

NUTRITIONAL COMPOSITION AND ANTINUTRIENTS CONTENT OF RAW AND PROCESSED LIMA BEAN (*PHASEOLUS LUNATUS*)

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Abstract

Lima bean (Phaseolus lunatus) is one of the underutilized food legumes in Nigeria. The raw is rich in nutrients but contains some antinutrients. The study thus evaluated the nutrients and antinutrients content of both the raw and processed lima bean to ascertain its utilization potential. Lima bean seeds were processed using different processing methods (roasting, germination, cooking and fermentation). The processed samples were evaluated for proximate composition, mineral and antinutrients content. Protein, fat, crude fibre and ash were highest in the raw lima bean (22.24 %, 1.22 %, 6.85 % and 4.68% respectively). Amongst the processed samples, fermented lima bean contained the highest protein and fat, germinated lima bean contained the highest crude fibre and ash while the highest carbohydrate was found in roasted lima beans. Germination significantly increased the mineral content of the raw lima bean. Cooked lima bean was found to contain the least minerals. All the processing methods significantly reduced ($p < 0.05$) all the antinutrients (tannin, phytate, trypsin inhibitor, lectin, oxalate and cyanide) evaluated. Fermentation was found to have the highest level of reduction effect on the antinutrients. Processing improved the nutritional value of the beans by reducing the antinutrients content. Processed lima beans are potential nutrient rich food material for food formulation.

Keywords: Lima bean, nutrients, antinutrients, processing, utilization

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1. INTRODUCTION

Protein energy malnutrition is still one of the major nutritional problems in the developing world (FAO, 2000). Adequate supply of animal proteins is difficult due to high cost and changing in consumers' attitudes towards animal based proteins as consumers are now more conscious in the food selection due to increased awareness about nutritional dependent health problems such as hypertension, gall-stone formation, hearth and kidney malfunctioning (Moses *et al.*, 2012). Plant protein from legumes have been found useful for alleviating malnutrition as well as providing health benefits. Legumes are important component of diets in many parts of the world. The seeds have protein content twice as much as that of cereals and usually contain more balanced profile of essential amino acids (Vijayakumari *et al.*, 1997). The protein content of legume grains range from 17 to 40 g/100 g, much higher than that in cereals (7-

11.8 g/100 g) and approximately equal to the protein content of meat 18-25 g/100 g (De Oliveira, 2006).

Some legume are under- utilized because they are either not popular (Olanipekun *et al.*, 2017) or there are constraints with their utilization (Adeniran *et al.*, 2013; Farinde *et al.*, 2017). Lima bean (*Phaseolus lunatus*) is one of the underutilized legumes in Nigeria. Lima bean is an herbaceous plant belonging to the family Fabaceae in the genus *Phaseollus*. Lima beans are sometimes referred to as: haba beans, sugar beans, butter beans, Guffin beans, civet beans, Hibbert beans, Pallar beans, Sieva beans, Madagascar beans, and Burma beans in America. It is also known as Maharage in East Africa, Haricot de lima in South Africa, Kokondo in West Africa and Awuje in Nigeria (Kay, 1979). Varietal differences exist in sizes, shapes and colour of lima beans, Shapes could be spherical, curve or kidney, colour usually ranges from green to creamy white, grey, light brown and dark brown with a starchy flavor.

Lima beans like other legumes are important source of protein, carbohydrate and dietary fibres but low in fat. It also contains thiamin, riboflavin, niacin and vitamin B6 which are co enzymes for protein, carbohydrate and fat metabolism (Holland *et al.*, 1991). Lima bean could be consumed as whole beans and it can also be made into flour which can be added to conventional flour for improved nutrient content. Matured lima bean seeds contain about 12.07% moisture, 20.62% protein, 0.90% fat, 62.83% carbohydrate, 5.71% crude fibre and 3.55% ash per 100g (USDA, 1986).

Despite the nutritional benefits of this legume, lima bean is still underutilized because of inadequate processing and antinutritional factors content. Like other legumes, lima beans contain some anti-nutrients such as trypsin inhibitors, phytic acid, haematoglutinins, oxalate, tannins and cyanide which interfere with absorption and utilization of important minerals as well as reducing protein digestibility and the nutritive value of foods (Sharma and Sehgal, 1992; Yellavila *et al.*, 2015). Reduction to a safe level or total elimination of these antinutrients will improve the nutritional quality of the food and also confer effective utilization potential on such foods for human consumption. Various authors have reported that antinutritional factors in legumes/beans could be reduced or eliminated through various processing techniques (Bau *et al.*, 1997; Oboh *et al.*, 2000; Farinde *et al.*, 2011; Mada *et al.*, 2012; Adeniran *et al.*, 2013; Audu and Aremu, 2011; Farinde *et al.*, 2014; Adebayo, 2014; Olanipekun *et al.*, 2011). Farinde *et al.* (2017) also reported improvement on lima bean utilization through steamed cooking and baked cooking. Understanding proximate composition, minerals, and antinutrients contents of processed lima bean will elucidate its utilization potentials. This study therefore evaluated the nutrients and antinutrients content of both the raw and processed lima bean for enhanced utilization of the legume.

2. MATERIALS AND METHODS

2.1 Source of materials

Matured dry lima bean seeds (brown variety) were purchased from a local market at Ita-Ogbolu, via Akure, Ondo State Nigeria. Processing materials such as cooking utensils and oven were obtained from the Food processing laboratory of Product Development Programme, Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria.

2.2 Processing of the beans

Processing of the lima bean seeds was carried out at food processing laboratory of Product Development Programme, Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria. Raw lima bean seeds were sorted by removing dirt and broken beans. The clean beans were soaked in tap water (1:5 w/v) for 12 h at room temperature ($28 \pm 2^\circ\text{C}$). The soaked lima bean seeds were then subjected to various traditional processing methods (roasting, cooking, germination and fermentation). Raw (un-soaked) lima beans served as control while all other processing first underwent soaking.

2.2.1 Processing of Raw lima beans: Clean beans (500g) was milled into powder, packed in a polythene bag and sealed. The powdered bean was kept in the refrigerator (4°C) for analysis.

2.2.2 Processing of roasted lima beans: Processing of roasted lima bean seeds was carried out following the method described by Oraka and Okoye (2017) with slight modification. Soaked Lima bean seeds were dehulled manually by rubbing between palms. The dehulled beans (500g) were spread on a tray and dried in hot air oven (Apex B35E, London) at 60°C to a constant weight. The dried beans were then roasted in open frying pan with constant stirring on a gas cooker using moderate flame for 15 – 20 min. The roasted beans were milled into powder, packed in a polythene bag and sealed. The powdered bean was kept in the refrigerator for analysis.

2.2.3 Processing of cooked lima beans: Cooked lima bean seeds processing was carried

out according to the method used by Farinde *et al.* (2017b). Soaked lima bean seeds (500g) was drained, rinsed in portable tap water twice and cooked in 2000 mL of portable tap water on a gas cooker for 2 h 30 min. The cooked beans were drained, placed on tray and dried in an hot air oven (Apex B35E, London) at 60°C to a constant weight. The dried cooked beans were milled into powder, packed in a polythene bag and sealed. The powdered bean was kept in the refrigerator (4°C) for analysis.

2.2.4 Processing of germinated lima beans:

Germination of the lima bean seeds was carried out following the method described by Akonor *et al.* (2014). Soaked lima bean seeds (500g) were spread on a tray lined with moistened cotton wool. Germination was carried out at $28 \pm 2^\circ\text{C}$ out of direct sunlight for 72 h. The seeds were kept moist by sprinkling with 100 ml of water at 12 h interval to keep the seeds moist, active and prevent mould growth. After germination, the seeds were dried in hot air oven (Apex B35E, London) at 60°C to a constant weight. The dried seeds were milled into powder, packed in a polythene bag and sealed. The powdered bean was kept in the refrigerator (4°C) for analysis.

2.2.5 Processing of fermented lima beans:

Lima bean fermentation was carried out using modified method of Farinde *et al.* (2014). Soaked lima bean seeds were dehulled manually in water to separate the cotyledons from the seed coat. The dehulled beans were washed in water and cooked for 1 h on a gas cooker during which the beans became soft enough to split into two. The cooked beans were drained and poured while still warm into clean plastic container with tight screw cover. The container with the content was placed in an incubator at $32 \pm 2^\circ\text{C}$ for 72 h to ferment. The fermented beans were dried in an oven (Apex B35E, London) at 60°C to a constant weight. The dried seeds were milled into powder, packed in a polythene bag and sealed. The powdered bean was kept in the refrigerator (4°C) for analysis.

2.3 Chemical Analysis

All analysis were carried out on dry weight basis and were determined in triplicates.

2.3.1 Proximate composition and Mineral content

Proximate composition of the lima bean samples was determined using the standard method of AOAC (2005). Percent Nitrogen was converted to crude protein by multiplying with a factor of 6.25. Fat content was determined by continuous solvent extraction method using Soxhlet apparatus. Crude fibre was determined gravimetrically. Total ash content was determined by furnace incineration. Carbohydrate was calculated by difference. Mineral content in the lima bean samples was determined using wet digestion method of AOAC (2000).

2.3.2 Anti nutrients content

Tannin content of the lima bean samples was determined using Folin-Denis Colorimetric method described by Harbone (1973). Sample (5.0 g) was mixed with distilled water in the ratio of 1:10 (w/v). The mixture was shaken for 30 min at room temperature and filtered to obtain the extract. Standard tannin acid solution was also prepared. Sample extracts (2 ml) was put in their respective flasks and labeled. The content of each flask were mixed with 35ml distilled water and 1ml of the Folin-Denis reagent added. Saturated Na_2CO_3 solution (2.5 mls) was added and the solution was diluted to the 50ml mark with distilled water and incubated for 90min at room temperature. Absorbance was measured at 760 nm in a colorimeter (Kerronic 20D, Germany) with the reagent blank at zero. Tannin content was calculated as shown below:

$$100\% \text{ Tannin} = \frac{100}{w} \cdot \frac{a_u}{a_s} \cdot \frac{c}{1000} \cdot \frac{v_t}{v_a} \quad (1)$$

where w = weight of sample; a_u = absorbance of test sample; a_s = absorbance of standard tannin solution; c = concentration of standard tannin solution; v_t = total volume of extract; v_a = volume of extract analyzed.

Phytate was determined using the Bipyrimidine colorimetric method described by Onwuka and Olopade (2005). Sample (2 g) was soaked in 50 ml of 0.2 NHCl solution and shaken for

30 min in a shaker. The solution was filtered to obtain the extract. Extract (0.5 mls) was dispensed into a test tube and 1ml of acidified ferrous ammonia sulphite solution added, boiled for 30min and then cooled to room temperature. The mixture was centrifuged at 3000 rpm for 5min and the supernatant collected for analysis. 1ml of the supernatant was mixed with 1.5 ml of 2.2 Bipyridine solutions. 1ml of the standard solution was treated the same way as the sample extract as described above. The absorbance of the standard and the sample were read in a spectrophotometer (Spectrumlab 752x, Japan) at a wavelength of 519 nm against a blank. Percent phytate was calculated as follows:

$$\% \text{ Phytate} = \frac{100 \cdot a_u \cdot c \cdot v_t}{w \cdot a_s \cdot 100 \cdot v_a} \quad (2)$$

where:

a_u = absorbance of sample;
 a_s = absorbance of standard solution;
 c = concentration of the standard;
 v_t = total volume of extract;
 v_a = volume of extract analyzed;
 w = weight of sample.

Trypsin Inhibitor was determined using spectrophotometric method described by Arntfield *et al.* (1985). Samples were extracted by weighing 1 g of the sample and dissolving it in 50ml of 0.5M NaCl solution. The mixture was stirred for 30 min at room temperature and then centrifuged. The supernatant was filtered through Whatman No. 1 filter paper. The filtrate (extract) was used for the determination. To 10 ml of the filtrate in a test tube or beaker was added 20 ml of 0.1% Trypsin solution. The content of the test tube was allowed to stand for at least 5 min after which its absorbance was measured at a wavelength of 410 nm against a blank (distilled water) in a spectrophotometer (Spectrumlab 752x, Japan). Trypsin activity was expressed as number of trypsin unit inhibited (TIU) per unit weight (g) of the sample analyzed and calculated as follows:

$$TUI/mg = \frac{b-a \cdot F}{0.01} \quad (3)$$

where:

b = absorbance of sample;
 a = absorbance of blank;
 F = experimental factor.

$$F = \frac{1}{w} \cdot \frac{V_f}{V_a} \cdot D \quad (4)$$

where:

w = weight of sample;
 V_f = total volume of extract;
 V_a = volume of extract used;
 D = dilution factor.

Lectin/Hemagglutinin was determined using spectrophotometric method described by Onwuka (2005), Sample (1 g) was weighed and dispersed in a 10 ml normal saline solution buffered at pH 6.4 with a 0.01M phosphate buffer solution. The mixture was allowed to stand at room temperature for 30 min and then centrifuged to obtain the extract. One milliliter of 2% (v/v) trypsinized rabbit blood erythrocyte suspension in saline phosphate buffer (pH 7.0) was added to 0.1 ml of the extract diluents in a test tube. A control sample was also prepared containing only the blood cells. The test tubes containing the sample mixture and the ones containing the control sample were allowed to stand for 4 h at room temperature. Normal saline (1 ml) was added to all the test tubes and they were allowed to stand for 10 min, after which their absorbance were read in a colorimeter (Kerronic 20D, Germany) at 620 nm. The hemagglutinin units per milligram of the sample was calculated as:

$$\text{Hemagglutinin}/mg = (b - a) \cdot F \quad (5)$$

where b = absorbance of test sample solution;
 a = absorbance of the blank control;
 F = experimental factor given by:

$$F = \frac{1}{W} \cdot \frac{V_f}{V_a} \cdot D \quad (6)$$

where V_f = total volume of extract;
 V_a = volume of extract used in the essay;
 W = weight of the sample used;
 D = dilution factor.

Oxalate was determined titrimetrically using the method described by Falade *et al.* (2004). Sample (5g) of the sample was weighed into a

100ml beaker, 20 ml of 0.30 NHCl was added and warmed on magnetic plate and stirred for one hour. The extract was filtered into a 100ml volumetric flask and diluted to the 100 ml mark of the flask. Extract (5 ml) was pipette into a conical flask and made alkaline with 1.0 ml of 5 N ammonium hydroxide. Indicator paper was placed in the conical flask to show the alkaline regions. Phenolphthalein indicator (2 or 3 drops) was added, 1 ml glacial acetic acid was added (excess acid decolourised the solution). One milliliter of 5 % CaCl₂ was then added and the mixture was allowed to stand for 3h after which it was centrifuged at 3000rpm for 15min. The supernatants were discarded and the precipitates, washed three times with hot water with thorough mixing and centrifuging each time. Two milliliters of 3NH₂SO₄ was added to each tube and the precipitates dissolved by warming in a water bath (70 – 80°C). The content of the tubes were carefully poured into a clean conical flask and titrated with freshly prepared 0.05M KMnO₄ at room temperature until the first pink colour appeared and till the solution became colourless. The solution was then warmed to 70 – 80°C and titrated until a permanent pink colour that persisted for at least 30 seconds was attained. The oxalate content was calculated as sodium oxalate equivalent.

Cyanide was determined by alkaline picrate colorimetric method described by Balagopalan *et al.* (1988). Sample (2g) was dispersed in 50ml of distilled water in a 25ml conical flask. An alkaline picrate paper was hung over the sample mixture and the blank in their respective flasks. The set up were incubated overnight and each picrate paper eluted or dipped into a 60ml of distilled water. A standard cyanide solution was prepared and diluted to a required concentration. The absorbance of the eluted sample solutions were measured with colorimeter (Kerronic 20D, Germany) at 540 nm wavelength with the reagent blank at zero. Cyanide content was calculated as shown below.

$$HCN(mg/kg) = \frac{1000}{w} \cdot \frac{a_u}{a_s} \cdot C \cdot D \quad (7)$$

where w = weight of sample analyzed;
 a_u = absorbance of test sample;
 a_s = absorbance of standard HCN solution;
 C = concentration of the standard in mg/d;
 D = dilution factor where applicable.

2.3.3 Statistical analysis

Statistical Package for the Social Sciences (SPSS version 17.0) was used for analysis. One way ANOVA was used to compare the means of data obtained. Duncan multiple range test was used to separate the means. Results were accepted at 5% significant level.

3. RESULTS AND DISCUSSION

3.1 Proximate composition of the lima bean samples

The result of the proximate composition of the lima bean samples are shown in Table 1. All the lima bean samples are low in moisture and fell within the recommended range of 0 – 13.5% as reported by James (1995). The moisture content of the samples ranges between 7.01% in the roasted lima bean to 8.05 % in the fermented lima bean sample. Moisture content in a food is an index of its water activity, foods with high moisture content are prone to quick spoilage due to microbial activities (Onyeike *et al.*, 1995; Aruah *et al.*, 2012). Roasting reduced the moisture content in the raw lima bean by 6.53 % .This might be due to the fact that two types of dry heat were applied to the beans i.e. drying and roasting. The low moisture content in the roasted beans is an advantage for the keeping quality of the beans. Germination increased the moisture content of the beans by 4.66%. This might be due to absorption of water during soaking and germination of the beans (Nonogaki *et al.*, 2010). Cooking was found to increase the moisture content by 6.93%, the beans must have absorbed water during cooking. There was no significant difference ($p>0.05$) in the moisture content of cooked and fermented lima bean samples, although the value for moisture content in the fermented lima bean (8.05%) was a little higher than that of cooked lima bean (8.02%). The highest value for moisture

content of fermented lima bean compared to the other processed lima bean samples might be due to absorption of water by the beans during soaking and cooking, and subsequent degradation of the fermenting beans with the release of moisture as a result of microbial activities (Oladumoye, 2007; Fadahunsi *et al.*,

2011). The moisture content in all the lima bean samples are similar to the reports of Olanipekun *et al.* (2015) and Oraka and Okoye (2017) on processed kidney bean and lima bean respectively.

Table 1: Proximate composition of lima bean samples (%)

| Sample | Moisture | Protein | Fat | Crude fibre | Ash | Carbohydrate |
|------------|--------------------------|---------------------------|--------------------------|--------------------------|--------------------------|---------------------------|
| Raw | 7.50 ± 0.02 ^c | 22.24 ± 0.05 ^a | 1.22 ± 0.09 ^a | 6.85 ± 0.01 ^a | 4.68 ± 0.05 ^a | 58.56 ± 0.09 ^d |
| Roasted | 7.01 ± 0.01 ^d | 19.79 ± 0.10 ^d | 1.05 ± 0.02 ^d | 5.02 ± 0.10 ^d | 4.51 ± 0.01 ^c | 62.62 ± 0.01 ^a |
| Germinated | 7.85 ± 0.11 ^b | 21.05 ± 0.10 ^c | 1.09 ± 0.09 ^c | 6.22 ± 0.10 ^b | 4.56 ± 0.10 ^c | 59.23 ± 0.05 ^c |
| Cooked | 8.02 ± 0.01 ^a | 19.35 ± 0.01 ^e | 1.14 ± 0.01 ^b | 6.00 ± 0.05 ^c | 3.84 ± 0.02 ^b | 61.65 ± 0.02 ^a |
| Fermented | 8.05 ± 0.01 ^a | 21.67 ± 0.10 ^b | 1.17 ± 0.01 ^b | 5.11 ± 0.02 ^d | 3.90 ± 0.02 ^d | 60.10 ± 0.01 ^b |

Means are values of three replicates. Means followed by the same superscript within a column are not significantly different at 5% level

Protein content in the lima bean samples ranged between 19.79% in the roasted lima beans to 22.24% in the raw lima beans. All the processing methods reduced the protein content of the raw lima beans. Similar result on reduction of protein content during processing such as cooking and roasting have been reported by Ndidi *et al.* (2014) and Adegunwa *et al.* (2012) in African yam bean and Beni seeds respectively. Processing has also been found to reduce protein content but increase protein digestibility. Martin-Cabrejas *et al.* (2009) and Rehmon and Shah (2005) found that protein content reduced while protein digestibility increased with soaking and cooking in chickpea and lentils respectively. Reduction in the protein content might be due to the processing techniques particularly heat treatment which may denature some of the protein. Cooked lima bean contained the least protein content (19.35%), this might be as a result of leaching of some of the soluble protein into the cooking broth. However, fermentation was found to have the minimum reduction in the protein content. It was reduced by 2.56% followed by germination which was reduced by 5.35%. Higher protein content in fermented samples compared with other processed samples might be as a result of microbial activities during fermentation, the

microorganisms must have probably synthesized proteinase enzymes which hydrolyzed the complex plant protein to amino acids and peptides resulting in an increase in the total nitrogen. Fogarty and Griffin (1973); Abiose *et al.* (1986); Omafuvbe *et al.* (2000, 2002) and Farinde *et al.* (2017a) reported that *Bacillus species*, majorly involved in natural fermentation of beans are known to be important producer of the protenaise (extracellular enzyme). Synthesis of new protein during fermentation of African locust bean was also reported (Obeta, 1983). The result of the protein content of the fermented sample in this study is similar to that reported by Adegbehingbe *et al.*, 2014).

Both the raw lima beans and the processed lima bean contained low fat ranging between 1.05% in the roasted lima bean to 1.20 % in the raw lima beans. Lima bean has been reported to be low in fat (USDA, 1986; Farinde *et al.*, 2011). Low fat content in the beans is an advantage as this will reduce the risk of heart attack and increased blood cholesterol level (Hassium *et al.*, 2009). The least fat content observed in the roasted lima bean sample might be as a result the dry heat that was applied which removed oil from the beans. Roasting has been reported to improve nutritive value of food material and also add desirable flavor to the food (Nnam,

1997)). There was no significant difference ($p>0.05$) in the fat content of the cooked and fermented lima bean samples. Decreased fat content in the processed lima bean samples might be due to loss of total solids during soaking prior to further processing (Wang *et al.*, 1997) and denaturation of the fat by heat processing and leaching into the processing water. Higher fat content in the fermented sample might be due to microbial activities, *Bacillus species* for example which are known to be majorly involved in beans fermentation have been reported to synthesize fat (Akindumola and Glatz, 1998).

Processing also reduced the crude fibre in the lima beans. Amongst the processing methods, crude fibre was highest in germinated sample (6.22%). Roasting and fermentation were significantly low ($p<0.05$) in crude fibre probably because the lima bean seeds were dehulled after soaking prior to roasting or fermenting. Beans seed coat has been reported to contain more fibre than the cotyledon (Mungudi *et al.*, 2010). Crude fibre consists of indigestible carbohydrates in foods. Studies have shown that fibre reduces constipation, lowers cholesterol and help reduce the risk of diseases associated with colon such as piles, appendicitis, and cancer (Anderson, 1990; Galisteo *et al.*, 2008). The crude fibre content for both the raw and processed lima bean samples in this study is higher than the value reported by Ndidi *et al.* (2014) and Olanipekun *et al.* (2015) for crude fibre content of raw and processed kidney beans and bambara groundnuts respectively.

There was no significant difference ($p>0.05$) in the ash content of roasted and germinated lima bean samples. Cooked lima bean contained the least ash, this might be due to leaching of salts and minerals into the cooking water. Similar report was given by Reebe *et al.* (2000).

All the processing methods increased the carbohydrate content in the lima beans. High carbohydrate content in this study is similar to the report of Agiang *et al.* (2010) that suggested that processing softens the cellulose, causes starch granules to break down and

makes starch more available. The least carbohydrate content in the processed lima bean samples was found in the germinated lima bean (59.23%). This might be due to breaking down of the seed carbohydrate into simple sugars which the embryo uses as its major energy source for growth. Germination has been reported to decrease the calorific content in the germinated seeds (Colmenares de Ruiz and Bressani, 1990).

3.2 Mineral content of the lima bean samples

The result of the mineral content of the lima bean samples is shown in Table 2. The raw lima beans and the processed lima beans contained appreciable amount of minerals with potassium being the most abundant. Lima bean has been reported to be a very good source of potassium (World Healthiest Foods). The content of all the minerals evaluated (Phosphorous, Magnesium, Potassium, Calcium, Sodium, Iron and Zinc) were significantly increased ($p<0.05$) with germination. Germination is a period in the life cycle of plants when they start emerging from the seed. It increases the availability of nutrients in seeds, grains and legumes.

Roasting, cooking and fermentation decreased the mineral content in the raw lima bean. Decrease in the mineral content of the roasted samples might be as a result of dehulling of the beans. Seed coat removal has been implicated in reducing mineral content in grains (Damodaran *et al.*, 2008). However roasting had higher values for the minerals compared to cooking and fermentation, this correlates with the higher ash content observed for the germinated and roasted lima bean samples in this study. Reduction of mineral content in cooked and fermented lima bean samples might be due to leaching of the minerals into the cooking water during cooking. Phosphorous content increased from 295.28 mg/100 g in the raw lima bean to 315.02 mg/100g in the germinated lima bean sample. In the processed samples, phosphorous content ranged between 224.60 mg/100g in the cooked lima bean sample to 315.02 mg/100g in the germinated

lima bean. Phosphorous has been known to contribute to blood formation and also act as supportive structure for building and protection of bone and teeth (Ogunlade *et al.*, 2005). Magnesium content increased from 312.12 mg/100g in the raw lima bean to 324.12 mg/100g in the germinated lima beans. The value of magnesium of the raw lima bean is higher than the values reported by Oshodi *et al.*, (1997) and Aremu *et al.* (2008). Magnesium enhances enzymatic activities and maintains the electrical potential in the nerves (Shills and Yong, 1992). Potassium content in the lima bean samples ranged between 887.10 mg/100g in the cooked lima bean to 1102.10 in the germinated lima bean. Potassium plays vital role in maintenance of cellular water balance, it regulates the pH of the body and enhances protein and carbohydrate metabolism (Onibon *et al.*, 2007). Diets rich in potassium have been associated with reduction in kidney stone risk (World Healthiest Foods). Similarly, calcium was highest (264.02 mg/100g) in

germinated lima bean and least (189.58 mg/100g) in the cooked lima bean. Calcium helps in bones and dental development, and also helps in enzymes and hormonal release (Beto, 2015). Sodium content was low in both the raw and processed lima bean samples compared with the phosphorous, magnesium, potassium and calcium. There was no significant difference ($p > 0.05$) in the sodium content of cooked and fermented lima bean samples. Sodium and potassium have been found to regulate and maintain osmotic pressure of the body fluid. They also regulate glucose absorption and protein retention during growth ((NRC, 1989; Graham and Welch, 1996). High level of potassium and low level of sodium is an advantage for consumers with high blood pressure who require high potassium but low sodium for their body fluid electrolyte balance (Bamibgoye and Adepoju, 2015).

Table 2: Mineral content of the lima bean samples (mg/100g)

| Sample | Phosphorous | Magnesium | Potassium | Calcium | Sodium | Iron | Zinc |
|------------|----------------------------|----------------------------|-----------------------------|----------------------------|----------------------------|--------------------------|--------------------------|
| Raw | 295.28 ± 0.09 ^c | 312.12 ± 0.06 ^b | 986.90 ± 0.11 ^b | 225.10 ± 0.02 ^b | 110.22 ± 0.09 ^b | 8.70 ± 0.10 ^b | 4.50 ± 0.10 ^b |
| Roasted | 302.10 ± 0.05 ^b | 306.21 ± 0.11 ^c | 955.12 ± 0.05 ^c | 216.14 ± 0.02 ^c | 106.06 ± 0.09 ^c | 7.68 ± 0.06 ^c | 4.10 ± 0.02 ^c |
| Germinated | 315.02 ± 0.09 ^a | 324.12 ± 0.05 ^a | 1102.10 ± 0.02 ^a | 264.02 ± 0.10 ^a | 112.12 ± 0.06 ^a | 8.95 ± 0.10 ^a | 5.12 ± 0.11 ^a |
| Cooked | 224.60 ± 0.06 ^e | 225.61 ± 0.11 ^e | 887.10 ± 0.06 ^e | 189.58 ± 0.05 ^e | 101.06 ± 0.10 ^d | 7.25 ± 0.05 ^e | 4.00 ± 0.11 ^e |
| Fermented | 280.67 ± 0.11 ^d | 298.15 ± 0.05 ^d | 922.13 ± 0.02 ^d | 198.76 ± 0.02 ^d | 101.90 ± 0.10 ^d | 7.58 ± 0.09 ^d | 4.06 ± 0.05 ^d |

Means are values of three replicates. Means followed by the same superscript within a column are not significantly different at 5% level

Germinated and roasted lima bean samples also contained higher iron and zinc compared to the cooked and fermented lima beans. Iron content ranged from 7.25 mg/100g in the cooked lima bean to 8.95 mg/100 g in the germinated lima bean while zinc content also ranged from 4.00 mg/100 g in the cooked lima bean to 5.12 mg/100 g in the germinated lima bean samples. The values of iron and zinc content in this study are higher than the values of iron and zinc content in baby Lima reported by USDA (1986). Iron is required for oxygen to travel to tissues and organs. It helps to carry oxygen throughout the body in form of hemoglobin and myoglobin, it is an integral part of many

proteins and enzymes and it also helps in energy metabolism (Nestle Good Foods). Zinc helps in enhancing immune activities and it is important for proper sense of taste and smell (Nestle Good Foods). Though mineral content were reduced in the fermented lima beans compared to the roasted and germinated, their bioavailability in the fermented beans must have increased due to reduced antinutrients content. Fernandes *et al.* (2010) reported that mineral contents decreased with processing in common beans (*Phaseolus vulgaris L.*), but the bioavailability of the minerals increased.

3.3 Antinutrients content of the lima bean samples

The result of the antinutrients content in the lima bean samples is presented in Table 3. Antinutrients are natural or synthetic plant compounds that reduce the body's ability to digest and absorb essential nutrients such as protein, vitamins and minerals. They include trypsin inhibitor, lectin, tannin, phytate and oxalate (Bouchenak and Lamri-Senhadji, 2013; Campos-vega and Oomah, 2010). Their positive or negative effects seem to be associated with their concentration in the beans which depends on type of bean, as well as their interaction with other components of the diet. Anti-nutrients are found at some level in almost all foods. However, their levels are reduced through various traditional processing methods such as fermentation, cooking and malting (Gibson *et al.*, 2007). Such processing methods are widely used in those parts of the world where cereals and legumes form the major part of the diet (Phillips, 1993).

The result of this study shows that all the processing methods significantly reduced anti nutrients content in lima bean. Tannin content ranged between 2.12 mg/100g in the fermented lima bean to 9.80 mg/ 100g in the raw lima bean samples. The tannin content in the lima beans samples in this study is far lower than the 150 – 200 mg/100g of safe level reported by Schiavone, *et al.* (2007). Tannins, also known as polyphenols are able to form complexes with protein and various organic compounds including amino acids (Ranilla *et al.*, 2006). They inhibit the activities of digestive enzymes thus making the protein insoluble and indigestible (Carnovale *et al.*, 1999). Roasting and fermentation significantly ($p < 0.05$) reduced tannin content of the lima beans. Roasting reduced tannin content by 77.97 %, while fermentation reduced the tannin content by 77.55 %. This is probably as a result of dehulling of the beans after soaking before further processing in the two methods. A lot of tannin must have been removed with the seed coat as tannin has been reported to be more concentrated on beans seed coat (Ranilla *et al.*,

2006). Tannins and other polyphenols in legumes and cereals may be reduced during germination as a result of the formation of polyphenol complexes with proteins and the gradual degradation of oligosaccharides (Camacho *et al.*, 1992).

Phytate (phytic acid) forms insoluble complexes with minerals in both cereals and legumes thus rendering the minerals poorly digested and unavailable for absorption. Phytate was significantly ($p < 0.05$) reduced from 141mg/100g in the raw lima bean to 21.44mg/100g, 18.10 mg/100g, 16.70 mg/100g and 15.45 mg/100g in roasted, germinated, cooked and fermented lima bean samples respectively. The highest percent reduction level was found in the fermented lima bean sample (89.04%). The level of phytates observed in this study is lower than the phytate content of 10 – 60 mg/g that could pose health problem to humans (Thompson, 1993). Soaking, cooking, germination and fermentation have been reported to reduce phytate level in dry beans (*Phaseolus vulgaris L.*) to as much as 50 – 80% (Deshpande and Cheryan, 1983). Phytate however is beneficial on the other hand, it was found that it slows down digestion rate of carbohydrate and lowers the blood glucose (Thompson, 1988). Ramírez-Ca´rdenas *et al.* (2008) also pointed out that some studies have shown that low concentrations of phytates and phenolic compounds can be protective against cancer and cardiovascular diseases.

Trypsin inhibitor decreased significantly ($p < 0.05$) with heat treatment. It decreased from 24.82 TIU/g in the raw lima bean to 1.69 TIU/g, 0.90 TIU/g and 0.76 TIU/g in roasted, cooked and fermented lima bean respectively. Trypsin inhibitor interferes with protein digestion in animals and humans, it could cause enlargement of the pancreas and enhance chemically induced tumors (Grant, 1989). Amongst the processing treatment, germination was found to have the least reduction effect on the trypsin inhibitor content of the lima bean. This is an indication that heat treatment particularly moist heat is very effective in

reducing trypsin inhibitor to minimal acceptable level. Cooking and fermentation reduced the trypsin inhibitor content in the lima bean by 96.37% and 96.93% respectively. The high reduction of trypsin inhibitor by heat treatment in this study is in line with the report of Alagbaoso *et al.* (2015) and Truengo *et al.*

(1990) in which trypsin inhibitor was reduced by 98% and 90% in jack bean and kidney beans respectively. Duarte-Rayas *et al.* (1992) also reported that heat treatment reduced trypsin inhibitor in dry beans by 80 – 90 %.

Table 3: Antinutrients content of the lima bean samples

| Sample | Tannin (mg/10g) | Phytate (mg/100g) | Trypsin inhibitor (TIU/g) | Lectin (mg/kg) | Oxalate (mg/kg) | Cyanide (mg/kg) |
|------------|--------------------------|----------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Raw | 9.80 ± 0.12 ^a | 141.00 ± 0.10 ^a | 24.82 ± 0.11 ^a | 12.20 ± 0.11 ^a | 10.72 ± 0.09 ^a | 76.70 ± 0.10 ^a |
| Roasted | 2.16 ± 0.11 ^d | 21.44 ± 0.11 ^b | 1.69 ± 0.12 ^c | 4.48 ± 0.10 ^c | 0.71 ± 0.09 ^b | 10.10 ± 0.10 ^b |
| Germinated | 4.21 ± 0.11 ^b | 18.10 ± 0.10 ^c | 1.95 ± 0.09 ^b | 4.51 ± 0.12 ^b | 0.70 ± 0.07 ^b | 10.40 ± 0.08 ^b |
| Cooked | 3.72 ± 0.11 ^c | 16.70 ± 0.12 ^d | 0.90 ± 0.11 ^d | 3.22 ± 0.08 ^d | 0.59 ± 0.09 ^c | 8.10 ± 0.05 ^c |
| Fermented | 2.12 ± 0.10 ^d | 15.45 ± 0.10 ^e | 0.76 ± 0.11 ^e | 3.06 ± 0.11 ^d | 0.52 ± 0.08 ^d | 7.08 ± 0.091 ^d |

Means are values of three replicates. Means followed by the same superscript within a column are not significantly different at 5% level

Lectin content was also reduced significantly with processing. It reduced from 12.20 mg/kg in the raw lima bean to 4.48 mg/kg, 4.51 mg/kg, 3.22 mg/kg and 3.06 mg/kg in roasted, germinated, cooked and fermented lima beans respectively. There was no significant difference ($p < 0.05$) in the lectin content of cooked and fermented lima bean samples. Lectins are natural compounds found in plant particularly legumes. They are protein or glycoprotein, they agglutinate red blood cells (Hudson, 1984). Lectin toxicology may lead to nutritional deficiencies and immune reactions (Enwere, 1998).

All the processing methods significantly ($p < 0.05$) reduced the oxalate content of the lima bean. Oxalate content was reduced from 10.72 mg/kg in the raw lima bean to 0.71 mg/kg, 0.70 mg/kg, 0.59 mg/kg and 0.52 mg/kg in roasted, germinated, cooked and fermented lima bean samples respectively. There was no significant difference ($p > 0.05$) in the oxalate content of roasted and germinated lima bean samples. Cooking and fermentation were found to have high level of oxalate reduction in the lima bean (94.49 % and 95.14 %) respectively. The high level of reduction of oxalate in this study is similar to the report of Alagbaoso *et al.* (2015) who reported that

cooking reduced oxalate content in jack bean by 92 %. The values for oxalate content in all the samples were within safe level (3-5mg/kg) as reported by Ndidi *et al.*, 2014. Oxalate reduces the availability of essential nutrients. It has been found to prevent absorption of calcium and iron in foods (Dresbash, 1980). Diet high in oxalate has been reported to increase the risk of development of kidney stone (Chai and Liebman 2004).

Cyanide content in the lima bean was also significantly reduced ($p < 0.05$) by all the processing methods. Cyanide content in the raw lima beans was reduced from 76.70 mg/kg to 10.10 mg/kg, 10.40 mg/kg, 8.10 mg/kg and 7.08 mg/kg in roasted, germinated, cooked and fermented lima bean samples respectively. Soaking lima beans in water overnight before further processing has been reported to eliminate the hydrocyanic acid toxicity in the beans (Holland *et al.*, 1991). Cyanide is an effective cytochrome oxidase inhibitor, it interferes with aerobic respiratory system. Acute hydrogen cyanide poisoning could cause difficulties in breathing which can result into death (Osuntokun, 1973). The values of cyanide content observed in this study are far below the permissible level/safe dose of 50-200mg/kg as reported by (Richard, 1991).

Fermentation was found to also have the highest percent reduction for cyanide (90.76 %).

Cooking and fermentation were found to significantly reduced ($p < 0.05$) all the antinutrients evaluated in the lima bean samples. Carlin and Udebible (1997) reported that moist heat was more efficient as method of processing for antinutrients reduction than dry heat during soybean processing.

Significant reduction of all the antinutrients in the lima bean by fermentation in this study might be attributed to the release of the antinutrients into the processing water during soaking and cooking coupled with the activities of the microorganisms during the fermentation process. Microorganisms such as lactic acid bacteria and *Bacillus* species have been reported to degrade toxins in foods during fermentation (Holzapfel, 2002; Chelule *et al.*, 2010; Nwosu, 2012; Farinde *et al.*, 2017a.) Chelule *et al.* (2010) reported that starter culture of Lactic acid bacteria was able to reduce phytate and phenolic compounds level during fermentation of maize into mahewu.

4. CONCLUSION

The study showed that both the raw lima bean and the processed lima beans are rich in nutrients (protein, carbohydrate, fibre, ash and minerals). Both the raw and processed lima bean samples are low in fat which is an advantage as this will reduce the risk of heart attack and reduce blood cholesterol level. Although the raw lima bean contained the highest protein, fat, ash and fibre, lima bean cannot be consumed raw because of the antinutrients content. All the processing methods (roasting, germination, cooking and fermentation) were effective in reducing the antinutritional factors evaluated in the lima beans thus improving the nutritional value of the beans. Fermentation was found to have the highest significant effect on the reduction of all the antinutrients evaluated. Utilization of lima bean could be enhanced through adequate and appropriate processing techniques. Processed

lima beans are potential nutrient rich food material for food formulation.

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