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Microbial, Nutritional and Sensory Qualities of Baked Cooked and Steamed Cooked Lima Beans

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Abstract Lima bean (*Phaseolus lunatus*) is a nutritious under- utilized food legume in Nigeria. The need for utilization of lima beans for a healthy and functional food informed this study. Hence the study assessed the microbial, nutritional and sensory qualities of baked cooked and steamed cooked lima beans. Lima beans were processed into baked beans and cooked beans. The time for bake cooking and steam cooking for the lima beans samples were compared. Microbial counts in the two lima beans samples were determined. The processed lima beans samples were evaluated for proximate composition, phytochemicals and antioxidants content. The products were also subjected to sensory evaluation. Total processing time for baked cooked lima beans (BCLB) was shorter (10 h) compared to steamed cooked lima beans (SCLB) (12h). The total viable count was higher in SCLB (3.66 cfu g^{-1}). Baked cooked lima beans (BCLB) recorded the highest protein (14.97 %), the least fat (1.02%) and the highest fibre content (5.88 %) when compared with steamed cooked lima bean (SCLB). Phenolics, flavonoids and lycopene content were higher in BCLB (18.5 GAE/g, 104.5 mg/100g and 12.7 mg/100g) respectively compared to SCLB. SCLB was significantly higher ($p < 0.05$) in tannin, phytate and trypsin inhibitor compared to BCLB. BCLB samples were the most preferred in all the sensory attributes tested when compared with SCLB samples. The study shows that both baked cooked and steamed cooked lima beans have great utilization potentials, but BCLB has a higher potential in terms of shorter cooking time as well as antioxidant and phytochemicals benefits. Baked cooked lima beans could be explored at household and commercial level for improved nutrition and income generation.

Keywords: *baked lima beans, total viable count, antioxidant, proximate, anti-nutrients*

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1. Introduction

Malnutrition such as protein and iron deficiencies is still prevalent in developing countries such as Nigeria [1]. This is because most of the staple foods consumed are carbohydrate based. Legumes are known to be a good and cheap source of protein. However some legumes, though available, are under-utilized. Lima bean is one of the grain legumes that are under-utilized in Nigeria. This is because lima beans are not popularly known [2] and also the seeds are hard to cook [3]. It belongs to the family Fabaceae in the genus *Phaseollus*. Lima beans are usually cultivated for their edible seeds. 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 B_6 which are co enzymes for protein, carbohydrate and fat metabolism [4]. Like other legumes, lima beans contain some anti-nutrients including trypsin inhibitors, phytic acid, haematoglutinns, oxalate, tannins and cyanide which interfere with absorption and utilization of important minerals such as calcium, iron, zinc and magnesium [5]. These anti-nutrients reduce protein digestibility and the nutritive value of foods [6]. However these anti-nutrients can be reduced through different processing methods [7,8].

In Nigeria, Lima beans are traditionally consumed as cooked beans either as sole or in combination with cereals such as rice or tuber such as yam. Utilization of lima beans this way started to reduce due to its hard seed coat which makes the beans to take too long to cook. There is therefore the need to find alternative ways of utilizing this legume which is of nutritional and health benefits. Some authors have started work on utilizing lima beans to produce *daddawa* [8,9,10] and lima bean flour [11].

Baked beans could be tested as another way for lima bean utilization. Baked beans are oven cooked by baking the beans in tomato sauce. The tomato sauce in which baked beans are cooked and served is rich in lycopene. Lycopene is free radicals fighting antioxidant [12]. Free radicals are known to cause damage in the body systems causing different diseases.

The development of value-added bean-based products such as baked beans will assist the food industry, fast-food restaurants, and domestic consumers. Baked beans are nutritious, rich in protein (including the important amino acid lysine). Baked beans contain energy giving low glycaemic index carbohydrates. They are also very good source of dietary fibre which help to reduce constipation and bad cholesterol in the body [13,14,15]. It is also a very good source of B vitamins (vitamin B6, niacin and folate). Consumption of baked lima beans will improve the nutritional status of the consumers. Baked beans are international food, hence development of baked beans from lima beans in the country will provide dietary diversity and will also serve as an international product for boosting the country's economy. Utilization of lima bean in this way will also enhance its cultivation and production need thus improving the livelihood of farmers and food processors.

2. Materials and Methods

2.1. Processing of Baked and Cooked Lima Beans

Matured dry lima beans were purchased from the market at Ita-Ogbolu, via Akure, Ondo State Nigeria. The beans were sorted to remove stones and broken seeds. Baked and cooked lima beans were processed following the method described by [16] with slight modification. Whole lima bean seeds (1 kg) were soaked in water for 8 h at room temperature. The beans were then drained and portioned into two. One part (500 g) was baked cooked while the second part was steamed cooked. Baked cooked lima beans were developed by pre-cooking the soaked beans with 500 ml of water for 30 min prior to baking. This was to soften the beans a little before adding the baking sauce. The semi-cooked beans and mixture of grinded tomatoes (250 g), onions (100 g), ginger (5 g), honey (25 ml), soya bean oil (20 ml) and salt to taste were placed in hot air infra red oven (PL-6 GB4706.39-2003 MAXCHEF) already preheated to 180°C. The beans were baked cooked for 1 hour, 30 min. Steamed cooked beans were processed by cooking the soaked beans in water (1500 ml) on a gas cooker for 3 h 30 min and traditional sauce of grinded dried pepper (2 g), palm oil (20 ml), grinded onions (100 g) and salt to taste were added. The beans and the traditional sauce were further cooked together for another 30 min.

2.2. Microbial Analysis

Microbial count in the samples was determined using pour plate method as described by [17]. Samples (2.0g each) of the baked and cooked lima beans were aseptically taken and diluted serially in 0.1% peptone water. The aliquots (1.0ml) of appropriate dilution were plated in triplicate on nutrient agar (NA), MacConkey agar (MCA) and potato dextrose agar (PDA) to determine the total viable count, total coliform count and fungi count respectively. Nutrient agar and macConkey agar plates were incubated at 35±2°C aerobically for 24 h while potato dextrose agar plates were incubated at 28 ± 1°C for five days. Colonies were counted and expressed as colony forming unit per gram (cfu g^{-1}) of the sample. The counts were done in triplicates and the mean taken.

2.3. Proximate Composition of the Lima Bean Samples

Proximate composition of the baked and cooked lima bean samples was determined using the standard method of [18]. Percent Nitrogen was converted to crude protein by multiplying with a factor of 6.25. Carbohydrate was calculated by difference.

2.4. Determination of Anti nutrients in the Beans Samples

Tannin content of the samples was determined using Folin-Denis Colorimetric method described by [19]. Absorbance was measured at 760nm in the colorimeter with the reagent blank at zero. Phytate was determined using the Bipyrimidine colorimeter method described by [20]. Absorbance was read at a wavelength of 519 nm. Trypsin Inhibitor was determined using the spectrophotometric method described by [21]. Absorbance was read at 410 nm wavelength. One trypsin activity unit inhibited was given by an increase of 0.01 absorbance unit at 410nm. Cyanide was determined by alkaline picrate colorimetric method described by [22]. Absorbance of the eluted sample solutions were measured with colorimeter at 540 nm wavelength with the reagent blank at zero. Oxalate was determined titrimetrically as described by [23]. The oxalate content was calculated as sodium oxalate equivalent.

2.5. Antioxidant and Phytochemicals Determination

The antioxidant activity in the beans samples was determined using the 1,1-diphenyl-2-pycrylhydrazyl (DPPH) method, as reported by [24]. The results were expressed as milligrams of Trolox equivalents antioxidant capacity (TEAC g^{-1}) extract. Total phenolic content (TPC) in extracts from the samples was determined by a Folin-Ciocalteu method described by [25], using garlic acid as standard, Flavonoids were determine using the method described by [19]. Lycopene content was determined by extracting the lycopene in the samples with hexane [26] and measuring the content spectrophotometrically at 503 nm [27,28].

2.6. Sensory Evaluation of the Beans Samples

Baked and cooked lima bean samples were processed and coded. The coded samples were presented to twenty semi trained panel of judges who are familiar with baked and cooked beans. The judges were presented with water for mouth rinsing after each tasting and they were asked to score the samples for colour, appearance, flavour, texture, taste and overall acceptability using 9 point hedonic scale where 9 = like extremely, 5 = like moderately and 1 = dislike extremely [29].

2.7. Statistical Analysis

Data obtained were analyzed using descriptive and inferential statistics. Analysis of Variance (SPSS version 17) was used and means were separated by Duncan multiple range test. Results were accepted at 5% significant level.

3. Results and Discussion

3.1. Processing Time of the Beans

The processing time for baked cooked (BCLB) and steamed cooked lima beans (SCLB) samples is presented in Table 1. BCLB was found to have reduced processing time (10 h) compared with the processing time for SCLB (12 h). Soaking for 8 h was found to soften the beans before baking or cooking. Soaking is an integral part of processing to facilitate shorter cooking time. Studies have shown that legumes with thick bean seed coats usually show a slow rate of water absorption in comparison to thin beans [16]. Aside from shorting of cooking time, soaking beans with salt solution, discarding the soaking solution and cooking with fresh water has an improved effect on the flavor and protein quality of beans [30].

Table 1. Processing time for the baked cooked and steamed cooked lima beans samples

Sample	Soaking time (h)	Cooking/Baking time (h)	Total processing time (h)
BCLB	8	2	10
SCLB	8	4	12

BCLB = Baked cooked lima beans, SCLB = Steamed cooked lima beans.

Table 2. Microbial count of the baked cooked and steamed cooked lima beans samples

Sample	Total viable count (cfu g^{-1})	Total coliform count (cfu g^{-1})	Fungi count (cfu g^{1})
BCLB	3.56 ± 0.11^a	Nil	Nil
SCLB	3.66 ± 0.08^a	Nil	Nil

Means are values of three replicates ± standard error. Means in the same column followed by the same superscript are not significantly different (p< 0.05). BCLB = Baked cooked lima beans SCLB = Steamed cooked lima beans.

3.2. Total Viable Count of Microorganisms in the Processed Lima Beans Samples

The results of the microbial count of the baked cooked and steamed cooked lima beans samples are shown in Table 2. The total viable count in baked cooked lima beans (BCLB) and steamed cooked lima beans (SCLB) are 3.56 and 3.66 cfu g^{-1} respectively. There was no significant difference (p>0.05) in the total viable count of BCPB and SCLB. The result of the total viable count in this study is less than the total viable count of cooked beans (4.74 cfu g^{-1}) before fermentation into daddawa in [10] study and 4.19 cfu g^{-1} observed by [31]. There was no coliform and fungi in both the baked and cooked lima bean samples. This is an indication of absence of coliform in both samples due to the hygienic conditions under which the beans were processed. Similarly, the absence of fungi (yeasts and moulds) in the beans samples might be due to the appropriate temperature used in the processing of the beans. That is processing under unconducive temperature for the growth of fungi.

3.3. Proximate Composition of the Lima Beans Samples

The results of the proximate composition of the lima beans samples are shown in Table 3. There was no significant difference (p > 0.05) in the protein content of baked cooked lima bean (BCLB) and steamed cooked lima beans (SCLB). The protein content in both the BCLB and SCLB are similar to the protein content of cooked and canned pinto beans reported by [32]. Moisture content was highest in SCLB. Higher moisture in steamed cooked beans might be as a result of the cooking medium that is water, while on the other hand, the cooking medium for the baked cooked beans is tomato sauce which is of less water content. BCLB contained the least fat (1.02%) compared to SCLB (1.67%). It is assumed that addition of traditional cooking sauce for the steamed cooked beans resulted in the higher oil content observed for the steamed cooked lima beans. Fibre content in BCLB and SCLB were 5.88 and 5.71 % respectively. The result of the fibre content in the lima beans samples is close to that reported by [33] for cooked beans. 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 [13,14,15].

3.4. Antinutritional Factors in the Baked Cooked and Steamed Cooked Lima Beans Samples

Table 4 presents the result of the antinutrients content of the lima beans samples. Anti nutrients are substances that may have negative effect on the nutritive value of food material by impairing protein digestibility and mineral absorption in the body. However these antinutrients are easily destroyed or inactivated by processing methods such as soaking, germination, boiling, roasting, autoclaving and fermentation [7,8,34,35,36]. Soaking and cooking have been found to minimizes the presence of antinutrients such as trypsin inhibitors, volatile compounds such as HCN, and off-flavor volatile components [4,16] in legumes which can cause severe toxic effects.

Table 3. Proximate composition of the baked cooked and steamed cooked lima beans samples (%)

Sample	Moisture	Crude protein	Crude fat	Crude fibre	Ash	Carbohydrate
BCLB	42.89 ± 0.05^b	14.97 ± 0.05^a	1.02 ± 0.10^b	5.88 ± 0.08^a	2.65 ± 0.08^a	31.59 ± 0.02^a
SCLB	45.97 ± 0.03^a	13.56 ± 0.03^a	1.67 ± 0.08^a	5.71 ± 0.05^a	2.46 ± 0.07^b	32.81 ± 0.01^a

Means are values of three replicates ± standard error. Means in the same column followed by the same superscript are not significantly different (p< 0.05). BCLB = Baked cooked lima beans, SCLB = Steamed cooked lima beans.

Table 4. Anti-nutritional factors in the baked cooked and steamed cooked lima beans samples

Sample	Anti-nutrients content				
	Tannin (mg/kg)	Phytate (mg/kg)	Trypsin inhibitor (TIU/g)	Oxalate (mg/kg)	Cyanide (mg/kg)
BCLB	5.75 ± 0.11^b	10.10 ± 0.06^b	3.01 ± 0.12^b	10.10 ± 0.05^b	7.27 ± 0.01^b
SCLB	8.19 ± 0.25^a	12.06 ± 0.05^a	4.03 ± 0.11^a	9.15 ± 0.11^b	6.12 ± 0.04^a

Means are values of three replicates ± standard error. Means in the same column followed by the same superscript are not significantly different (p< 0.05). BCLB = Baked cooked lima beans, SCLB = Steamed cooked lima beans.

Tannins are known to inhibit the activities of digestive enzymes, they form complexes with protein and make the protein insoluble and indigestible [37]. Tannin content in the baked cooked (BCLB) and steamed cooked lima beans (SCLB) samples were 5.75 and 8.19 mg/kg respectively. The tannin contents observed in this study are slightly lower than the tannin content of 3.65mg/g observed by [33].

Phytates form complexes with nutritionally important minerals such as calcium, magnesium, zinc, copper and iron [38]. Samples, BCLB and SCLB contained 10.10 and 12.0 mg/kg of phytates respectively. The level of phytates observed in this study is lower than the phytate content of 10 – 60 mg/g that could pose health problem through reduction of mineral absorption if consumed over a long period of time [39]. Matured dried beans normally contain phytates in water soluble form [40] and this might be responsible for significant reduction of phytates in beans when soaked in water. Soaking has been found to decrease phytate content in legumes For example, soaking for 6 h at room temperature, at 50°C and 60°C respectively reduced the phytate in cowpea by 32.6%, 44.8% and 54.30% respectively [41].

Trypsin inhibitor are widely distributed among plants, especially in seeds, grains and legumes. They interfere with protein digestion by inhibiting digestive enzymes. This can lead to reduced availability of amino acids thus affecting growth rate [42]. Trypsin inhibitor is known to be heat labile. Trypsin inhibitor content in BCLB and SCLB were 3.01 and 4.03 TIU/g respectively. The value of trypsin inhibitor obtained in this study is higher than the trypsin inhibitor content of 1.10 mg/100g observed in boiled African yam bean reported by Ndidi *et al*. (2014). Complete destruction of trypsin inhibitor activity after autoclaving and boiling has been reported in African yam bean and lima bean [43,44,45,46].

Oxalate form complexes with minerals such as calcium thus interfering with the absorption of this mineral. Diet high in oxalate has been reported to increase the risk of development of kidney stone [47]. Oxalate content in the baked and cooked lima beans samples were 10.10 and 9.15mg/kg respectively. There was no significant difference ($p > 0.05$) in the oxalate content of baked and cooked lima beans.

Cyanides occur as toxic substance (hydrogen cyanide) in foods particularly tubers and legumes. Cyanide is an an effective cytochrome oxidase inhibitor, it interferes with aerobic respiratory system. Acute cyanide toxicity can thus result into death [48]. Cyanide content in the baked coked and steamed cooked lima beans samples were 7.27 and 6.12 mg/kg respectively. The cyanide content of the lima beans samples are lower than the cyanide content of boiled African yam bean reported by [33]. Soaking lima beans in water overnight before further processing has been reported to eliminate the hydrocyanic acid toxicity in the beans [4]. The World Health Organization established a safety threshold of 10 mg/kg of total cyanide in cassava flour, to protect consumers against adverse effects of chronic cassava intakes [49]. The values of cyanide content observed in this study are far below the permissible level/safe dose of 50-200mg/kg as reported by [50].

3.5. Antioxidant Capacity and Phytochemicals Content in Baked Cooked and Steamed Cooked Lima Beans Samples

The result of the antioxidant capacity and the phytochemicals contents in the lima bean samples are shown in Table 5. Baked cooked lima beans (BCLB) was significantly higher ($p<0.05$) in antioxidant capacity and phytochemicals content compared to steamed cooked lima beans (SCLB). Tomato and ginger sauce which form the main cooking broth for BCLB might have contributed to the increase in the phytochemicals content and baking could be a better processing method for retention of phytochemicals. The results obtained in this study is similar to the observation of [51] who concluded that the baked beans had a higher capacity of removing free radicals compared to the raw beans. Ginger (*Zingiber officinale*) has been reported to be a good source of flavonoids and phenolic compounds. Antioxidant activities of ginger and other plant extracts have been linked to their phenolic content. Phenolic compounds are able to donate hydrogen ion which is responsible for the inhibition of free radical induced lipids [52]. The phenolic compounds content in the baked and cooked lima beans are 18.5 and 13.9 gallic acid equivalent /g (GAE /g) respectively. The polyphenolic content of ginger has been found to be between 0.8 to 42.1 mg of gallic acid equivalent /g [53]. Phenolics have been reported to have both anticancer and antioxidant properties [54]. Baked lima beans was significantly high ($p<0.05$) in flavonoid and lycopene (104.5 mg/100g and 12.7mg/100g) compared to the cooked beans (62.5 mg/100g and 1.2 mg/100g) respectively. Tomatoes have been reported as a good source of lycopene and both tomatoes and ginger have been found to be good source of flavonoids. Like other phytonutrients, flavonoids are powerful antioxidants with anti-inflammatory and immune system benefits. Diets rich in flavonoid-containing foods have been reported to likely prevent cancer, neurodegenerative and cardiovascular diseases [55]. Flavonoids may lower the risk of atherosclerosis by protecting the body cells from free radical damage. They may also improve the quality of blood vessel walls [55]. Lycopene is also a free radical fighting antioxidant. Studies have shown that lycopene helps to prevent prostate, lung, and stomach cancers [56]. It has also been reported that increase intake of lycopene in diets could reduce the risk of cancers of the pancreas, colon, rectum, esophagus, oral cavity, breast, and cervix [56]. This antioxidant may also help reduce risk of developing cardiovascular disease by reducing low density lipoprotein (LDL) cholesterol and lowering blood pressure [57].

3.6. Sensory Evaluation of Baked Coked and Steamed Cooked Lima Beans

The result of the sensory evaluation of the baked cooked (BCLB) and steamed cooked lima beans (SCLB) samples is shown in Table 6. Sample BCLB recorded the highest value for all the sensory attributes tested (colour, appearance, flavor, texture, taste, overall acceptability). The sensory scores of BCLB for all the sensory attributes tested were also significantly higher ($p < 0.05$) than the those of SCLB. The two cooked lima beans samples were accepted by the sensory panelists but BCLB was most preferred.

Table 5. Antioxidant capacity and phytochemicals content in the baked cooked and steamed cooked lima beans samples

Sample	Antioxidant capacity (TAEC/g)	Phenolics (GAE/g)	Flavonoids (mg/100g)	Lycopene (mg/100g)
BCLB	26.5 ± 1.2^a	18.5 ± 0.11^a	104.5 ± 0.02^a	12.7 ± 0.12^a
SCLB	19.6 ± 1.0^b	13.9 ± 0.11^b	62.5 ± 0.01^b	1.2 ± 0.10^b

Means are values of three replicates ± standard error. Means in the same column followed by the same superscript are not significantly different ($p < 0.05$). BCLB = Baked cooked lima beans SCLB = Steamed cooked lima beans.

Table 6. Sensory evaluation of the baked cooked and steamed cooked lima bean samples

Sample	Colour	Appearance	Flavour	Texture	Taste	Overall acceptability
BCLB	7.9 ± 0.06^a	7.6 ± 0.05^a	6.6 ± 0.10^a	7.7 ± 0.10^a	7.5 ± 0.05^a	7.7 ± 0.01^a
SCLB	4.8 ± 0.02^b	5.4 ± 0.05^b	5.4 ± 0.08^b	5.0 ± 0.10^b	6.0 ± 0.05^b	5.1 ± 0.05^b

Values are mean scores ± standard error where n = 20. Means followed by different superscript within the same column are significantly different ($p < 0.05$). BCLB = Baked cooked lima beans SCLB = Steamed cooked lima beans.

4. Conclusion

Lima beans could be processed into baked and cooked beans. Processing time for baked cooked lima beans (BCLB) was shorter than that of steamed cooked lima beans (SCLB). BCLB was found to have higher nutrients and phytochemicals content than SCLB. BCLB and SCLB were both acceptable but the BCLB was most preferred with respect to all sensory attributes tested.

In conclusion, both BCLB and SCLB have great utilization potentials, but BCLB has a higher potential in terms of antioxidant and phytochemicals benefits as well as shorter cooking time. Development of baked beans from Lima beans in the country will provide dietary diversity, improve lima bean utilization, improve nutritional status of consumers and boost the country's economy. Further studied should be carried out on shelf-life of baked cooked lima bean for enhanced commercialization.

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Statement of Competing Interest

The authors have no competing interest.

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