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Effects of Processing Methods (Boiling, Soaking and Fermentation) on Nutrient and Anti-nutrient Composition of African Oil Bean (*Pentaclthra macrophylla)* Seeds.

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Abstract

This study investigated the nutrient and anti-nutrient compositions of African oil bean seed (*Pentaclthra macrophylla*) samples subjected to three processing methods (boiling, soaking and fermentation). It was an experimental study. The samples were subjected to different processing methods (soaking, boiling and fermentation). Association of Analytical Chemist (2010) and other analytical methods were used to determine the nutrient and anti –nutrient composition of the samples. Data were analyzed using means, standard deviation and ANOVA at 0.05 level of significance. Duncan's new multiple range test was used to separate the means. The result reveal that fermented sample had highest protein (16.26%) and fibre (5.24%), appreciable fat (20.66%), and ash (5.10%) with low carbohydrate (21.47%). For the minerals and vitamins, the soaked sample contained 289.73±2.16mg/100g of calcium, 3.25±0.02mg/100g of iron, and 37.80±0.00mg/100g of phosphorus and 377.38±0.52µg/100g for pro vitamin A. The result of the anti-nutrients showed that the tannin content of all the samples were within the acceptable limits of 0.201 to 0.296 mg/100g, while the boiled sample had the least value of oxalate (39.00±1.41mg/100g) and phytate (38.79±1.39mg/100g). This study showed that soaking and fermentation possessed superior nutritional benefits while boiling reduced the levels of anti-nutrients.

Keywords: Nutrients, Anti- nutrients, Processing, Boiling, Soaking, Fermentation, African Oil, Bean, Seeds

Introduction

The African oil bean tree (*Pentaclethra macrophylla)* is a large leguminous woody plant that belongs to the sub-family *Mimosoidae* (Enujiugha, 2003). It is popular in Nigeria where it is known by several names such as *Apara* in Yoruba, *Ukana* in Efik, and, the most prominent, *Ugba/Ukpaka* in Igbo. The seed pulp of *P. macrophylla* is consumed boiled or roasted, and more frequently undergo fermentation prior to their consumption (Ugbogu *et al.,* 2020). It is native to tropical Africa and holds significant potential as a versatile food source due to its rich nutrient composition. Traditionally, the seed is consumed in various forms across West Africa, this seed is valued for its high protein and fat content, contributing significantly to local diets (Oyeleke *et al*., 2014). The seed is a good source of minerals, vitamins, fibre and contain many phytochemicals. African oil bean seed is

rich in essential amino acids as well as fatty acids and minerals. The seed contains more than 52 percent oil in its cotyledons, with polyunsaturated fatty acid especially linoleic and oleic acids constituting more than 82 percent of the fatty acids in the seed (Nwachukwu et al., 2018). Among the Igbos of Nigeria, its commonest folklore culinary application is the fermented seed product popularly called *Ugba*. which has a meaty taste and is served both as a delicacy and a soup flavoring agent (Ugbogu *et al.,* 2020).

Recently, there has been a significant increase in research focused on tapping into the nutritional benefits of lesser-known legumes and oil seeds due to limited availability and high costs of animal protein sources and some plant protein sources (Hoehnel, et al., 2022). Nigeria as well as other countries in Africa depend on these foods to satisfy their nutritional needs. The growing population has put enormous pressure on the staple food availability of the people, and imported foods are relatively expensive and unaffordable to the majority of the populace due to poverty (Igbozuliki *et al*. 2020). Despite the nutritive value of the African oil bean seed, its high oil content, short shelf life and the presence of antinutrients are some of the reasons limiting its use as a food supplement.

Different food processing methods have various effects on nutrients present in the food including both positive and negative effects. Food processing techniques affects the food in various ways including taste augmentation, texture retention etc. (Singh *et al.* 2023). Treatment such as soaking, cooking, fermenting, roasting and malting have been found to increase the nutritive quality of African oil bean seed (Ajeigbe *et al*., 2012). However, there is no streamlined method, safety guideline or standards and so production practices and packaging are on individual/family basis. The non-standardized processing methods is a major concern that needs adequate research (Eluchie *et al*., 2021).

African oil bean goes through several primary processes before it is used in different food preparations. They are processed to detoxify the antinutritional factors, increase palatability and improve bioavailability of the nutrient (Oly-Alawuba & Anunukem, 2018). The methods of processing and also the length of fermentation vary from one producer to another with the final intended use resulting in non-uniform products (Nwokeleme & Obeta-Ugwuanyi, 2015). Over the years, fermentation has become part of the culture and traditions of most indigenous communities in developing countries especially in Africa (Okechukwu *et al*., 2015). Fermentation is a metabolic process which involves the conversion of carbohydrate by microorganism into an alcohol or an acid. It is a simultaneous process which involves several catabolic and anabolic reactions that depends on several conditions, including substrate, micro flora, and environmental factors (Nwachukwu *et al*., 2018). This metabolic conversion in food is owed greatly to microbial enzymic reactions (Okechukwu *et al*., 2015).

Soaking affects legumes either adversely or beneficially. Soaking reduces anti-nutritional factors such as trypsin inhibitors of legumes; water-soluble minerals, vitamins, amino acids, proteins and sugars are solubilized by the soaked water (Oly-Alawuba & Anunukem, 2018). Prolonged soaking causes enzyme driven changes in proteins, starch and cell wall

materials, thereby inducing changes in physico-chemical and functional properties of foods (Sokari & Wachukwu, 2007). Understanding the balance between these nutrients and antinutrients is crucial to optimizing the bean's nutritional benefits and mitigating any adverse effects. This study aims to explore the nutrient and antinutrient composition of the African oil bean seed, highlighting its potential as a functional food. Therefore, there is need to evaluate the different methods of processing high grade of edible African oil beans seeds to minimize the loss of nutrients and also reducing the anti-nutritional factors.

Objectives of the study

The broad objective of this study is to investigate effects of processing methods (boiling, soaking and fermentation) on nutrient and anti-nutrient composition of African oil bean (*Pentaclthra macrophylla*) seeds. Specifically, the study determined:

1. proximate composition (crude protein, ash, fiber, fat, moisture and carbohydrate) of the boiled, soaked and fermented African oil bean (*Pentaclthra macrophylla*) seed samples,
2. mineral composition (iron, zinc, calcium, phosphorus) of the different samples,
3. vitamin composition (vitamin A, B_1, B_2) of the different samples,
4. anti-nutrient composition (phytate, oxalate, tannin) of the different samples.

Materials and methods

***Study design*:** This study adopted an experimental design

***Procurement of raw materials*:** African oil bean seed was purchased from Orie Orba main market in Udenu Local Government Area of Enugu State, Nigeria.

***Sample preparation*:** This involved the following;

***Processing of African Oil Bean Seed*:** Five kilogram of African oil bean seed was sorted to remove dirt and damaged ones. It was washed with portable water, boiled for six hours, dehulled, sliced into 2.0 mm thick with knife and re-washed. It was then divided into three portions and labeled as B, S and F. All samples were each boiled for two hours, drained and cooled. Sample B was dried for 12 hours and packaged in a well labeled air tight container. Sample S was soaked for 10hrs, drained, sun dried for 12 hours and packaged. Sample F was soaked and fermented for 72 hours, sun dried for 12 hours and packaged. All samples were labelled (B, S, F) appropriately. Each sample was thus divided into four portions for four sets of analysis based on the four specific objectives of the study and taken to the laboratory for analysis.

***Proximate analysis*:** Association of Analytical Chemist AOAC (2010) and Pearson (2005) method were used to determine the proximate composition of the samples.

***Protein*:** The micro Kjeldahl method was used for the determination of protein. One milliliter of each sample was digested with concentrated sulphuric acid, distilled and titrated. The crude protein was obtained by multiplying N by the conversion factor of 6.25 (cP =TN x 6.25).

***Fat*:** Two milliliters of each sample was extracted with acetone (BP 400C – 600C) using "Sohxlet extractor" for 1 hour. The solvent free samples were dried in an oven, cooled in a dissector and reweighed prior to calculation of crude fat content.

***Ash*:** Two milliliters of each sample was weighed into already weighed crucibles, labelled and put into the furnace, heated gradually until the temperature was maintained or 550 - 6000C was reached for 6 hours. After ashing, the furnace was

switched off; temperature was allowed to drop prior to removing the crucibles. Crucibles was put in desiccators and cooled, samples were reweighed and percentage ash calculated.

***Crude Fiber*:** 100ml of 1.25% sulphuric acid (H2SO4) was added into the flask and made to boil over a heater for about 30minutes, filtered using a Buckner funnel and filter flask. The residue was put back into flask and diluted with 100ml of 1.25% NaOH and heated for another 30minutes and filtered using suction method. The residue was rinsed with 1% HCl, and added to neutralize the NaOH present, washed with methylated spirit to remove any trace of acid. The residue was put into a weighed crucible, dried in an oven set at 100 C for 30minutes, cooled in a desiccator and reweighed. The residue was put into a muffled furnace set at 550 C for 2hours for complete ashing. The ash was weighed and the percentage fiber in the sample calculated.

***Moisture*:** The Petri dishes were washed, dried in hot air oven at 1000C for about 25 minutes, and cooled in desiccators for 10minutes. The dishes were reweighed. Two milliliters of sample was added to each dish in hot air oven, dried for two hours, removed, cooled in desiccators, reweighed and dried until a constant weight was obtained. The percentage moisture was calculated.

***Carbohydrate*:** This was determined by Difference that is, subtracting the sum of the percent of protein, fat, moisture, and ash from 100 percent. Carbohydrate percentage was calculated.

***Mineral Analysis*:** Association of Analytical Chemist AOAC (2010) was used to determine the mineral composition of the samples

Iron: Five milliliters of Phenanthroline solution and two milliliters of concentrated HCl were added in the test-tube. One milliliter of hydroxylamine solution was added and left to boil for 2mins. Nine milliliters of ammonium acetate buffer solution was added and diluted with 50ml of water. The absorbance was read at 510nm wavelength.

Calcium: Previously ashed sample was dissolved in 5ml of 30% HCl and 45ml of distilled water. The diluted samples were filtered and the filtrates were used to analyze for calcium using atomic absorption spectrophotometer.

Zinc was separated from other metals by extraction with dithizone and then determined by measuring the colour of the zinc dithizone in carbon tetrachoric. Two grams of the digested sample was pipette into test tube and 5ml of acetic buffer was added. 1ml of sodium thiosulphate solution was added and mixed after which 10ml of dithizone solution was added. The mixture was shaken for 40 minutes, and the reading taken at 535nm, the standard was prepared and zinc concentration of the sample was calculated.

***Phosphorus*:** Two grams of each sample was pipette into 50ml of graduated flask. Ten milliliters of molybdate mixture were added and diluted to the mark with water. It was allowed to stand for 15minutes for colour development. The absorbance at 400nm was measured against the blank. A standard graph was plotted relating absorbance to phosphorus concentration. Concentration of phosphorus (mg/ml) was extrapolated from the graph.

***Vitamin Analysis*:** Pro Vitamin A was determined using Pearson (2005) method while Vitamin B_1 and Vitamin B_2 were determined using Association of Analytical Chemist AOAC (2010).

***Pro Vitamin*:** Two (2) milliliters of each sample was put into a film container and 20ml of petroleum ether was added. The solution was filtered through Whatman filter paper No 42. The filtrate was evaporated to dryness, later dissolved with 0.2mls of chloroform acetic anhydride, 2mls of TCA chloroform was added and read at 620nm using a spectrophotometer.

Vitamin B_1: Five (5) grams of samples are homogenized with 50ml of ethanoic sodium hydroxide solution. This will be filtered into a 100ml flask. A 10ml of the filtrate was pipetted into a beaker and color developed by the addition of 10ml potassium dichromate. The absorbance was read at 360nm. A blank sample was prepared and read at same wavelength. The values were extrapolated from a standard curve.

***Vitamin B_2*:** Five (5) grams of each of the samples was extracted with 100ml of 50% ethanol solution shaken for 1 hour. This was filtered into a 100ml of 30% hydrogen peroxide (H_2O_2) and allowed to stand over hot water bath for 30mins. 2ml of 40% sodium sulphate was added to make up the 50ml mark and absorbance read at 510nm in a spectrometer.

***Anti-nutrient Composition*:** This was determined by using the method described by Association of Analytical Chemist AOAC (2010)

Phytate: About 0.5gram of the sample was weighed into 500ml of 2.4% HCl for 1hour at room temperature, poured out and filtered. From the filtrate, 5 milliliters were pipette and diluted to 25ml of water. From the diluted sample, 10ml was pipetted into a test-tube through amber liters in grade 200 – 400 mesh to elude inorganic phosphate and added 15mililiter of 0.7m sodium chloride. The absorbance was taken at 520nm.

Tannin: Five grammes of the sample was extracted with 300ml diethyl ether for 20 hours at room temperature. The residue was boiled for 2 hours with 100ml distilled water, cooled and filtered and the extract was adjusted to a volume of 100ml in a volumetric flask. Then, calorimetrically using Folin- Denis reagent, the tannins content was determined by measuring the absorbance of the solution at wavelength of 760nm.

Oxalate: One gram of the sample was weighed into 100mL conical flask. Then, 75mL of 3M H2SO4 was added and the solution stirred intermittently with a magnetic stirrer for about one hour. It was filtered and the filtered collected and titrated hot O.1N potassium permanganate (KMnO4) solution t temperature of (80-90 c) until a faint pink color appeared and persisted for at least 30 seconds.

Data Analysis: Data generated from the study were analyzed with means and standard deviation.

Results

Table 1: Proximate composition of the samples of cooked, soaked and fermented African oil bean samples

Samples	Protein (%)	Ash (%)	Crude fibre (%)	Fat (%)	Moisture (%)	Carbohydrate (%)
Boiled AOB	$13.16^{b}\pm0.164$	$1.92^{c}\pm0.120$	$3.82^{b}\pm0.028$	$20.66^{a}\pm0.148$	$38.99^{c}\pm0.021$	$21.47^{a}\pm0.482$
Soaked AOB	$12.27^{c}\pm0.325$	$5.10^{a}\pm0.141$	$2.56^{c}\pm0.021$	$17.74^{c}\pm0.064$	$46.30^{b}\pm0.092$	$16.05^{b}\pm0.516$
Fermented AOB	$16.26^{a}\pm0.215$	$3.85^{b}\pm0.212$	$5.54^{a}\pm0.014$	$18.54^{b}\pm0.170$	$47.27^{a}\pm0.021$	$8.55^{c}\pm0.174$

***Keys**: AOB- African oil bean. Values are mean ± Standard deviation; values with different superscripts across the rows are significantly different.*

Table 1 shows the result of proximate composition of cooked, soaked and fermented African oil bean samples. The protein content of the samples ranged from 12.27- 16.16%. The ash content of the sample ranged from 1.92 - 5.10%. The moisture content of the samples varied from 38.99 -47.27%. The fat contents varied from 17.74 - 20.66%. The crude fibre content of the samples oscillated between from 2.56 to 5.54%. The carbohydrate content of the samples ranged from 8.55 to 21.47%.

Table 2: Mineral Composition of the Samples of Cooked, Soaked and Fermented African Oil Bean Samples

Samples	Calcium (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)	Phosphorus (mg/100g)
Boiled AOB	230.00^{b}±14.142	2.25^{b}±0.021	1.37^{a}±0.099	20.92^{b}±0.021
Soaked AOB	289.73^{a}±2.164	3.25^{a}±0.021	1.32^{a}±0.141	37.80^{a}±0.000
Fermented AOB	203.11^{b}±3.684	2.14^{c}±0.042	0.44^{b}±0.085	14.74^{c}±1.251

***Keys**: AOB- African oil bean. Values are mean ± Standard deviation; values with different superscripts across the rows are significantly different.*

Table 2 shows the mineral composition of the samples. Soaked AOB sample contained the highest calcium content while fermented AOB sample had the lowest calcium mean value. The iron content of the samples ranged from 2.14 to 3.25 mg/100g. The zinc content of the samples ranged from 0.44 to 1.37 mg/100g. The phosphorus content of the samples ranged from 14.74 to 20.92 mg/100g.

Table 3: Vitamin Composition of the Cooked, Soaked and Fermented African Oil Bean Samples

Samples	Pro Vitamin A (µg/100g)	Vitamin B_1 (mg/100g)	Vitamin B_2 (mg/100g)
Boiled AOB	162.99^{c}±0.84	0.50^{a}±0.01	0.43^{b}±0.01
Soaked AOB	377.38^{a}±0.52	0.29^{c}±0.02	0.24^{c}±0.00
Fermented AOB	302.72^{b}±0.73	0.34^{b}±0.02	0.57^{a}±0.01

***Keys**: AOB- African oil bean. Values are mean ± Standard deviation; values with different superscripts across the rows are significantly different.*

Table 3 shows the vitamin composition of the samples. The pro vitamin A content of the samples analyzed ranged from 162.99 to 377.38µg/100g. The vitamin B_1 content of the samples ranged from 0.29 to 0.50 mg/100g. The vitamin B_2 content of the samples ranged from 0.24 to 0.57 mg/100g.

Table 4: Anti-nutrient Composition of Cooked, Soaked and Fermented African Oil Bean Samples

Samples	Tannin (mg/100g)	Oxalate (mg/100g)	Phytate (mg/100g)
Boiled AOB	0.243^{a}±0.04	39.00^{c}±1.41	38.79^{c}±1.39
Soaked AOB	0.201^{a}±0.04	67.00^{b}±2.82	188.15^{a}±0.02
Fermented AOB	0.296^{a}±0.04	58.00^{a}±2.82	49.41^{b}±0.00

***Keys**: AOB- African oil bean. Values are mean ± Standard deviation; values with different superscripts across the rows are significantly different.*

Table 4 shows the anti-nutrient composition of the samples. Tannin content of the samples ranged from 0.201 to 0.296 mg/100g. The oxalate content of the samples oscillated from 39.00 to 67.00 mg/100g. The phytate content of the samples ranged from 38.79 to 188.15 mg/100g.

Discussion

The result of the proximate analysis shows that the protein content of the fermented African oil bean sample was higher when compared to the boiled and soaked samples. This is in line with the values (15.46%) reported by Nwachukwu et al. (2018) on fermented African oil bean. The high protein content in the fermented sample may confer nutritional advantage on the fermented oil bean. This could be attributed to net synthesis of protein by fermenting seeds which might have resulted in the production of some amino acids during the fermentation process (Adebowale & Maliki 2011). The percentage value of protein will definitely meet the daily protein requirement of individuals (Igwenyi *et al.* 2015).

Soaking increased the ash content when compared to the boiled and fermented samples. The value gives an indication of the level of macro and micro nutrients in the seeds. The values obtained in this study were in line with the findings (6.12%) reported by Eze *et al* (2014) and slightly higher than the values 3.47% reported by Ikhuoria *et al.* (2008) for raw African oil bean seed.

The result of the moisture content is within the range reported by Ikhuoria *et al.* (2008) who recorded 39.05% moisture and slightly lower than the values reported by Nwachukwu (2018) on the effect of fermentation time on the proximate and mineral composition of fermented African oil bean seed. Moisture has been reported to increase during fermentation. The moisture content of the boiled and soaked samples was lower than that of fermented sample, which might be due to its low dry matter content. The crude fibre values reported in this work were in agreement with 3.66% reported by Balogun (2013) in processed African oil bean. Fiber has a physiological effect on the gastrointestinal function by promoting the reduction of traclonic pressure which is beneficial in diverticular disease such as cancer of the colon and hemorrhoids (Okwu &Morah 2004).

The fat values reported in this study was highest in the boiled samples when compared to soaked and fermented samples. The values were similar to the findings of Eze *et al.* (2014) that reported 19.72% on the proximate composition, biochemical and microbiological changes associated with fermenting African oil bean. However, it was a bit higher when compared to the reports of Okorie *et al* (2013) on controlled fermentation and preservation of African oil bean seed. The carbohydrate content of the samples ranged from 8.55 to 21.47%. The carbohydrate content of the samples was in line with 12.15% reported by Nwachukwu *et al.* (2018) in fermented African oil bean. The lowest carbohydrate content in fermented sample could be attributed to the conversion of complex starch to disaccharide by amylase enzyme and breaking down of complex starch into alcohol and carbon dioxide by the activities of micro-organisms. These values were similar to the report of

Enujiugha and Akanbi (2005) on oil bean seed with 28.25% carbohydrate.

The calcium content in this study was higher when compared to the value 89.58 mg/100g reported by Nwachukwu *et al.* (2018). Calcium is essential for many metabolic processes including nerve function, muscle contraction and blood clothing, De et al., (2019). The iron content of the samples ranged from 2.14 to 3.25 mg/100g. The iron content in this study was slightly lower than the findings of Enujiugha and Akanbi, (2005) who reported 4.23mg/100g in fermented oil bean seed. The variation could be due to the age of the crop and location. This mico-nutrient is known to be important in human body because it is a component of haemoglobin (Asoegwu *et al.,* 2006).

The zinc content of oil bean seed samples was in line with the findings of Ikhuoria *et al.* (2008) who worked on oil bean with value 1.31mg/100g. The zinc content could mean that the seeds can play a valuable role in the management of diabetes, which results from insulin malfunctioning (Okaka, 2001). The phosphorus content of the samples ranged from 14.74 to 20.92 mg/100g. The result showed that boiling of oil bean increases the phosphorus content. The value reported in this study was higher than the research work of Okwu and Aluwo (2008) that reported 1.51 and 0.77mg/100g for raw and fermented samples respectively. It was however; lower than 102.48 mg/100g reported by Nwachukwu *et al.* (2018) in African oil bean.

Pro vitamin A value obtained in this study were lower than 50 percent reported by Devi (2015) in oil bean seed. Vitamin A is valuable for the promotion of growth of cells and tissues, resistance to diseases and for delaying the ageing process. The RDA requirement for vitamin A for a normal healthy, active adult man and non-pregnant woman is 0.3mg/day and 0.27mg/day respectively (FAO 2001). The values obtained for vitamin B_1 are slightly lower than 0.62 – 2.10 mg/100g reported Akinlabu *et al.* (2019) in fermented African oil bean. They are also in line with the recommended minimum daily intake of 0.2 to 1.0mg for vitamin B_1. The value of vitamin B_2 content in this research is comparable to the value 0.09 – 0.18 mg/100g recorded by Akinlabu *et al.* (2019) in African oil bean. Again, the values obtained were within the recommended daily intake of 0.3 to 1.6mg (Belitz *et al*., 2009). Vitamin B1 aids in the prevention of Alzheimer's Disease, and boost body immunity Gibson et al., (2016). Vitamin B2 is also beneficial for eye health, migraines, energy production, decreasing cardiovascular risk, and boosting antioxidant status (Mahabadi et al., 2022).

The levels of tannin obtained in this study were slightly higher than the value 0.042mg/100g obtained by Balogun (2013) in oil bean seed. The presence of tannins in the seeds serves as nutritional inhibitor because they combine with proteins and this makes them indigestible and unavailable to the body. Adequate processing (cooking, soaking and fermentation) reduces tannins to trace amounts. The reduction of oxalate in the boiled and fermented samples could be as a result of leaching of the anti-nutrients into water. The value of reported in this study was higher than the findings of Oly-Alawuba *et al.* (2018). The mean phytate values among the samples were higher than 0.04 mg/100g reported by Balogun (2013) in fermented oil bean seed. The variation could be due to difference in the nature soil, processing method, and age of the seeds. Phytate in food renders minerals unavailable for absorption. It has

been reported that calcium absorption increases with low phytate (3.01mg/g) (Onuekwe, 2012).

Conclusion

Based on the results of this study, fermentation improved the nutrients composition of African oil bean seed (*Pentaclethra macrophylla*). The fermented sample had the highest protein and fibre, moderate fat and ash with low carbohydrate values and appreciable amounts of vitamins. The protein level in the fermented sample will go a long way in alleviating protein deficiency among the poor with the aim of meeting up with the FAO protein requirement recommended levels. The fermented sample had appreciable levels of minerals. However, the soaked sample contained the highest calcium, iron and phosphorus with all samples having minimal anti-nutrient levels within the acceptable limit. All processed samples proved satisfactory in both nutritional requirements and anti-nutritional reduction. However, fermentation proved to be the best processing method for African oil bean.

Recommendations

1. Individuals and families should adopt fermentation as the only method for the processing of African oil bean as it conserved more nutrient.
2. Fermented African oil bean should be used for the production of food supplements or nutrient concentrates.
3. It should be incorporated into local dishes to enrich their nutritional quality.

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