Influence of Different Processing Methods on the Chemical Analysis, Nutritional and Phytochemical Composition of Lima Beans (Phaseolus lunatus); An Underutilized Edible Crop

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ABSTRACT

The influence of different processing methods on the chemical, nutritional and phytochemical composition of an underutilized lima beans was evaluated. For this study. mature dried lima beans were obtained from a local farm in Egbeda, Ibadan, Oyo State, Nigeria. The sample was sorted and separated into three (3) portions: baseline (sample as control), fermented sample and de-hulled sample. The results showed that polyphenol content of the fermented samples and de-hulled samples was significantly higher (P<0.05) when compared to the control. However, similar result was also observed in the flavonoids and ascorbic acid contents which were also significantly higher in the fermented and de-hulled samples than the control. A significant reduction (P<0.05) was seen at the levels of antinutritional factors (Phytate, Tannin and Saponin) of fermented and de-hulled samples compared to the control. However, de-hulled samples have a better reduction in anti-nutritional factors compared with fermented sample. In addition, fermentation significantly (P<0.05) reduced the cyanogenic compound to a safe level compared with the control and de-hulled samples. A significant (P<0.05) increase in magnesium (Mg) and iron (FE) was observed in the mineral analysis of fermented and de-hulled samples as compared to the control while there was no significant difference in other mineral elements. Result of the proximate analysis showed improved protein content in processed lima beans as compared to the control. It can therefore be concluded that fermentation of lima beans improves the quality, safety and nutritive values.

Keywords: Lima bean (Phaseolus lunatus), De-hulling, Fermentation, Flavonoid and Polyphenols.

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INTRODUCTION

Lima beans (Phaseolus lunatus L Walp) belonging to the family Fabaceae and genus Phaseolus, is a leguminous plant which originates from Peru. Lima bean is the second most economically important of the species of Phaseolus and is one of the 12 mostly used legumes in the World (Ogechukwu and Ikechukwu, 2017). It is an annual or short-lived perennial pulse species, widely cultivated in both temperate and subtropical regions (Aremu et al., 2016). Its cultivation in Africa spans through a wide range of ecological conditions viz warm temperate zones, arid and semi-arid tropical regions (El-Gohery, 2021). Lima beans are usually cultivated for their edible seeds.

Lima beans are commonly consumed among the rural dwellers of Yoruba land (Southwestern Nigeria) and Igbo land (Southeastern Nigeria), where it is called "kapala" and "ukpa" respectively (Seidu et al., 2014). It can either be consumed solely or cooked in combination with cereals such as rice or tuber such as yam (Farinde et al., 2017). Lima beans can also serve as substitute for the expensive soy meal and groundnut meal which constitute the major portion of conventional protein sources used in composite livestock feeds (Tope, 2014). It differs in size, shape and colour, as the shape can be spherical, curve or kidney, while colour ranges from green to creamy white, grey, light brown and dark brown with a starchy flavor (Farinde et al., 2018).

Lima beans possess good nutritional profile being a good source of minerals, dietary fibers, carbohydrates and proteins but, it is low in fat (Farinde et al., 2018; El-Gohery, 2021). Its B-complex vitamins content includes

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pyridoxine, thiamine, pantothenic acid, riboflavin, and niacin. While its mineral content includes molybdenum, iron, copper, manganese, calcium and magnesium (Ogechukwu and Ikechukwu, 2017; Nguyen *et al.*, 2020). Lima bean is a cheap source of protein when compared with animal products such as meat, fish, and egg (El-Gohery, 2021). The seeds contain protein twice as much present in cereals with more balanced profile of essential amino acids including lysine which is lacking in cereals (Bonita *et al.*, 2020). Lima bean seeds are also rich in bioactive compounds which promote human health (Ibeabuchi *et al.*, 2019) and their regular dietary intake contribute to healthy living (Bonita *et al.*, 2020).

As much as Lima bean is beneficial, it is still classified as underutilized grain legume in Nigeria. Underutilization of Lima bean is as a result of certain reasons in which its rare popularity, hard seed coat which prolongs its cooking, inadequate processing methods and presence of anti-nutritional factors are inclusive (Farinde et al., 2017; Taiwo et al., 2017; Farinde et al., 2018). Antinutrients are components which interfere with absorption and utilization of important minerals as well as reducing protein digestibility and the nutritive value of foods thereby causing a level of damage to the consumers (Adeniran et al., 2013; Farinde et al., 2018). Antinutritional factors in Lima bean include trypsin inhibitors, phytic acid, haematoglutinins, oxalate, tannins and cyanide (Yellavila et al., 2015).

According to El-Gohery, inactivation or total elimination of antinutritional factors will improve the nutritional quality of lima beans thereby, increasing its acceptability and utilization as a food. Processing techniques required in reduction or removal of these antinutrients include roasting, cooking, boiling, soaking, autoclaving, germination, blanching, dehulling and fermentation (Bonita *et al.*, 2020; El-Gohery, 2021).

MATERIALS AND METHODS

1. Materials

1.1 Collection and preparation of Lima beans

Matured dried Lima beans were collected from a local farm in Egbeda town, Ibadan, Oyo State, Nigeria for this research. The sample was sorted and divided into three (3) portions which include controls, fermented sample (undergone five days fermentation which involves daily continual change of water) and de-hulled sample (outer covering seed coat was removed). After all processing methods were achieved, the samples were homogenized into powder using motorized blender.

2. Extraction of flavonoids and polyphenols

Extraction of flavonoid and polyphenols was carried out according to Dietrych-Szostak and Oleszek (1999). Ten ml of methanol 70 % were introduced into a glass

test-tube containing 0.1 g of each ground sample. This was followed by heating in a water bath for 2 hrs at 37 °C with shaking every 15 min. The mixture was then allowed to cool to room temperature (23 °C) before being centrifuged at 3000 revolution per minutes for 10 min. The supernatant was collected for total flavonoids determination or stored at -20 °C until used. In the case of total polyphenols, absolute methanol was used as an extractant.

3. Anti-oxidant analysis

3.1 Determination of total polyphenolic content

The Folin-Ciocalteu method was used to determine the amount of total phenolic compound (Singleton *et al.*, 1999) as 100 µl of each diluted extract were mixed with 2.8 ml of deionized water and 2 ml of 50 % Folin-Ciocalteau's phenol reagent. The mixture was incubated for 30 min at room temperature, and absorbance was read at 765 n m and result was expressed as milligram gallic acid equivalent per gram extract (mg GEA/g extract). A standard curve made from gallic acid at different concentrations (50, 100, 150, 200, 250 mg/ml).

3.2 Determination of total flavonoid content

The total flavonoid content was determined using the aluminum chloride colorimetric method (Chang *et al.*, 2002). Each extract (100 μ l) polyphenols was mixed with 1.5 ml of 95 % ethanol, 100 μ l of 1M potassium acetate and 2.8 ml of deionized water and the absorbance of the reaction mixture was measured at 415 n m after incubation at 45 °C for 2 hrs. The total flavonoid content was expressed as milligram quercetin equivalent per gram extract (mgQE/g extract)

3.3 Determination of ascorbic acid

The concentration of ascorbic acid was determined by redox titration using standardized iodine solution, according to a slightly modified method (Galani *et al.*, 2017). Vitamin C in sample reacts with iodine to produce dehydroascorbic acid and iodide ions. Excess iodine then reacts with the starch indicator to produce violet color and indicate the end point of titration as ascorbic acid is converted to dehydroascorbic acid.

4. Anti-nutrient determination

4.1 Estimation of Saponins

The method described by Gracelin *et al.*(2013) was used to determine the quantity of saponins. Briefly, 5 g of each sample were put into a conical flask and 25 ml of 20 % aqueous ethanol were added. The samples were heated in a hot water bath at 55 °C for 4 h with continuous stirring. The mixture was filtered and the residue re-extracted with another 50 ml 20 % ethanol. The combined extracts were reduced to 8 ml over water bath at 90 °C. The concentrate was transferred into a separatory funnel and 4 ml of diethyl ether were added and shaken vigorously. The aqueous layer was

recovered while the ether layer was discarded. The purification process was repeated and 15 ml of n-butanol were added. The combined n-butanol extracts were washed twice with 5 ml of 5 % sodium chloride. The remaining solution was heated in a water bath at 55 °C After evaporation, the samples were dried in the oven at 102°C to a constant weight and the saponin content was calculated as percentage.

% Saponin = Weight of saponin X 100
Weight of sample

4.2 Estimation of Tannins

The amount of tannin was determined by the method of Hagerman and Butler (1989). One-half gram of each sample was weighed into a 50 mL plastic bottle. Fifty mL of distilled water were added and shaken for 1 h in a mechanical shaker. The mixture was filtered into a 50 mL volumetric flask and made up to the mark. 5 mL of the filtrate were transferred to test tube and mixed with 2 mL of 0.1 M FeCl₃ in 0.1 M HCl and potassium ferrocyanide. After 10 minutes, the absorbance was read at 765 n m using a spectrophotometer. Tannic acid was used as a standard, and calculated as mg/g.

4.3 Estimation of Phytates

Phytates were estimated using the method described by Sotelo *et al.* (2010) 1.0 g of each sample was extracted with 10 mL 3 % trichloro acetic acid to precipitate the phytate as ferric phytate with 10 ml of 0.1 % ammonium ferric sulphate. The ferric phytate is converted to ferric hydroxide and sodium phytate by boiling with 10 mL (0.5 M) sodium hydroxide. The precipitate was dissolved with 1 mL of 0.65 M HCl and phytate was determined using spectrophotometer at 519 n m. Phytic acid, Dodecasodium salt (Sigma P-8810) was used as standard.

4.4 Estimation of cyanide content

Cyanogenic content was determined according to the procedure of Ogungbemi *et al.* (2021) one hundred grams samples were added to a small plastic bottle, a buffer/enzyme paper was added, followed by 1 L of 1 M pH 6 phosphate buffer, a picrate paper and a screw cap

lid. The bottles were allowed to stand overnight at $30\,^{0}$ C, the picrate papers were removed from the plastic support and $5.0\,$ L of water were added to elute the colour. The absorbance was measured in a spectrophotometer at $510\,$ n m and the total cyanide content in mg cyanogenic equivalents/ $100\,$ g fresh weight was evaluated and values were reported in triplicate.

5. Proximate/ Chemical Analysis

The proximate composition of the samples includes the moisture, crude protein, crude fat, crude fibre and ash contents, which were determined according to the standard analytical method by the Association of Official Analytical Chemists (AOAC, 2005). The total carbohydrate was determined by difference.

6. Mineral Analysis

Minerals' constituent determination was carried out following the modified procedure of James and Emmanuel (2011). Concerntrated hydrochloric acid (9 ml) was added into 1.5 g of the sample, followed by 3 ml of conc. HNO₃ and heated on a hot plate slowly at first, until frothing ceases. Heating was continued until HNO₃ evaporated and a while fumes were observed, then allowed to cool and filtered. This was diluted and made up to 100 ml with distilled water. The following elements were determined using Atomic Absorption Spectrophotometer: calcium, chromium, manganese copper, zinc, iron, magnesium and nickel.

RESULT

1. Effect of different processing methods on antioxidant screening of Phaseolus lunatus.

Table (1) shows the effect of processing methods on anti-oxidant present in lima beans. There were significant differences in all the anti-oxidants screened for the investigated samples. Polyphenol content of the fermented samples was significantly (P<0.05) higher when compared with control and de-hulled samples. However, these were statistically similar (P<0.05).

Table 1. Effect of different processing methods on the phytochemical (Anti-oxidant) screening of lima beans.

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Samples	Polyphenols (mg GEA/g)	Flavonoids (mg QE/g)	Ascorbic acid (mg/g)	
Control	2.648 ± 0.212^{a}	1.413 ± 0.09^{c}	0.385 ± 0.013^{b}	
Fermented	3.202 ± 0.142^{b}	1.936 ± 0.03^{a}	0.523 ± 0.011^{a}	
De-hulled	2.821 ± 0.101^{a}	0.813 ± 0.05^{b}	0.530 ± 0.091^{a}	

Values are analyzed in triplicates and represented as (Means \pm SD) with different superscript in the same column are significantly different (P<0.05)

Flavonoids and ascorbic acid contents were also significantly (P<0.05) higher in the fermented samples; however, the ascorbic acid present in fermented sample was not statistically different (P<0.05) from the dehulled sample.

2. Effect of different processing methods on the phytochemical (Anti-nutrient) screening of lima beans

Results of the anti-nutritional factors of processed samples of lima beans are presented in Table 2. There were significant differences (P<0.05) among the anti-nutrients present in the samples, phytate and tannin were significantly higher (P<0.05) in the control. The phytate content ranged from 0.185 mg/100g in the control to 0.154 mg/100g in the fermented sample. For the tannin content, it ranged from 1.378 mg/100g in the control to 0.4225 mg/100g in the dehulled sample. The result also showed that saponin was significantly lower (P<0.05) in the fermented and the de-hulled samples.

3. Effect of different processing methods on the Cynogenic compound (HCN) of lima beans

The effects of different processing methods on cyanide concentration in lima beans are shown on Fig. (1). The control exhibited a higher level of cynogenic compound of above 0.5 mg/100g, while the different processing methods (Fermentation and de-hulled) showed a significant reduction in HCN with fermented sample being the least concentration.

4. Effect of different processing methods on the mineral composition of lima beans.

The data given in (Table 3) indicate that the fermented samples had higher values of potassium, calcium and sodium; however, it was not statistically different (P<0.05) from the control and de-hulled samples. The control had higher manganese and iron content while the fermented and dehulled samples were not significantly different (P<0.05) from each other. The manganese and iron present in fermented and dehulled samples were not significantly different (P<0.05) from each other.

Table 2. Effect of different processing methods on the phytochemical (Anti-nutrient) screening of lima beans.

Samples	Phytate (mg/g)	Tannins (mg/g)	Saponin (mg/g)
Control	0.185 ± 0.003^{a}	1.378 ± 0.011^{a}	0.453 ± 0.072^{b}
Fermented	0.154 ± 0.011^{b}	1.1731 ± 0.092^{b}	0.221 ± 0.011^{a}
De-hulled	0.156 ± 0.065^{b}	0.4225 ± 0.021^{c}	0.221 ± 0.009^{a}

Values are analyzed in triplicates and represented as (Means \pm SD) with different superscript in the same column are significantly different (P<0.05)

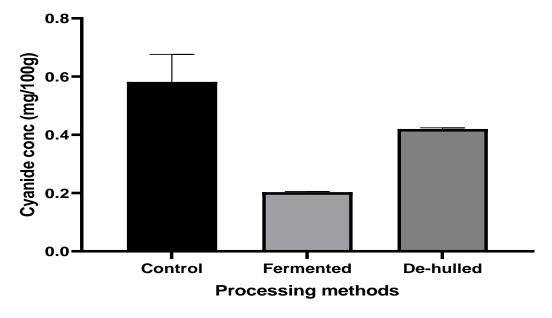


Figure 1. Effect of different processing methods on the cynogenic compound (HCN) of lima beans.

Table 3. Effect of different processing methods on the Minerals Analysis (mg/100g) of lima beans

Samples	Potassium	Calcium	Sodium	Manganese	Iron
Control	212.24 ± 0.23^{a}	310.22 ± 7.63^{a}	248.31 ± 8.12^{a}	170.35 ± 9.54^{a}	234.01 ± 2.12 ^a
Fermented	216.35 ± 8.92^{a}	312.14 ± 8.75^{a}	251.29 ± 9.63^{a}	185.31 ± 7.52^{b}	251.13 ± 8.86^{b}
De-hulled	209.32 ± 7.48^a	306.32 ± 9.75^{a}	245.32 ± 7.58^a	190.12 ± 9.54^{b}	254.03 ± 9.45^{b}

Values are analyzed in triplicates and represented as (Means \pm SD) with different superscript in the same column are significantly different (P<0.05)

5. Effect of different processing methods on the Proximate/Chemical Analysis of lima beans.

The chemical composition of processed samples of lima beans is presented in Table (4). There were significant differences (P<0.05) in moisture content for all the samples where the fermented sample was significantly (P<0.05) higher than other samples. The fermented and dehulled samples had significantly (P<0.05) higher protein content when compared with the control. Lipid, ash and fiber contents in the control and fermented samples were not significantly (P<0.05) different from each other. However, there were no differences in carbohydrate content among the investigated samples.

DISCUSSION

The results indicated that fermented sample has significant impact on the quality of lima beans (Oboh and Omoregie, 2011) from the stand point of the high polyphenol content as a result of fermentation. Xu and Chang (2008) reported a substantial amount of phenolic compounds to be associated with the hull. This agree with results obtained by Oboh and Omoregie (2011). The reduction of polyphenol content in the dehulled sample when compared with the fermented sample may be due to removal of the hull. Similar trend was observed in the findings of Oyeyinka et al. (2017) on dehulled sample of bambara grain. The same result was observed with flavonoids reduction in the dehulled sample when compared with the control and fermented samples. According to Oboh and Akindahunsi (2004), ascorbic acid is a good reducing agent and exhibits its antioxidant activities by electron donation. Oboh and Omoregie (2011) reported that vitamin C contribute to the antioxidant activities in plant foods.

Fermentation and dehulling had reducing effect on the anti-nutrients contents of lima beans. In agreement with Nwosu (2010), the reduction observed in the antinutritional contents of the fermented sample could be as a result of leaching and microbial activities. Olaleye et al. (2020) reported that phytates as an anti-nutrient which binds multivalent cations resulting into bioavailability of mineral elements present in legumes. Phytate content was reduced from 0.185mg/100g in the baseline sample to 0.154 mg/100g and 0.156 mg/100g in the fermented and dehulled samples respectively. The reduced content of phytate could be due to its hydrolysis into inositol and orthophosphate, resulting from the enzymatic action of the fermenting microorganisms. Dehulling reduces tannin content as it is located in the hull of seeds and this could be the reason for the least tannin content observed in the dehulled sample. This result is similar to that obtained by Oke et al. (2013) in dehulled African yam bean sample. Reduction in cyanide content observed in the fermented sample could be as a result of hydrolysis of the cyanide content of the seeds and this agrees with the findings of Adegbehingbe (Tope, 2014).

The amount of potassium, calcium and iron content in lima beans is not dependent on processing method. This result is contrary to that found by Farinde *et al.*(2018) Magnesium and iron were more abundant in the dehulled sample as dehulling increased. Magnesium increased from 170.35 mg/100g in the control to 190.12 mg/100g and iron from 234.01 mg/100g in the control to 254.03 mg/100g. This corresponds to the fermented sample in this study as manganese and iron increased from 170.35 and 234.01 mg/100g in the baseline sample to 185.31 and 251.13 mg/100g in fermented sample respectively.

Table 4. Effect of different processing methods on the proximate / chemical composition of lima beans

Samples	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Fiber (%)	Carbohydrate (%)
Control	5.54 ± 0.32^{a}	14.23 ± 2.71^{b}	3.12 ± 0.18^{a}	2.35 ± 0.19^{a}	16.05 ±2.11a	57.70 ±6.53 ^a
Fermented	$9.45 \pm 0.53^{\circ}$	19.32 ± 1.28^{a}	3.22 ± 0.21^{a}	2.19 ± 0.11^a	16.45 ± 3.12^{a}	49.37 ± 0.32^a
De-hulled	7.80 ± 0.09^{b}	20.44 ± 2.15^{a}	4.96 ± 0.09^{b}	1.34 ± 0.18^{b}	14.63 ± 2.63^{b}	50.83 ± 3.98^{a}

Values are analyzed in triplicates and represented as (Means \pm SD) with different superscript in the same column are significantly different (P<0.05).

Abbas and Ahmad (2018) stated that dehulling and soaking showed the greatest retention of minerals with regard to any other processing techniques.

Moisture content is an indication of water activities in food substance and foods, as high moisture content is susceptible to rapid deterioration due to microbial activities. (Farinde et al., 2018) Moisture content of the samples varied between 5.54 % in the control and 9.45 % in the fermented sample. There was an increase in moisture content after fermentation. This is in agreement with the result obtained by Tope (2014). The moisture content obtained for all the samples can however be termed low as it is within the range 0-13.5% recommended by James (1995). The dehulled sample had lower moisture content (7.80 %) when compared with fermented sample. The protein content revealed that processing had impact on the amount of protein, that is, processing increased the protein content of lima beans. This is contrary to the findings of Oke et al. (2013) who found different processing methods to reduce the protein content of African Yam Bean and lima bean flours. The highest protein value (20.44 %) was found in the dehulled sample while the control had the least value (14.23 %). El-Gohery (2021) reported similar trend in dehulled and raw lima beans. The reduced ash content in the dehulled sample is comparable to that recorded by El-Gohery, Farinde et al.(2017,2018) where the dehulled sample had the least ash content. The reduction in ash content may be a result of loss of endospermic matter that contains the micro and macro elements during removal of the seed coat in water.

CONCLUSION

Over time, lima beans crop has been regarded as under-utilized or neglected. This study has therefore revealed that it can be subjected to different processing methods prior consumption or usage. Dehulling and fermentation were found to reduce the anti-nutrient and increase the anti-oxidants content of lima beans. This is an indication that processing the seeds of lima beans can improve its quality and nutritive value.

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