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Effect of heat processing treatments on the nutrient and anti-nutrient contents of lima bean (*Phaseolus lunatus*) flour

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Abstract

The effect of heat processing treatments on the nutrient and anti-nutrient contents of lima bean (*Phaseolus lunatus*) flour were investigated. The lima bean seeds were sorted, cleaned and processed into boiled, roasted and autoclaved lima bean flours. The flours obtained were analysed for proximate, vitamin and anti-nutrient contents using standard methods. The proximate composition of the samples revealed that the flours had a range of moisture, 8.25±0.01 - 10.21±0.06%, crude protein, 18.24 ±0.02 - 22.72±0.00%, ash, 3.94±0.13 - 4.82 ±0.02%, fat, 1.47±0.03 - 1.68±0.00%, crude fibre, 3.62±0.00 - 4.27±0.02%, carbohydrate, 57.31±0.13 - 62.89±0.01%, and energy 334.11±0.30 - 339.91±0.18kJ/100g, respectively. The vitamin contents of the flours showed that the samples contained 3.40±0.00 - 4.57±0.05mg/100g ascorbic acid, 0.03±0.00-0.07±0.00mg/100g thiamine, 0.02±0.00 - 0.08±0.00mg/100g riboflavin, 0.19±0.00 - 0.25±0.00mg/100g niacin, 2.77±0.04 - 5.72±0.00mg/100g vitamin A and 1.86±0.00 - 2.94±0.00mg/100g vitamin E, respectively. The result of the anti-nutrient composition of the flours also showed that the alkaloid, phytate, saponin, tannin, trypsin inhibitor, oxalate levels of the samples were significantly ($p \leq 0.05$) reduced by autoclaving and boiling treatments compared to the sample processed by roasting. In addition, the saponin content of the flours was relatively higher in boiled sample than in roasted and autoclaved flours. However, the nutrient and anti-nutrient contents of the flours observed that the flours have the potentials to be used as nutritional supplements in the preparation of a variety of food products than the raw sample.

Keywords: lima bean, boiling, roasting, autoclaving, proximate, vitamin, anti-nutrient content

Introduction

Lima bean (*Phaseolus lunatus*) is a legume that is originated from Peru. Lima bean is the second most economically important species of *Phaseolus* and is also one of the twelve primary grain legumes known in the world (Fofana *et al.*, 1999) [5]. The lima bean is an annual or short-lived perennial species, with a mixed mating system that is predominantly autogamous but without crossing levels up to 48% (Baudoin *et al.*, 1998) [3]. The grain of Lima bean has high protein content and can be used for human alimentation thus decreasing over dependence on common bean (Vieira, 1992) [18]. According to Oliveira *et al*. (2004), the lima bean is actually an alternative food source for human alimentation in the Northeast region of Brazil. It is an important source of plant protein and is also rich in antioxidants, vitamins, minerals, and plant sterols. Lima beans are a very rich source of B-complex vitamins, especially vitamin B_6 (pyridoxine), thiamine (vitamin B_1), pantothenic acid, riboflavin and niacin. Most of these vitamins function as co-enzymes in carbohydrate, protein, and fat metabolism in the body. Lima bean is also one of the excellent sources of minerals like molybdenum, iron, copper, manganese, calcium, magnesium. Lima beans are relatively high in potassium(1724mg) compared to red kidney beans (1359mg), broad beans (1062mg) and black beans (1483mg). Potassium is important electrolyte of cell and body fluids. It helps to counter the pressing effects of sodium on heart and blood pressure.

Lima bean is one of the lesser known and underutilized edible grain legumes that is widely cultivated in Africa (Vieira, 1992) [18].

Like other grain legumes, lima bean is one of the underutilized legumes that is relatively rich in protein but low in fat and crude fibre. The utilization of lima beans in human and animal nutrition is limited by the presence of naturally occurring antinutritional factors such as alkaloids, saponins, trypsin inhibitors, tannins, phytates and oxalates and these anti-nutrients can be drastically reduced or eliminated during processing by the application of simple processing techniques such as boiling, roasting, autoclaving, blanching, fermentation, soaking and germination. (Sandberg, 2002) [15]. The objective of this study is to investigate the effects of boiling, roasting and autoclaving on the nutrient and anti-nutrient contents of lima bean flours.

Materials and Methods

Mature dried lima bean *Phaseolus lunatus* seeds used for the study were purchased from Afor Nmaku Market, Enugu State, Nigeria. The seeds were sorted, cleaned and divided into four equal portions of 500g each. Three portions were subjected to different processing treatments (boiling, roasting and autoclaving) while the fourth batch was processed raw.

Preparation of Raw Lima Bean Flour.

The raw lima bean flour was prepared according to the method of Ugwu and Oranye (2006) [17]. During preparation, five hundred grams (500g) of Lima bean seeds which were free from dirt and other extraneous materials were weighed, cleaned and soaked in 2 litres of potable water at room temperature (30 ± 2^0C) for 12 h. The soaked seeds were drained and dehulled manually by rubbing in between palms to remove the hulls. The dehulled seeds were rinsed, spread on the trays and dried in a hot air oven (Model DHG 9101 ISA) at 60^0C for 6 h with occasional stirring of the seeds at intervals of 30 min to ensure uniform drying. The dried seeds were milled into flour using the locally fabricated attrition mill and sieved through a 500 micron mesh sieve. The flour produced was packaged in an airtight plastic container and kept in a freezer until needed for analysis.

Preparation of Boiled Lima Bean Flour.

The boiled lima bean flour was prepared according to the method of Ugwu and Oranye (2006) [17]. During preparation, five hundred grams (500g) of lima bean seeds which were free from dirt and other extraneous materials were weighed, cleaned and soaked in 2 litres of potable water at room temperature (30±2^{0}C) for 12 h. The soaked seeds were drained, rinsed and dehulled manually by rubbing in between palms to remove the hulls. The dehulled seeds were boiled with 2 litres of potable water in a hot plate at 100^{0}C for 30 min. The boiled seeds were drained, spread on the trays and dried in a hot air oven (Model DHG 9101 ISA) at 60^{0}C for 6 h with occasional stirring of the seeds at intervals of 30min to ensure uniform drying. The dried seeds were milled into flour using the locally fabricated attrition mill and sieved through a 500 micron mesh sieve. The flour produced was packaged in an airtight plastic container and kept in a freezer until needed for analysis.

Preparation of Roasted Lima Bean Flour.

The roasted lima bean flour was prepared according to the method of Ugwu and Oranye (2006) [17]. During preparation, five hundred grams (500g) of lima bean seeds which were free from dirt and other extraneous materials were weighed, cleaned and soaked in 2 litres of potable water at room temperature (30±2^{0}C) for 12 h. The soaked seeds were drained, rinsed and dehulled manually by rubbing in between palms to remove the hulls. The dehulled seeds were rinsed, spread on the trays and roasted in a hot air oven (Model DHG 9101 ISA) at 240^{0}C for 40 min with occasional stirring of the seeds at intervals of 5 min to ensure uniform roasting. The roasted seeds were milled in the attrition mill and sieved through a 500 micron mesh sieve. The flour produced was packaged in an airtight plastic container and kept in a freezer until needed for analysis.

Preparation of Autoclaved Lima Bean Flour.

The autoclaved lima bean flour was prepared according to the method of Ugwu and Oranye (2006) [17]. During preparation, five hundred grams (500g) of lima bean seeds which were free from dirt and other extraneous materials were weighed, cleaned and soaked in 2 litres of potable water at room temperature (30±2^{0}C) for 12 h. The soaked seeds were drained, rinsed and dehulled manually by rubbing in between palms to remove the hulls. The dehulled seeds were placed in a beaker and autoclaved in an autoclave (Model 75xG) at temperature of 121^{0}C and pressure of 6 atmosphere for 40 min. The autoclaved seeds were spread on the trays and dried in a hot air oven (Model DHG 9101 ISA) at 60^{0}C for 4 h with occasional stirring of the seeds at intervals of 30 min to ensure uniform drying. The dried seeds were milled into flour using the locally fabricated attrition mill and sieved through a 500 micron mesh sieve. The flour produced was packaged in an airtight plastic container and kept in a freezer until needed for analysis.

Chemical Analysis

The moisture, crude protein, fat, ash and crude fibre contents of the samples were determined in triplicate according to the method of AOAC (2006). Carbohydrate was determined bydifference (Anton *et al., 2008*). The energy content of the flours was calculated from the proximate composition using the At water factor 4×protein, 9×fat, 4×carbohydrate (Shumaila and Mahpara, 2009). The ascorbic acid and niacin contents of the samples were determined according to the method of AOAC (2006). The thiamine and riboflavin contents of the flours were determined according to the flourimetric method of Onwuka (2005) [13]. Vitamin A and Vitamin E were determined by the method of Ene-Obong and Obizoba (1996) [4]. The alkaloid, tannin, saponin, oxalate and trypsin inhibitor levels of the samples were determined using the spectrophotometric method of Onwuka (2005) [13]. Phytate was determined by the solvent extraction gravimetric method of AOAC (2006) [2].

Statistical Analysis

The data generated after the analysis were subjected to Analysis of Variance (ANOVA) using special package for social sciences (SPSS version 20, 2013) to detect significant differences among the sample means at ($p \leq 0.05$). Significant means were separated using Turkey's Least Siginificance Difference (LSD) test.

Results and Discussion

Proximate Composition

The proximate composition of the samples is presented in Table 1. The moisture content of the sample was significantly ($p \leq 0.05$) higher in boiled sample compared to the samples processed by autoclaving and roasting. The increase could be attributed to the inhibition of large quantity of water by the seeds as a result of boiling during processing. The observation is in agreement with the report of Nsa and Ukachukwu (2009). The high moisture affects the storage stability of legume and other flour products. The crude protein content of the flour was significantly ($p \leq 0.05$) lower in boiled and autoclaved samples than in roasted flour sample. The reduction in the protein content of the boiled flour could be attributed to leaching of some soluble protein into the boiling water during processing (Obasi and Wogu, 2008) [9]. Dietary proteins are needed for the synthesis of new cells enzymes and hormones required for the development of the body. (Okaka *et al.*, 2006). The ash content of the raw sample which was originally 4.82% was found to be significantly ($p \leq 0.05$) reduced to 3.94% by autoclaving treatment compared to the values of 4.66% and 4.32% recorded by roasted and boiled samples, respectively. The high ash content by boiled and roasted flour is an indication that they are food sources of minerals than in the autoclaved sample. (Okoye and Mazi, 2012). The fat content of the flours which ranged from 1.47 to 1.68% was significantly ($p \leq 0.05$) reduced by autoclaving followed by boiling and roasting treatments. Fat is important in human diets because it is a high energy-yielding nutrient. Legumes seeds are generally low in fats and oil (Okaka *et al.*, 2006). The crude fibre content of the samples was significantly ($p \leq 0.05$) reduced by autoclaving compared to boiling and roasting treatments. Fibre has been credited for promotion of increased excretion of bile acids, sterols and fats which have been implication in the etiology of certain ailments in humans (Okaka *et al.*, 2006). The carbohydrate content of the flours was significantly ($p \leq 0.05$) lower in boiled and autoclaved flour than in the sample processed by roasting. The decrease could be attributed to thermal decomposition of some carbohydrate components into carbonic acid and carbon dioxide by boiling and roasting treatments (Obasi and Wogu, 2008) [9]. The energy content of the sample was significantly ($p \leq 0.05$) increased by autoclaving and roasting treatments compared to the sample processed by boiling. The increase in energy content of the sample could be a reflection of their high protein and carbohydrate content (Obum *et al.,* 2006). Generally boiling, roasting and autoclaving treatments greatly increased some nutrient contents of lima bean flours by reducing their anti-nutrient contents.

Vitamin Contents

Table 2 shows the vitamin contents of lima bean flours. The ascorbic acid content of the flours ranged from 3.40 to 4.57mg/100g with the roasted and autoclaved samples having the highest (4.25mg/100g) and least (3.40mg/100g) values, respectively. The differences could be due to oxidation and leaching of the vitamin into boiling water during boiling, roasting and autoclaving of the legumes. Generally, the raw sample had higher ascorbic acid content than the processed samples (boiled, roasted and autoclaved samples). Ascorbic acid plays an important role in the prevention of scurvy. It also promotes the wound healing, healthy immune system and prevents cardiovascular diseases (Okaka *et al.,* 2006). Ascorbic acid is easily destroyed by oxidation, especially at high temperatures and it is the vitamin most easily lost during food processing (Potter and Hotchkiss, 2006). Roasting improves the amount of Ascorbic acid more than boiling and autoclaving which are crucial for body performance, prevents scurvy, aid wound healing, healthy immune system and cardiovascular diseases (Lee and Kader, 2000). The thiamine content of the samples which ranged from 0.03 to 0.07mg/100g was significantly ($p \leq 0.05$) reduced by boiling and autoclaving followed by roasting treatment. Thamine functions as a co-enzyme in energy metabolism. It helps in the functioning of peripheral nerves and treatment of beriberi (Potter and Hotchkiss, 2006). The riboflavin content of the flours was significantly ($p \leq 0.05$) increased by autoclaving than the boiling and roasting treatments. The autoclaved sample recorded higher riboflavin content than the raw sample. Riboflavin functions as part of a group of enzymes called flavoproteins. Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) assist in the respiratory chains of cellular metabolism more especially in the oxidation-reduction reaction involving the release of energy. More so, the presence of this vitamin improves growth, reproduction and prevents anaemia and abnormal gait (Potter and Hotchkiss, 2006).

The niacin content of the samples varied from 0.19 to 0.25mg/100g, a range that is lower than the level of niacin (3.86 to 4.58mg/100g) reported by Okoye and Mazi (2012) for boiled and roasted groundnut flours. Niacin which is equally a member of the B-complex vitamin functions as a co-enzyme (NAD and NADP) in the body. It also has specific effect on the growth and plays an important role in reducing the levels of blood cholesterol (Potter and Hotchkiss, 2006).

The vitamin A content of the raw sample which was found to be 5.72mg/100g was significantly ($p \leq 0.05$) reduced by autoclaving followed by boiling and roasting treatments. Vitamin A helps in maintenance of normal vision of the eyes (Okaka *et al.*, 2006). A deficiency of vitamin A leads to blindness, failure of normal bone and tooth development in the young children. (Potter and Hotchkiss, 2006). The Vitamin E content of the samples was significantly ($p \leq 0.05$) reduced by autoclaving than the boiling and roasting treatments. The values obtained in the study were higher than those (1.45-2.68mg/100g) reported by Ibrahim *et al.* (2002) for cooked and soaked cowpea flours. Vitamin E is a strong antioxidant which functions as such in human metabolism. It is also able to spare carotene and vitamin A from oxidative destruction. Generally, the result showed that the processed lima bean flours could be used as nutritional supplements in the formulation of a number of food products than the raw sample.

Anti-nutrient Contents

Table 3 shows the anti-nutrient contents of lima bean flours. The alkaloid content of the samples which ranged from 0.08 to 1.23% was significantly ($p \leq 0.05$) lower in autoclaved and boiled flours compared to the sample processed by roasting. The decrease is an indication that autoclaving and boiling have greater reduction effect on the alkaloid level of the raw lima bean than the roasting treatment (Murray, 1996). The phytate content of the raw sample which was found to be 0.65% was significantly ($p \leq 0.05$) reduced to 0.04% by autoclaving followed by boiling (0.47%) and roasting (0.49%) treatments, respectively. According to Udensi *et al.* (2003) phytate lead to hard cook phenomenon in pulses, which increased the cooking time of legume grain. The apparent decrease in phytic acid during boiling and autoclaving could be attributed to the formation of insoluble complexes between phytates and other components such as phytate protein and phytate mineral complexes (Sandberg, 2002) [15]. Phytate related compounds have been reported to have beneficial effect as an antioxidant (Echendu *et al.,* 2009). Phytate lowers the bioavailability of certain minerals such as zinc, calcium and iron thus making them nutritionally unavailable to the body. The reduction in phytate contents is in agreement with the work of Ene-Obong and Obizoba (1996) [4] who reported reduction of phytic acids in boiled bambara groundnut by up to 74%. The saponin content of the samples was significantly ($p \leq 0.05$) higher in boiled sample compared to the samples processed by autoclaving and roasting. The observation is in agreement with the report of Ugwu and Oranye (2006) [17] for boiled and autoclaved African breadfruit flour. Saponin has cholesterol binding properties and haemolytic activity (Sadipo *et al.,* 2000). This cholesterol binding activity of saponin could be harnessed in the treatment of cancer, cardiovascular disease and any other malady affecting the health or well-being of an individual (Murray, 1996). The tannin content of the sample which ranged from 0.08 to 0.98% was significantly ($p \leq 0.05$) lower in autoclaved seed flour compared to the sample processed by boiling and roasting treatments. The presence of tannin in food has been reported to have deleterious effect in inhibiting the absorption of certain minerals such as zinc, calcium and magnesium in humans (Oke, 1996). Tannins are known to reduce protein quality directly by forming complexes thereby decreasing its digestibility and palatability. Tannins can equally participate in oxidation-reduction reactions which results in the loss of ascorbic acid (Nget-Hong *et al.*, 1983) [8]. Reduction in tannin during processing will however improve the utilization of such nutrients. The trypsin inhibitor level of the samples ranged from 2.45 to 18.41% with autoclaved and roasted flours having the least and highest values, respectively. The observation is in agreement with the report of Obasi and Wogu (2008) [9] for roasted and boiled yellow maize flour. The oxalate content of the raw lima bean flour which was found to be 0.27% was drastically reduced to 0.02% by autoclaving followed by boiling compared to the roasting treatment (0.03%). Oxalates affect calcium and magnesium metabolism, and react with proteins to form complexes which have an inhibitory effect in peptic digestion (Akande *et al. 2010*) [1].

Generally, autoclaving which is otherwise known as pressure cooking has a more significant effect on the reduction of an anti-nutrient content of lima beans than the boiling and roasting treatments. Therefore, autoclaving and boiling could be employed for total destruction of the toxins without destroying the nutritional value of the food.

Conclusion

Autoclaving followed by boiling generally resulted in reduction in the protein, ash, fat, fibre, niacin and riboflavin contents of the products. The use of roasting in the treatment of lima beans yielded product with increased carbohydrate, thiamin, ascorbic acid, vitamin A and vitamin E contents. Furthermore, of all the heat processing treatments used, autoclaving drastically reduced the anti-nutrient contents of lima beans than the boiling and roasting treatments. It is therefore recommended that any of the processes can be used for processing lima beans, however, due to the fact that autoclaves are quite expensive and msy not be affordable by local producers or manufacturers of lima bean products, boiling and roasting can be easily employed.

Table 1: Proximate Composition of Lima Bean Flours

	Samples			
Parameters	**Raw**	**Boiled**	**Roasted**	**Autoclaved**
Moisture (%)	$9.21^{c} \pm 0.13$	$10.21^{a} \pm 0.06$	$8.25^{d} \pm 0.01$	$9.85^{b} \pm 0.01$
Crude protein (%)	$22.72^{a} \pm 0.00$	$18.72^{c} \pm 0.04$	$19.80^{b} \pm 0.00$	$18.24^{d} \pm 0.02$
Ash (%)	$4.82^{a} \pm 0.02$	$4.34^{c} \pm 0.03$	$4.66^{b} \pm 0.06$	$3.94^{d} \pm 0.13$
Fat (%)	$1.68^{a} \pm 0.00$	$1.53^{c} \pm 0.01$	$1.61^{b} \pm 0.01$	$1.47^{d} \pm 0.03$
Crude fibre (%)	$4.27^{a} \pm 0.02$	$3.84^{c} \pm 0.00$	$4.13^{b} \pm 0.00$	$3.62^{d} \pm 0.00$
Carbohydrate (%)	$57.31^{d} \pm 0.13$	$61.37^{c} \pm 0.07$	$61.56^{b} \pm 0.08$	$62.89^{a} \pm 0.01$
Energy (kJ/100g)	$335.24^{c} \pm 0.51$	$334.11^{d} \pm 0.30$	$339.91^{a} \pm 0.18$	$337.73^{b} \pm 0.17$

Values are mean + standard deviation of triplicate determinations.
Means in the same row with different superscripts are significantly different ($p \leq 0.05$).

Table 2: Vitamin Contents of Lima Bean Flours.

	Samples			
Parameters	**Raw**	**Boiled**	**Roasted**	**Autoclaved**
Ascorbic acid (mg/100g)	$4.57^{a} \pm 0.05$	$3.72^{c} \pm 0.00$	$4.25^{b} \pm 0.00$	$3.40^{d} \pm 0.00$
Thiamin(mg/100g)	$0.07^{a} \pm 0.00$	$0.03^{a} \pm 0.00$	$0.05^{a} \pm 0.00$	$0.03^{a} \pm 0.00$
Riboflavin (mg/100g)	$0.03^{b} \pm 0.00$	$0.02^{b} \pm 0.00$	$0.02^{b} \pm 0.00$	$0.08^{a} \pm 0.00$
Niacin(mg/100g)	$0.25^{a} \pm 0.00$	$0.19^{a} \pm 0.00$	$0.23^{a} \pm 0.01$	$0.19^{a} \pm 0.00$
Vitamin A (mg/100g)	$5.72^{a} \pm 0.00$	$3.47^{c} \pm 0.01$	$4.82^{b} \pm 0.00$	$2.77^{d} \pm 0.04$
Vitamin E (mg/100g)	$2.94^{a} \pm 0.00$	$1.92^{c} \pm 0.03$	$2.77^{b} \pm 0.05$	$1.86^{d} \pm 0.00$

Values are mean ± standard deviation of triplicate determinations.
Means in the same row with different superscripts are significantly different ($p \leq 0.05$).

Table 3: Anti-Nutrient Contents of Lima Bean Flour.

	Samples			
Parameters	**Raw**	**Boiled**	**Roasted**	**Autoclaved**
Alkaloid (%)	$1.23^{a} \pm 0.00$	$0.10^{c} \pm 0.00$	$1.17^{b} \pm 0.01$	$0.08^{c} \pm 0.00$
Phytate (%)	$0.65^{a} \pm 0.00$	$0.47^{b} \pm 0.55$	$0.49^{b} \pm 0.00$	$0.04^{c} \pm 0.00$
Saponin (%)	$16.29^{a} \pm 0.05$	$1.80^{b} \pm 0.03$	$1.22^{c} \pm 0.01$	$1.20^{c} \pm 0.03$
Tannin (%)	$0.98^{a} \pm 0.71$	$0.19^{c} \pm 0.01$	$0.35^{b} \pm 0.00$	$0.08^{d} \pm 0.00$
Trypsin Inhibitor (%)	$18.41^{a} \pm 0.01$	$7.63^{c} \pm 0.04$	$14.31^{b} \pm 0.01$	$2.45^{d} \pm 0.00$
Oxalate (%)	$0.27^{a} \pm 0.02$	$0.03^{c} \pm 0.00$	$0.18^{b} \pm 0.00$	$0.02^{c} \pm 0.00$

Values are mean ± standard deviation of triplicate determinations.
Means in the same row with different superscripts are significantly different ($p \leq 0.05$).

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