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## Evaluation of the Effect of Processing Methods on the Nutritional and Anti-nutritional Compositions of Two Under-utilized Nigerian Grain Legumes

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Abstract: The nutritional and anti-nutritional compositions of African Yam Bean (AYB) and Lima bean flours under different processing methods were determined. Nutritional and anti-nutritional properties studied include moisture content, crude protein, crude fibre, ash content, ether extract, carbohydrate, tannin, protease inhibitor and phytate. The moisture content of AYB flours ranged from 9.31 to 9.61% while that of lima beans ranged from 9.32 to 9.56%. There is a significant different among the samples when the unprocessed AYB (control) and the processed AYB were compared. The same trend was also observed with lima bean flours. However, some nutrient did not show significant variations with processing. It was observed that samples of soaked/de-hulled AYB have the least protease inhibitor of 0.73 mg/100 g and it is significantly different from the unprocessed samples. Soaked/de-hulled flours of both AYB and lima beans have the most percentage decrease in antinutritional content. Lima bean flours were observed to have higher anti-nutritional content than AYB. The percentage decrease of anti-nutritional factors in the samples is proportionally higher than that of the nutrients. The nutritional and anti-nutritional compositions of the samples suggest that processed African Yam Bean (AYB) and Lima bean flours would have useful application in fabricated foods.

**Key words:** Nutritional, anti-nutritional, African yam bean, lima bean, flour

#### INTRODUCTION

Protein of animal origin in Nigeria is of greater demand than its supply (Ojewole, 1993). Going by principles of economics, greater demand against supply brings increased point of sale prices of animal protein, therefore, making it unaffordable by low-income earners. In tropical Africa, mixture of cereals or starchy foods and grain legumes is usually the stable diet among low-income groups. The legumes and protein-rich seeds are added as substitutes to high protein foods of animal origin which are too expensive for the majority of the low-income earners (Wokoma and Aziagba, 2001). Thus, leguminous plants have met the need of the consumers as sources of protein. This is due to their contributions of about 18% of the world's protein requirement in Nigeria. The most popularly consumed protein-rich foods include cowpea (Vigna unguiculata), African locust bean (Parkia biglobosa), melon (Citrullus vulgaris), African oil bean (Pentaclethra macrophylla) and groundnut (Arachis hypogea).

Cowpea consumption, for example, has gained popularity in Nigeria because it is a good source of protein for many who cannot afford protein from foods

such as fish and meat (Osho and Dashiell, 1998). African Yam Bean (AYB) (Sphenostylis stenocarpa) is one of the under-utilized legumes cultivated in various parts of Africa for its edible seeds and potato-like spindle shaped tubers. It belongs to the family, Papilionacea which is sometimes classified in the sub-family Leguminosae (Evans and Boulter, 1974). The prices of African yam beans and lima beans, an underutilized legume, are considerably lower than their popular counterpart, cowpea (Frank-Peterside et al., 2002). This comparison is similar for pigeon pea and lima beans too. Unlike cowpea, these minor underutilized legumes (lima beans and African yam beans) have not received much popularity and wider acceptance, although most of them are widely distributed and with better yields (Amoatey et al., 1997). These neglected pulses of tropical origin have attracted research interest in recent times due to their nutrient contents and have also been reported that they have higher antinutritional content as compared to the major legumes (Fasoyiro et al., 2006). The anti-nutrients found in these legumes reduce the availability of nutrients for absorption from these legumes but a number of processing and preparation methods helps in reducing their effect (Ene-Obong and Obizoba, 1996). The

underutilized grain legumes are considered to be functional foods in addition to their traditional role of providing dietary protein because of their relatively higher free radical scavenging ability and also their cholesterol lowering activity.

In order to contribute to the search of a nutritious and more affordable food source, the present study analyzed two (2) underutilized legumes, lima beans and African yam beans flours, for their proximate composition and various anti-nutrients like tannins, phytate and trypsin inhibitor under different processing methods.

#### MATERIALS AND METHODS

**Sample collection:** African yam bean (*Sphenostylis stenocarpa*) and Lima bean (*Phaseolus lunatus*) seeds were used in this study. The dry seeds were purchased from a local market, Uhanmi Market, Isua Akoko, Ondo State, Nigeria and Waso Market, Ogbomoso, Oyo State, Nigeria, respectively. All bean seeds were stored in air-tight plastic containers in the refrigerator, prior to use. Storage in the refrigerator is to avoid weevil infestation.

**Sample preparation:** Three processing methods were applied before milling the seeds which includes the following: A portion, each of African yam bean and lima beans, were soaked for 24 h and sun-dried before milling. The second portion each were soaked for 12 h and de-hulled before milling. The third portion each was sprouted then dried before milling. The last portion each were milled unprocessed and serve as control. The legumes seed were milled to pass through a 1.0 mm sieve. All the flour samples from each legume were stored in polythene bags at 4°C.

#### Analytical procedure

Moisture content determination: The moisture content of the samples was determined using AOAC (1990) method. The sample (2-5 g) was weighed into already-weighed clean drying cans. The cans with the sample were then placed in a well-ventilated oven (Fisher Scientific Isotemp oven, by Fisher Scientific Co. USA, model 655F) maintained at 105±2°C. After 16-18 hours, the drying cans were then transferred into a desiccator to cool after which the final weights were taken.

Percentage moisture content (MC) = 
$$\frac{W_1 - W_2}{s} \times 100$$

Where:

W<sub>1</sub> = Weight of empty moisture content can with sample before drying

W<sub>2</sub> = Weight of moisture content can with sample after drying

S = Weight of sample

Ash content determination: This was carried out using AOAC (1990) method. About 2 g of the samples were weighed into a crucible dish which had been previously ignited and weighed. The crucible dish was then placed on a hot plate inside the fume cupboard to char the organic matter, while the remaining residue (inorganic matter) was later transferred into the muffle furnace, (Fisher Scientific Isotemp Muffle furnace by Fisher Scientific Co. USA, model 186A) maintained at 600°C for 6 h to completely ash the sample. The crucible dishes were then transferred into a dessicator to cool and it was weighed thereafter.

The percentage ash content was calculated as follows:

Ash content % = 
$$\frac{W_1 - W_2}{W_2 - W_1} \times 100$$

Where:

 $W_1$  = Weight of crucible

W<sub>2</sub> = Weight of crucible+sample before ashing

W<sub>3</sub> = Weight of crucible+sample after ashing

Crude protein determination: This was carried out using HACH (1990) method. The sample (0.20-0.25 g) was weighed into the digesting tube and 4 mL each of concentrated tetraoxosulphate IV acid (H2SO4) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were added. One (1) tablet of Kjeldhal catalyst was added and the sample was digested on the digestion block at 375°C for three (3) h. The tube was then filled up with distilled water to the mark and then covered with aluminium foil and it was mixed well. Approximately 1 mL of the digest was pipetted into a 25 mL volumetric flask and 3 drops each of polyvinyl alcohol solution and mineral stabilizer were added and filled up to the 25 mL mark with distilled water. Then 1 mL of Nessler reagent was added. The mixture was then poured into the Hach spectrophotometer to determine the concentration of nitrogen at 460 nm.

Nitrogen% = 
$$\frac{\text{conc.} \times 0.0075}{\text{sampleweight}(g)}$$

Protein% = % Nitrogen×6.25

Crude fibre content determination: This was carried out using AOAC (1990). The sample (2.0 g) was weighed into a 600 mL long beaker and 200 mL of hot  $1.25\%\,H_2SO_4$  was

added to it The beaker was then placed on the digester apparatus that had been pre-heated. The content was then boiled and refluxed for 30 min. The content was then filtered through Whatman GF/A paper by gravity. The beaker was rinsed with distilled water and the residue was washed on the paper with distilled water until the filtrate was neutral. The residue was then transferred from the paper back into the beaker with the aid of 1.25% hot Sodium hydroxide (NaOH) and the volume of the NaOH was adjusted to 200 mL. The beaker was returned onto the digestion apparatus, boiled and refluxed for 30 min. It was then filtered and rinsed. The paper was then transferred with residue into a crucible and dried at 100°C overnight. It was then cooled in a desiccator and weighed (Weight A). The sample was then placed in a furnace at 600°C for 6 h. Then cooled in a desiccator and reweighed (Weight B). The loss in weight during incineration represents the weight of crude fibre in the sample.

Total dietry fibre% = 
$$\frac{\text{(Weight A - Weight B)}}{\text{Sample weight(g)}} \times 100$$

Ether extract content determination: The procedure of AOAC (1990) was employed using an automated system (Soxtec/Soxhlet system HT2). Three gram of dried sample was weighed into the thimble, plugged with cotton wool and inserted into the soxtec HTec. Fifty milliliter of solvent (Hexane) was poured inside the cups (with boiling chips). The cups was also inserted into the soxtec HT. Extraction was done for 15 min in "Boiling" position and for 30-45 min in "Rising" position before the solvent evaporates. The cup was released, dried at 100°C for 30 min, cooled in a desiccator and weighed.

The percentage fat content will be obtained as below:

$$Fat\% = \frac{ Weight \ of \ cup \ with \ extracted \ oil - weight \ of \ empty \ cup}{Sampleweight \ (g)}$$

Carbohydrate content was determined by difference (AOAC, 1990).

Anti-nutritional factors analysis: The condensed tannins were determined by method of Morrison *et al.* (1995) which was a modification of vanillin method of Burns (1971) using 1.0 mg mL<sup>-1</sup> of catechin in 1% HCl-MeOH as standard, the coloured substituted product was measured at 500 nm. Phytate was determined by the amon exchange method which is the modified Holts Method using HN<sub>4</sub>CNS as standard. Protease inhibitory activities were determined by Casein Digestion method as described by Kakade *et al.* (1970).

**Statistical analysis:** Data were subjected to analysis of variance and means were separated by Duncan multiple range test in accordance with Statistical Analysis System package (SAS, 2003).

#### RESULTS AND DISCUSSION

**Nutritional composition:** The comparative nutritional analysis of Africa yam beans and Lima beans under different processed forms are shown in Table 1. The moisture content of AYB flours ranged from 9.31 to 9.61% while that of lima beans ranged from 9.32 to 9.56%. There is a significant different among the samples when the unprocessed AYB (control) and the processed AYB were compared. The same trend was also observed with lima bean flours. All the samples of AYB and lima beans have moisture content that is considerably low and will not favor microbial growth. In the reported results, it was observed that moisture content of the different processes increased as compared to their control, for both Lima and AYB flours. The increase might be due to the addition of water involved in the processes, as sprouting involved sprinkling water on sample dry seed and de-hulling involved soaking in water. The moisture content could have been further reduced if samples were subjected to further sun-drying. Since moisture content is a function of water activity that determines shelf life, it is inferred that the processed samples would have a lower shelf life relative to their corresponding control if under the same humidity condition contents (Adeyeye, Eromosele et al., 2008).

The crude protein content of unprocessed AYB and Lima bean flours are 23.53 and 24.46%, respectively. They are slightly different from each other (p<0.05) had significant difference (p<0.05) with other flours that were processed. The crude protein contents of unprocessed AYB and lima bean observed in this study were within the range of values reported for cowpea by Ekpenyong and Borchers (1980) and Omueti and Singh (1987); although, Dovlo et al. (1976) however reported lower value of crude protein (22%) for cowpea. This gives an indication that the unprocessed samples of AYB and lima beans have higher protein content. It was observed that there is decrease in protein content with processing. Singh and Eggum (1984) reported that considerable nutritional loses occur during primary processing which includes removal of seed coat from raw pigeon pea to obtain dhal. Such losses occur due to the presence of protein rich outer layers and these outer layers are lost as a result of abrasive action of roller mill during processing. It is observed that soaked/de-hulled samples of both lima bean

Table 1: Comparative analysis of African vam bean nutrient composition with different processing methods

Samples	Moisture (%)	Crude protein (%)	Ether extract (%)	Ash content (%)	Crude fiber (%)	Carbohydrate (%)
Unprocessed AYB	9.27±0.0577°	23.53±0.1528 <sup>f</sup>	1.51±0.0000°	3.27±0.0577 <sup>b</sup>	2.27±0.0577°	60.16±0.0577°
Sprouted AYB	$9.46\pm0.1000^{d}$	$22.63\pm0.1528^{d}$	1.26±0.0577°	3.27±0.1155b	$2.11\pm0.0000^{d}$	61.27±0.1528 <sup>c</sup>
Soaked/de-hulled AYB	9.61±0.0000°	21.77±0.0577°	1.32±0.1000 <sup>b</sup>	$3.21\pm0.1000^a$	2.03±0.0577°	$62.83\pm0.1732^{h}$
Soaked (24 h) AYB	9.31±0.0577°	21.03±0.0577 <sup>a</sup>	1.33±0.0577°	3.23±0.0577a	1.97±0.0577 <sup>b</sup>	62.56±0.1732g
Unprocessed Lima	9.32±0.0000°	$24.46\pm0.1528^{h}$	5.93±0.0577g	$3.37\pm0.0577^{d}$	$2.51\pm0.0000^{f}$	54.43±0.1155a
Sprouted Lima	9.43±0.1528°	23.73±0.0577g	$5.31\pm0.0000^{d}$	$3.32\pm0.1000^{\circ}$	2.27±0.0577°	55.97±0.0577°
Soaked/de-hulled Lima	9.56±0.1155°	22.83±0.0577e	5.37±0.0577°	3.27±0.0577 <sup>b</sup>	1.97±0.0577 <sup>b</sup>	$57.66\pm0.0577^{d}$
Soaked (24 h) Lima	9.43±0.1528°	22.17±0.0577°	5.47±0.0577 <sup>r</sup>	3.23±0.1528a	1.89±0.1155a	57.13±0.2082°

Mean values with different alphabets in the same column are significantly different at p<0.05

and AYB flours have the greatest percentage decrease, about 10%, as compared with their respective unprocessed samples. Twenty four hours soaked samples also have crude protein values which are slightly different from the 12 h soaked/de-hulled samples. This may be due to the fact that the grains spent longer time in water and some proteins are soluble in water, globulins (Lehninger *et al.*, 2000).

The ether extract of unprocessed AYB, 1.51%, is significantly different from that of its counterpartunprocessed lima beans, 5.93% (p<0.05). These low fat contents observed in these flours however, higher than that of cowpea as reported by Fasoviro et al. (2006). Moreover, Lima bean flours, unprocessed and processed, have significantly higher fat content than all the samples from AYB. The values for all the unprocessed samples of African yam beans are not significantly different from each other and also not significantly different from their control (unprocessed African yam beans). Twenty-four hours soaked sample of lima beans have the highest crude lipid content among all the processed samples of lima beans, 5.47%, while sprouted samples have the least crude lipid content, 5.31% and are significantly different from other samples. The result indicates that the various processing applied in this research did not significantly affect the fat content of AYB lima beans which obviously is due to the insoluble nature of fat. Although not significantly different, sprouted samples of AYB however, have the least fat content. This may result from the usage of nutrient during germination.

The ash content of the unprocessed AYB, 3.27% is not significantly different from sprouted flour. Ash content of all the samples of these minor grain legumes were in the range of 3.21-3.37% and are all significantly different (p<0.05) from each other, except unprocessed AYB and sprouted flours. It is observed that soaked/dehulled sample of African yam beans have the least value for ash content (Table 1). It is inferred that the physical removal of the testa of the seed may have contributed to this lower value as the testa may contain some mineral constituents, however, a more detail research on 'effect of de-hulling on mineral content of a variety of seeds' need to establish a fact.

Crude fiber contents of all the samples ranged from 1.89 to 2.51%. The crude fiber content of the unprocessed AYB flour, 2.27%, is significantly different from that of the unprocessed lima beans, 2.51% (p<0.05). The result also shows that the crude fiber content of all the processed sample of African vam beans are significantly different from each other including the control (unprocessed samples), however with minimum numerical value. It was observed that 24 h soaked samples of both lima beans and AYB flours have least values of crude fibre content. Bazzano et al. (2003) reported that lima beans contain both soluble and insoluble fiber and thus suggest that soluble fiber may have gone into solution during soaking. However, these values are much lower than values that Bazzano et al. (2003) reported to describe the functionality of lima beans.

The carbohydrate contents of all the samples are shown in Table 1. Greater proportion of the grain of each of the sample is actually carbohydrate, the value of which is 60%. Carbohydrate content, obtained by difference (McDonald et al., 1973), of unprocessed lima beans has the least value, 54.43%, among all the samples of lima beans (Table 1) and it is significantly different from all of them. It is observed that the carbohydrate content of the samples increased with processing. This is because each process reduced significantly other nutrient contents of the samples and since carbohydrate content is obtained by difference of the other constituents from 100% (McDonald et al., 1973), hence the increase. Fasoyiro et al. (2006) reported that these values are significantly higher than values for soybean and groundnut as their fat content is very high. The carbohydrate contents of all the samples are in the same range reported for cowpea (Obatolu et al., 2001). Adeyeye et al. (1994) also reported carbohydrate contents in the range of 43.26-53.35% in three varieties of African yam bean. There is therefore wide variability for this trait among the grain legumes.

**Anti-nutritional composition:** The results obtained for anti-nutritional analysis of AYB and lima bean flours under different processing are shown in Table 2. The anti-nutritional contents were measured in mg/100 g of

Table 2: Comparative analysis of anti-nutritional constituents of African yam beans and Lima beans with different processing

Samples	Protease inhibitor (mg/100 g)	Phytate (mg/100 g)	Tannins (mg/100 g)
Unprocessed AYB	1.47±0.0577e	18.23±0.3055 <sup>f</sup>	5.13±0.0667°
Sprouted AYB	$1.21\pm0.0000^{\circ}$	15.17±0.2887°	3.83±0.1667°
Soaked/de-hulled AYB	$0.73\pm0.0577^{a}$	$11.33\pm0.2887^{a}$	$1.51\pm0.0000^a$
Soaked (24 h) AYB	$1.03\pm0.0577^{b}$	12.33±0.0887°	2.17±0.1667 <sup>b</sup>
Unprocessed Lima	$3.51\pm0.0000^g$	$22.33\pm0.2887^{h}$	$10.51\pm0.0000^{h}$
Sprouted Lima	2.67±0.1155 <sup>f</sup>	21.05±0.0000g	$7.51\pm0.0000^{g}$
Soaked/de-hulled Lima	$1.05\pm0.0000^{b}$	$16.33\pm0.2887^{d}$	$4.83\pm0.1667^{d}$
Soaked (24 h) Lima	$1.37\pm0.1528^{d}$	18.17±0.2887°	6.05±0.0000 <sup>f</sup>

Mean values with different alphabets in the same column are significantly different at p<0.05

samples. The tannin, phytate and protease inhibitor contents of all the samples of AYB and lima beans were significantly higher than those recorded for the major grain legumes like cowpea, as reported by Fasoyiro et al. (2006). It was observed that samples of soaked/de-hulled AYB have the least protease inhibitor of 0.73 mg/100 g and it is significantly different from the unprocessed samples. It was also observed that there was considerable decrease of anti-nutritional content with processing. Soaked/de-hulled samples of both AYB and lima beans have the most percentage decrease in anti-nutritional content. Ma and Bliss (1978) reported that tannins are located in seed hull and that de-hulling processes may be expected to reduce its levels. Fasoviro et al. (2006) and Ajibola et al. (2011) also reported reduced values for processed AYB when compared with unprocessed flours. They recorded higher values of anti-nutritional content with colored varieties as compared with white varieties but they however suggested that a germplasm collection comprising several accessions of varying colors will be required to establish association between color intensity and anti-nutritional content in these minor legumes. Seed hull is the carrier of color intensity; this suggests the reason for the greater percentage decrease in antinutritional content of soaked/de-hulled samples of both AYB and lima beans as compared with other processes (Oboh et al., 1998; Fasoyiro et al., 2006).

Among all the samples, unprocessed lima beans have the highest value of phytate, 22.33 mg/100 g and it is significantly different from all other samples (Table 2). Soaked/de-hulled African vam beans have the least value, 11.33 mg/100g. Unprocessed lima beans have the highest tannin content, 10.51 mg/100 g and it is significantly different from that of African yam beans, 5.13 mg/100 g. The result also revealed that sprouted AYB flour (3.83 mg/100 g) and sprouted lima beans flour (7.500 mg/100 g) have the highest tannin content among the processed samples while soaked/de-hulled sample, 1.51% have the least but have close values with 24 h soaked samples. The values of anti-nutritional content of 24 h soaked samples were found to be in close range with that of soaked/de-hulled samples. Vidal-Valverde et al. (1997) reported significantly high percentage decrease in anti-nutritional content of faba beans with soaking. He then observed that anti-nutrients were not detected after

cooking the soaked beans. Therefore, this study has established that processing has a significant decrease effect on the anti-nutritional contents of the minor legumes studied.

#### CONCLUSION

This study has shown that the anti-nutritional content of AYB and lima bean flours had a great deal of percentage decrease when processed and a slight decrease in their nutrient composition; although the proportionality of decrease is much higher with the antinutritional factors as compared with that of nutritional contents. However, additional secondary processing such as cooking would minimize the anti-nutritional content without further deteriorating the nutrient content.

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