

Effect of Germination and Cooking on the Nutrient and Poly-Phenol Content of Lima Bean (Phaseolus lunatus)

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Abstract
The present study focused on the effect of germination and cooking on the nutritive properties of lima bean seed. Lima beans cultivars were cooked at 1hr 30mins and germinated at 35^{o}C for 5 days. Dried pulverized samples of raw, cooked and germinated samples were analyzed for proximate composition, mineral content and polyphenol activity respectively. The results show germination has improve the protein content from 18.80 to 27.10 %. Mineral composition, increases with germination for calcium, potassium, phosphorus, iron, zinc and magnesium. The heavy metals reduce on germination for lead, cadmium, arsenic, nickel and selenium. The phenol content increases on germination for total flavonoids, phenol content and total antioxidant assay activity. Thus, the study indicated that germination has improved the nutritional composition of lima beans than cooking of the beans flour. Germinated lima bean flour can be successfully used in food formulation.
Keywords: Germination; cooking; lima bean; Polyphenol content.

1. Introduction

Legumes are major sources of dietary proteins in the developing countries as animal proteins are expensive. They are regarded as poor man's meat which serves as useful but cheap source of good quality proteins during hungry periods. Legume contributes dietary fiber, starch and expensive phytochemicals, vitamins and mineral element. Nowadays there is a wide interest in the effects of processing on the antioxidants compounds of legumes; this is because many bioactive compounds with antioxidants activity were present in legume seeds. Methods of food processing as germination, cooking, fermentation can cause significant changes in nutrient availability, texture and organoleptic characteristic of seeds (Fernandez –Orozco *et al*., 2009). In Nigeria, plant foods provide at least 50% of the dietary energy and nutrients, and lima beans is not one of the most important legumes (Ma *et al.*, 2005) commonly consumed in the country.

Germination increases nutritive value, improve protein digestibility, reduces anti nutrient and hydrolyses oligosaccharides (raffinose and starchyose) (Bau *et al*., 2000) and can also lead to modification of bioactive constituents of legumes (Paucar-Menacho *et al*., 2010).

Lima bean known as ''Butter'' beans, ''Burma'' bean are cultivated as pulse crops, both in temperate and sub tropical regions; classified as legume are good sources of vegetable protein which is used to supplement animal protein in the diet. Lima beans are rich in niacin, thiamine and riboflavin (Sathe *et al.*, 1984) and contain high levels of potassium, phosphorus, calcium and iron (Ologhobo and Fetuga 1984b). Lima bean proteins vary between 22-25% which make significant contribution to the protein and energy requirements of many Nigeria. Their role as a source of proteins is however affected by several factors including low protein digestibility (Aletor and Fetuga 1984).

Processing is necessary before the incorporation of the legume seeds into food formulations or animal diet this is due to the high levels of anti-nutritional components present in legumes; attempts to increase the utilization of legumes have employed a wide range of processing techniques such as soaking, germination, dehulling, cooking, roasting, autoclaving, fermentation, and recently extrusion cooking. The most common domestic processing methods include ordinary and pressure cooking. Microwave heating is increasing and its use for cooking is becoming popular due to the reduction of processing time (Habiba, 2000). Lima beans are hard to cook with pungent smell, and cooking involves discarding the cooking water many times before final cooking. The objective of this work is to determine the effect of germination and cooking on the nutrient and polyphenol content of Lima beans seed for food fortification.

2. Materials and methods

Collection and preparation of samples

Lima beans seeds (*Phaseolus lunatus*) (brown varieties) were purchased at Ikere central market, Ikere local Government area of Ekiti State Nigeria. All chemical reagent used for the experiment were of analytical grade.

Seed Pretreatment.

The dry cleaned lima beans seeds were divided into three sets. The first set was cooked until soft and the resulting material were dried in an air oven at 60^{o}C for 3hrs. Ground into flour in Weley mill sieved and package. The second set was dry-milled into flour as raw. The last batch was germinated (sprouted) at 20^{0}C, 99% relative humidity in the dark for 24hrs after soaking for 12hrs. It was dried and milled into flour sieved and packaged for

the next analysis.
Analytical procedure
The samples were evaluated for proximate composition and mineral components according to AOAC (2000).
Determination of Flavonoids
Aluminum chloride colorimetric method was used for flavonoids determination according to (Chang, 2002). Each extract (0.5mL of 1:10g/mL) in methanol were separately mixed with 1.5ml of methanol, 0.1ml of 10% aluminum chloride, 0.1ml of 1M potassium acetate and 28ml of distilled water. It remain at room temperature for 30min; the absorbance of the reaction mixture was measured at 415nm with a double beam Perkin Elmer UV/ visible spectrophotometer (USA). The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100gml-1 in methanol.
Determination of Total phenols.
Total phenols were determined by Folin Ciocalteu reagent according to (McDonald et al., 2001). A dilute extract of each plant extract (0.5ml of 1:10gml-l) or Gallic acid (saturated phenolic compound) was mixed with Folin Ciocalteu reagent (5ml 1:00 diluted with distilled water) and aqueous Na_2Co_3 (4ml, 1M). The mixtures were allowed to stand for 15min and the total phenol were determined by spectrophotometer (Optizen 2120, South Korea) at 765nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/L solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of mg Gallic acid per Kg of extract (mg/Kg of dry mass).
Determination of Total Anti-oxidants
DPPH Radical scavenging activity
DPPH scavenging activity was carried out by the method of (Blois, 1958). Different concentrations (1000, 500, 250, 125, 62.5 and 31.2 mg/ml) of *Phaseolus lunatus* (methanol) were dissolved in DMSO (dimethyl sulfoxide) and taken in test tubes in triplicates. Then 5ml of 0.1mM ethanol solution of DPPH (1, 1 Diphenyl-2-Picrylhydrazl) was added to each of the test tubes and were shaken vigorously. They were then allowed to stand at 37^0C for 20minutes. The control was prepared without any extracts. Methanol was used for baseline corrections in absorbance (OD) of sample measured at 517nm. A radical scavenging activity was expressed as 1% scavenging activity and was calculated by the following formula.

$$\% \text{ radical scavenging activity} = \frac{\text{Control O.D} - \text{Sample O.D}}{\text{Control O.D}}$$

Statistical Analysis
All determinations were carried out in triplicates and the data were subjected to analysis of variance (ANOVA) using SPSS 15 computer program me, while means were separated using Duncan multiple range test. Significance was accepted at 5% level of probability.

3. Results and Discussion

The intrinsic physical attributes of protein (composition, amino acid sequence, conformation and structure) has been affected during germination, these has attributed to the increased in the protein content of the seed as shown in Table 1. 27.10% for germinated sample and 19.50% for the raw seed. The increase will be of benefit when the germinated seed are used as functional ingredients in food formulation. The protein content reduces on cooking; therefore lima bean seeds when germinated at 24hrs could serves as protein supplement. The seed are low in fat thereby not suitable as an oiling food, but has appreciable crude fiber content and could be useful in lowering blood cholesterol level in rats as well as regulates bowel actions reducing the risk of developing diabetes, hypertension, cancer and hypercholesterolaemia (Oboh, 2008; Koulshon *et al*., 2005 and Gardner *et al*., 2005). The non-glycaemic content of lima bean in the sample analyzed (4.28-4.52 %) is higher than for soybean seed as reported by (Kizito, 2010) (4.21%)

The minerals in our diet are essential for a variety of bodily functions. They are important for building strong bones and teeth, blood, skin, hair, nerve function, muscle and for metabolic processes such as those that turn the food we eat into energy. They are needed for body growth and development and maintaining normal health. These are shown by the mineral composition as reported in Table 2. The macro elements calcium, phosphorus, potassium and magnesium in the table have been improved by the germination process. Therefore incorporating germinated lima beans flour into the meal of school children, pregnant and breastfeeding mothers will help to increase the proportions of mineral composition in their diet and improve nerve impulse and bone remodeling based on the amount of calcium preventing rickets.

Table 1: Proximate composition of cooked and germinated lima bean flour.

Parameters	Raw	Cooked	Germinated
Moisture content (%)	15.30^{b}	16.10^{a}	16.21^{a}
Fat content (%)	0.52^{a}	0.41^{a}	0.37^{a}
Ash content (%)	5.12^{a}	4.25^{c}	4.63^{b}
Crude fibre (%)	4.52^{a}	4.46^{a}	4.28^{a}
Protein content (%)	19.50^{b}	18.80^{c}	27.10^{a}
Carbohydrate (%)	55.06^{b}	60.23^{a}	47.41^{c}

Values are mean of triplicate determination. Values within the same column followed by different superscript are significantly different ($p< 0.05$).

Table 2: Mineral composition of cooked and germinated lima bean flour

Parameters (m/100g)	Raw	Cooked	Germinated
Sodium	285.10^{c}	376.15^{b}	575.35^{a}
Calcium	471.35^{c}	642.50^{b}	742.65^{a}
Potassium	390.65^{c}	561.05^{b}	846.50^{a}
Phosphorus	486.28^{c}	748.20^{b}	1048.70^{a}
Iron	4.71^{c}	5.20^{b}	7.41^{a}
Magnesium	148.75^{c}	164.54^{b}	188.63^{a}
Zinc	2.62^{c}	3.68^{b}	5.76^{a}
Manganese	6.83^{c}	7.94^{b}	9.80^{a}
Copper	4.62^{b}	3.22^{c}	4.76^{a}
Lead	2.72^{a}	1.59^{b}	0.74^{c}
Cadmium	3.60^{a}	2.45^{b}	0.18^{c}
Cobalt	0.39^{a}	0.21^{b}	0.11^{c}
Arsenic	0.04^{a}	0.02^{a}	0.01^{a}
Nickel	0.06^{a}	0.03^{a}	0.01^{a}
Selenium	0.02^{a}	0.01^{a}	0.01^{a}

Values are mean of triplicate determination. Values within the same column followed by different superscript are significantly different ($p < 0.05$).

The heavy elements were reduced grossly by germination and cooking of the beans respectively. The trace minerals were improved by germination of the seed. This has showed that germination processes has tremendously improve the nutritive property of the seed this is based on Table 1 and 2 above.

It has been recognized that consumption of foods with high phenolic content is correlated with reduced cardiovascular, inflammation, cancer mortality and some other disease rates (Sharms Ardekani et al., 2011). Table 3 shows the contents of total phenols that were measured by Folin Ciocalteu reagent method. The total phenol varied from 3.57 to 25.75 mg/100g of the seed flour in the extract powder. This indicates that the phenolic compounds in the analyzed sample can act as free radical terminators. According to (Lopez-Amoros et al., 2006); indicated that germination modifies the quantitative and qualitative phenolic compounds of legumes and the changes depend on the legume and the conditions of germination. The phenolic content was higher in the germinated sample than in the cooked.

Flavonoids have been recognized to show antioxidant activity and their effect on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler et al., 2003 and Cook and Shamma, 1996). According to this study, the high content of flavonoids in the germinated lima bean can explain its high radical scavenging activity. The flavonoid content is higher in germinated sample than in the cooked sample, Table 3. The results indicate that germination caused an increment in total phenolic content and total phenolic compound highly contributed to total antioxidant capacity of legumes. The result of this analysis indicated that with germination, the antioxidant properties of lima bean flour are enhanced and they can be used as desired ingredients for new functional food formulations than when the lima beans seed are cooked. In conclusion, lima bean have great potential as source of plant proteins due to their high content of crude protein as reported in most literature and improved by germination in this work. They could therefore be incorporated into food to serve as protein supplement.

Table 3: Poly phenol composition of cooked and germinated lima bean flour

Parameters	Raw	Cooked	Germinated
Phenol content	3.57^{c}	10.25^{b}	25.75^{a}
Flavonoid content	18.40^{c}	21.70^{b}	27.20^{a}
Antioxidant capacity	51.40^{c}	54.25^{b}	58.24^{a}

Values are mean of triplicate determination. Values within the same column followed by different superscript are significantly different ($p < 0.05$).

4. Conclusion

Lima bean seed nutrient has been improved on germination than when the seed is cooked; these have been identified in terms of the protein content, mineral composition and the polyphenol content. Diet formulation could be improved when the legume is germinated to exploits its use in food as protein hydrolysate.

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