein content at 86.46 ± 0.3453% which differs significantly ($p<0.05$) form that of full fat (26.13 ± 0.1966%) which was high compared with defatted flour sample (26.83 ± 0.2012%). The high protein content of protein isolate is as result of the production process which increases the composition of protein in the finished product. The proteins are polymer and amino acids and their relative proportion represents its quality that is dependent on the genetic makeup of legume. The variations in protein contents are attributed to genetic makeup of legumes along with some environmental factors [14]. Fat contents ranged in different legume flour sample from 0.0%.

The protein isolate to 0.8733 ± 0.0155% which is the highest amount noted in the full fat flour. The composition of fat in the samples was as a result of the processing method adopted. The crude fibre data shows that defatting insignificantly ($p<0.05$) increased the crude fibre content from 5.2267% (for full fat flour) to 5.3067% for defatted flour. There was no crude fibre in protein isolate due to the processing method where other nutrients are removed. Results for ash content demonstrated significantly higher amount (5.9667 ± 0.015%) in the protein isolate while that of full fat (5.4533+0.0115%) and defatted flour (5.5267+0.015%) are statistically equal at 5% level from each other. The carbohydrate content of both flours (full fat, 57% and defatted 56.43%) were statistically equal at $p<0.05$. Production of protein isolate significantly ($p<0.05$) reduced the carbohydrate content to 1.6%.

Functional Properties

The functional properties studied for full fat, defatted flour and lima bean protein isolate are shown in table 2.

Emulsifying ability

The solubility of a protein is usually affected by emulsifying activity. Protein isolate because of its hydrophilicity or hydrophobic balance, depending on surface activity agents, can form and stabilize the amino acid composition, particularly at the protein emulsion by creating electrostatic repulsion on oil surface [15]. The emulsifying ability of the samples is as follows; full fat (82.4+0.2879%), defatted flour (89.1833+0.4497%) and lima bean protein isolate (36.62+0.3646%). The results of the study are in concordance with those reported.

Earlier by Mwasaru et al. [16]; who reported 88.16% and 84.90% emulsifying ability of lima bean. The low emulsifying ability of the protein isolate disagrees with Nwoji [17] which reported that the higher the protein content, the higher the emulsifying ability. However, the protein isolate could be suggested to have less protein units moving to the interface and absorb less water and oil due to its genetic makeup.

Water and oil absorption

Protein has both hydrophilic and hydrophobic properties thereby can interact with water and oil in foods. Results for water absorption revealed significant differences between full fat (1.8667+0.06%), defatted flour (2.7667+0.06%) and lima beans protein isolate (4.233+0.1155%). Protein isolate showed high water and oil absorption capacity (3.69+0.052%) compared with full fat flour (2.75+0.9526) and defatted flour (3.26+0.052%) for oil absorption capacity variations in water and oil absorption activity may be due to different protein concentration, their degree of interaction with water and oil and possibly the conformational characteristics. The lower water absorption capacity of the product is due to less availability of polar amino acids [18] and low fat absorption may be due to the presence of large proportion of hydrophilic group and polar amino acids on the surface of the protein molecules.

Foaming capacity

The following properties are used as indices of the whipping characteristics of the lima bean products [16]. Maximum foaming capacity was observed in the defatted flour (12.23 ± 1.1547%). This differs significantly by $p<0.05$ from that of the full fat (7.33 ± 0.5774%) and the protein isolate (3.667 ± 1.1547%). According to Kinsella [19]; Cherry and McWatters [20], foaming is a surface active function of protein. A colloidal system which forms the incorporation of air (gas) into a soluble surface- active agent is foam. Also the process of whipping results in partial protein denaturation which aids foam formation by unfolding of protein molecules. The high and low foamability exhibited by the defatted flour and protein isolate respectively shows that fat and protein affects foaming capacity [11].

Swelling index

The swelling index of the full fat, defatted and lima bean protein isolate are as follows; full fat (1.3167 ± 0.0231%), defatted flour (1.16 ±

0.0436%) and protein isolate (2.8±0.0854%). Figure 2 shows the values of the swelling index of the three products. There were significantly difference ($p<0.05$) among the samples. However, the protein isolate exhibited higher swelling power compared to the other samples. Swelling power is the ability to increase in volume when foamed example, legume flour is mixed with water. The extent of swelling gin the presence of water depends on the temperature, availability of water, species of starch, extent of starch damage due to thermal and mechanical processes and other carbohydrates and protein such as pectins, hemicelluloses and cellulose etc. [21]. The higher protein content of the protein isolate could have resulted to its higher swelling power.

Conclusion and Recommendation

Conclusion

Findings from this project work have revealed great potential food uses of lima bean. The successful isolation of protein from lima bean with high protein content as well as its very good functional properties shows that it is a very good quality protein source which could be used in various food applications. This finding also shows that lima bean can play important role in the quest for alternative source of protein especially in the third world countries where animal protein source are becoming increasingly expensive and unaffordable. It is now possible for utilization of lima beans in diverse industrial food processing and production. There is therefore little doubt that Lima bean will play significant roles in the alleviation of good protein problems of the third world.

Recommendations

It is hereby recommended, based on the findings of this project work that increased efforts would be made to encourage the cultivation of lima beans as well as its consumption. Such practice will help in fighting the menace of the current existing dilemma of protein malnutrition through exploitation of indigenous plant based sources.

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