EFFECT OF PROCESSING METHOD AND FERMENTATION ON THE ANTINUTRITIONAL FACTORS/PHYTOCHEMICAL CONTENTS OF AFRICAN OIL BEAN SEED

OKORIE, P. C[1*.] AND OLASUPO , N. A[2.]

[1]Federal Institute of Industrial Research Oshodi, Lagos, Nigeria. Email: chimezieokorie@yahoo.co.uk Tel.: +2348053514782, [2]Department of Microbiology, Faculty of Science, Lagos State University Ojo, Lagos, Nigeria. Email: naolasupo@yahoo.com

*Corresponding author

ABSTRACT:

Samples of raw African oil bean seed were collected and screened for the presence of some ant nutritional factors (phytochemicals). Samples of the oil bean seed at different stages of fermentation were also screened for these anti-nutritional factors. Quantitative analysis for these phytochemicals was carried out on the samples that tested positive during the qualitative assay. Effect of soaking and washing of the seed before fermentation on these toxic factors/phytochemicals was also determined. The qualitative analysis for these phytochemicals showed the presence of tannins, saponins, alkaloids, steroids, glycosides, flavonoids and phytate. Soaking the seed overnight and washing in water before fermentation had a significant effect on all the phytochemicals/anti-nutritional factors identified, Tannin was reduced from 12.58 – 3. 65 mg/100g, saponin from 52.00 – 22.00 mg/100g, phytate from 25.63 – 14.47 mg/100g, glycosides from 34.76 – 11.33 mg/100g, alkaloids from 2.52 – 0.14 mg/100g, flavonoids from 4.66 – 2.49 mg/100g and steroids from 26.48 - 5.43 mg/100g. Fermentation further reduced drastically the content of these substances in the samples. Alkaloids and tannins were completely removed from the samples after 24hr and 48hr of fermentation respectively.

KEY WORDS; Effect, Processing, Fermentation, Phytochemical

.

INTRODEUCTION:

Fermented African oil bean seed is usually called ugba by the Ibos in the eastern part of Nigeria. It is consumed throughout the western and central Africa but predominantly by the Ibos and other ethnic groups of eastern Nigeria. It is a traditional food generally prepared in homes as a small family business. Ugba is consumed as a basic food or as a flavouring agent. It is consumed in one of two ways;

1. Bean fermented for less than three days are used as a delicacy or d'oeuvre by mixing them with oil, pepper, salt and fish to make a so called native salad or
2. Well fermented ugba is added to soup as a flavouring condiment

The unfermented oil bean seed is suspected to contain a number of anti-nutritional and/or toxic factors including saponins, alkaloids, sterols, glycosides, and growth depressant caffeolyputrescine (Pierson *et al*, 1986). The presence or absence of these toxic substances in the fermented beans has however not been investigated. It is possible that they are eliminated or reduced during the processing and fermentation of the bean.

Although saponins have been reported to be toxic, they may be beneficial since they have been found to lower plasma cholesterol (Oakenfull *et al*, 1979). Saponins and alkaloids have been found to show prominent antibacterial activity against *Staphylococcus aureus, Bacillus subtilis*, *Bacillus cereus* and *Klebsiella pneumonia* (Khan *et al*; 2012). Sterols have also been found to exhibit antimicrobial activity against *Staphylococcus aureus*. Some of these phytochemicals have been shown to have antioxidant properties (Khan *et al*, 2012). In some plants, saponins may serve as anti-feedants, and to protect the plant against microbes and fungi. Some plant saponins may enhance nutrient absorption and aid in animal digestion (Foester and Hartmut, 2006). Saponins are also often bitter to taste, and so can reduce plant palatability or even imbue them with life threatening animal toxicity (Foerster and Hartmut, 2006). They form complexation with cholesterol to form pores in cell membrane bi-layers, e.g. in red blood cell membrane, leading to red cell lyses. They also however have been promoted commercially as dietary supplements and nutriceuticals (Francis *et al*, 2002). Tannins have been reported to inhibit the activity of some enzymes like trypsin, amylase and lipase by forming insoluble complexes with protein and divalent ions such as Fe3+ and Zn2+ thereby reducing their absorption in the body (Griffith, 1979; Elegbede, 1998).

Inositol hexakisphosphate ($InsP_6$), commonly known as phytate, is a major component of plant storage organs such as seeds, roots and tubers, where it serves as a phosphate source for germination and growth. However, due to its ability to chelate and precipitate minerals, phytate can decrease the bioavailability of critical nutrients such as zinc, iron, calcium and magnesium in food substances such as whole grains, nuts and legumes (Thompson and Erdman, 1982; Nancy and Wendt, 2003).

There is no recorded information on the effect of processing and fermentation on the anti-nutritional factor/ phytochemical contents of the African oil bean seed. It is therefore the aim of this work to assess the effect of processing method and fermentation on the phytochemical/toxic factors contents of the oil bean seed. This was done with the hope of improving the aesthetic quality, taste and nutritional value of the fermented African oil bean seed.

MATERIALS AND METHODS:

Collection of oil bean seeds:

The oil bean seeds used for this work were collected from Umuahia in Abia state in the eastern part of Nigeria.

Processing and Fermentation of the oil bean seed:

The processing and fermentation of the oil bean seed were done according to the method described by Pierson *et al.,* (1986) and modified by Okorie and Olasupo, (2013). The processing of the large brown glossy seeds of the African oil bean to obtain 'Ugba' involved the following; the oil bean seeds were boiled in an autoclave at a temperature of 121^{o}C and a pressure of 15 pounds per square inch (psi) for 1hr to soften the hard brown testa (shell). The shells were removed and the kernels washed, drained and rewashed with cold water several times. The washed cotyledons were cut into long thin slices and soaked in water overnight. These slices were wrapped in small packets with leaves and lightly tied. These small packets were placed in a basket to ferment at room temperature for 3 days to yield 'Ugba'.

Preliminary (Qualitative) assay for anti-nutritional factors/ phytochemicals*:*

Samples of sliced oil bean seed were collected before they were soaked and washed, after soaking and washing, and after 24, 48 and 72 hrs of fermentation respectively. These samples were used for a preliminary analysis to determine the presence or absence of the following substances;

Saponins:

Two grammes of each of the samples were grounded and boiled in 10ml of distilled water in a water bath for 5minutes. The solution was filtered while hot and then cooled. 3ml of dilute tetraoxosulphate (vi) solution (H_2SO_4) was added to the filtrate and the mixture was boiled for 15minutes. The filtrate was filtered again and 3ml of 20% NaOH and Benedict solution were added and observed for colour reaction (Harborne, 1973).

Tannins*:*

The method of A.O.A.C, (2010) was employed in analyzing for tannins in the samples. Two grammes of each sample was grounded and boiled in 5ml of distilled water for 5minutes in a water bath. The solution was filtered while hot and allowed to cool. 1ml of 10% ferric chloride was added to 1ml of the filtrate and observed for colour reaction.

Flavonoids:

The method of Anhwange *et al*, (2006) was employed in the analysis. One gramme of grounded sample of each of the samples was boiled in 5ml of distilled water for 10minutes and filtered while hot. The filtrate was allowed to cool and one drop of 10% Ferric chloride solution added and thoroughly mixed. Dilute solution of NaOH (1ml) was added and observed for colour change. A greenish-brown colour is an indication of flavonoid presence.

***Alkaloids*:**

The method used was according to what has been described by Chinedu *et al*, (2011). Extraction of alkaloids was done in water. 2ml of the extract was heated and dissolved in 2ml of dilute hydrochloric acid solution and a few drops of solution of iodine in potassium iodide were added and the colour change noted. A brown precipitate indicates the presence of alkaloid.

***Glycosides*:**

Extraction of glycoside was done in water. 2ml of the extract was dissolved in 1ml of hydrochloric acid solution, neutralized with sodium hydroxide solution and a few drops of 10% ferric solution were added to the mixture. This was then underlayed with 1ml of concentrated sulphuric acid. A brown or reddish-brown ring obtained at the interface indicated the presence of glycoside (Harborne, 1973).

Steroids:

Extraction of the steroids was done in water. 2ml of acetic anhydride was added to 5ml of the extract and 2ml of concentrated sulphuric acid added and observed for colour change. A violet to blue-green colouration indicated the presence of steroid.

Quantitative assay for anti-nutritional factors (phytochemicals)*:*

The samples as used for the qualitative assay were used in the quantitative analysis of the phytochemicals mentioned above.

Saponin:

5g of grounded sample was weighed into a 250ml beaker, 52ml of 95% ethanol was then added and the mixture left for 48hr before being filtered with muslin cloth. The residues were dried in the oven at 50^{o}C. 25ml of 75% ethanol was added to dissolve the extract and left to stand for another 48hr. It was then filtered again with muslin cloth. The filtrates were combined, shaken with a benzene-ether mixture (1:1) and decanted. The alcoholic extract was concentrated under reduced pressure. The crystals that were deposited were removed by filtration. N-butanol was used to dissolved the crystals, filtered and washed with water and dissolved in phosphate buffer pH 7.0 and was re-crystallized with 95% ethanol to yield white crystals. The crystals were weighed and saponin was calculated in mg/g

***Tannins*:**

5g of grounded sample was weighed into a beaker and extraction done with 35ml of methanol. The sample mixture was left overnight, filtered and sample extract used for tannin analysis. 5ml of the sample extract was pipetted into a 50ml volumetric flask. 35ml of water was added. 5ml of standard tannin acid solution was pipetted into another flask and 35ml of water added. To each of the flask, 1ml of Folin and Denis reagent and 2.5ml of saturated sodium carbonate solutions were added. The volume was made up to 50ml and mixed thoroughly. After 11/2hr, the samples were filtered and the absorbance measured at 760nm against a reagent blank-prepared in the same way but without tannic acid.

$$\text{Tannin (mg/100g)} = \frac{A}{B} \times \frac{100}{1}$$

Where A = Absorbance of Sample
B = Absorbance of Standard

Flavonoids:

Total soluble flavonoids of the sample were determined with spectrophotometric method using aluminum chloride and rutin as standard respectively (Chinedu *et al*, 2011). 1ml of the sample extract was diluted with 4ml of water in 10ml volumetric flask. Initially, 5%w/v of $NaNO_2$ solution (0.3ml) was added to sample flask; at 5minutes, 10%w/v of aluminum chloride (0.3ml) was added and after 6min., 1.0M NaOH solution (2ml) was equally added. 2.4ml of distilled water was added to the reaction flask and mixed thoroughly. The absorbance of the reaction

mixture was read at 510nm. The total flavonoids content was determined as quercetin equivalent (mg/g) of dry weight.

Alkaloids:

10g of grinded sample was weighed and the alkaloid content extracted with 50ml of distilled water. The sample was filtered and the filtrate was used for the determination of alkaloids. 5ml of sample extract was measured into a test tube and boiled. 2ml of 0.2M sodium nitrate solution and 1% v/v sulphuric acid were added to the extract. The mixture was thoroughly mixed and 2ml of 20% ammonium solution was added and mixed. The resulting solution was shaken gently by repeated inversion and allowed to stand at room temperature. The red precipitate formed was dissolved by the addition of Nessler's reagent and the resulting extinction was read at 560nm wavelength using spectrophotometer. A known standard alkaloid (sigma product) 5mg/ml was equally prepared and treated as sample above. The alkaloid content in the sample was calculated as follows;

$$\text{Alkaloid (\%)} = \frac{A}{B} \times \frac{C}{D}$$

Where A = Sample Absorbance
B = Standard Absorbance
C = Standard Concentration
D = Weight of Sample

***Glycosides*:**

5mg of strychnine sulphate was weighed and was transferred into a 100ml volumetric flask. This was dissolved and diluted to volume with distilled water. 10ml was taken and diluted to 100ml with 0.07M phosphate buffer. 5ml of sample extract was adjusted to pH 8.0 by adding 0.1N NaOH solution. The sample and a 10ml aliquot of the standard solution were place into individual centrifuge tubes. To a third tube add 10ml of pH 8.0 phosphate buffer as a blank. To each of the tubes add 25ml of benzene and shake for 10minutes. The mixture was centrifuged and benzene layer was removed. To the separated benzene layer add 0.5ml of bromothylmol blue solution in 0.01M phosphate buffer adjusted to pH 7.4, shaken for 5minutes, centrifuge and remove the benzene layer. 4ml of 0.1N NaOH was added to each of the three individual benzene solutions and shaken. The layers were separated and read in a spectrophotometer at 570nm wavelength. The glycoside content was calculated with the following formula;

$$\text{Glycoside (\%)} = \frac{A}{B} \times \frac{C}{10} \times 100$$

Where A = Sample Absorbance
B = Standard Absorbance
C = Standard Concentration

Steroids:

The total steroids were determined colorimetrically with reference to the saponic content. The saponic crystals were dissolved in 50ml formaldehyde – concentrated sulphoric acid mixture and the absorption was measured at 470nm. The steroids content was calculated as follows;

$$\text{Steroid (\%)} = \frac{A}{B} \times 100$$

Where A = Sample Absorbance
B = Weight of Sample

***Phytate*:**

Phytic acid was extracted in 0.5M nitric acid using the method of Anhwange *et al.* (2006). 2g of sample was weighed into a beaker; 3ml of 0.5M nitric acid and hypochloride (0.5M) were added and digested for 1hr. Thereafter filtered and the digested sample was quantitatively diluted to 25ml with water. The solution (sample extract) 2.5ml was mixed with 0.5ml of nitric acid solution plus 2.5ml of distilled water and 2.5ml of vanadomolybdate reagent. The solution was mixed and absorbance read at 460nm on a spectrophotometer. The phytate content of the sample was estimated from the known concentration of potassium dihydrogen phosphate (3.834g KH_2PO_4/L) 25ml of stock diluted in 250ml of water.

1ml = 0.2mg P_2O_5

$$\text{Phytate (\%)} = \frac{A}{C} \times 100$$

B D

Where A = Sample Absorbance
B = Standard Absorbance
C = Standard Concentration
D = Weight of Sample

Effect of anti-nutritional factors/phytochemicals on fermentation of ugba:

To determine the effect of the phytochemicals/toxic factors on the fermentation process of the oil bean seed, samples of the sliced oil bean seed before subjecting it to the processing method as described above, and after the processing method has been applied were collected and subjected to fermentation process for 72hr. The method employed was as described by Pierson *et al.*, 1986 and modified by Okorie and Olasupo 2013.

RESULTS:

The result of the preliminary/qualitative analysis of African oil bean seed for the presence of anti-nutritional factors/phytochemicals showed the presence of the following substances; tannins, saponins, alkaloids, steroids, glycosides, flavonoids and phytate (table1).

Soaking the seed in boiled water overnight and washing in water had significant effect on all the phytochemicals identified (table 2). Tannins were reduced significantly from 12.58mg/100g – 3.65mg/100g, saponins from 52.00mg/100g – 22.00mg/100g, phytate from 25.63mg /100g – 14. 47mg/100g, glycosides from 34.76mg/100g -11.33mg/100g, flavonoidss from 4.66mg/100g – 2.47mg/100g and steroids from 26.48 - 5.43 mg/100g. Alkaloids were almost completely eliminated from the samples by soaking and washing with water (2.52mg/100g – 0.14mg/100g).

The process of fermentation further reduced drastically the presence of these phytochemicals in the fermented product (table 3). Alkaloids and tannins were completely removed from the samples after 24hrs and 48hrs of fermentation respectively. All the other phytochemicals identified in the unfermented oil bean seed were still present in the fermented samples after 72hrs of fermentation when the process was terminated. Their levels of content were however drastically reduced by the fermentation process.

The presence of these toxic factors/phytochemicals as contained in the raw African oil bean seed had a negative effect on the fermentation of the seed into ugba (table 4). Result obtained shows

that the unsoaked samples could not be fermented after 72hrs while the soaked samples were fermented to ugba after the same period of fermentation as shown by the key indicators of fermentation of ugba. The pH of the soaked sample rose steadily to above 7.0 against what was observed for the un-soaked samples where the pH was relatively steady. Also the moisture content of the soaked samples increased from about 56% to 73% while there was no appreciable increase in the moisture content of the unsoaked samples. There was also a steady increase in the temperature of the soaked sample from 30.8^{oc} to 36.5^{oc} while that of the un-soaked samples remained relatively unchanged. The characteristic aroma of ugba was developed in the soaked samples after 72hrs of fermentation while such aroma was not perceived in the unsoaked samples. The texture of the soaked samples was very typical of ugba at the end of the fermentation period while that of the unsoaked samples was atypical of the product.

DISCUSSION:

The result of the preliminary assay for the presence of anti-nutritional factors/phytochemicals in the African oil bean seed revealed the presence of the following substances; tannins, saponins, alkaloids, flavonoids, steroids, cynogenic glycosides and phytate. Duke (1981), Achinewhu (1983) and Onwuliri *el al,* (2004) suggested the likely presence of some anti-nutritional factors like cyanide, phytate, tannin and oxalate in the raw seed of African oil bean. Apparently there is no record in literature to confirm the presence of these toxic factors in the oil bean seed as suggested by these workers. Also the presence of these toxic substances in the fermented oil bean seed has not been investigated. The result of this work has however confirmed the presence of some toxic substances/phytochemicals (tannins, saponins, alkaloids, flavonoids, cynogenic glycosides, steroids and phytate) in the oil bean seed as feared by these workers. Ruiz-Teran and Owens (1999) however detected the presence of such substances during soya bean tempeh production and observed that such substances are leached out during soaking. Momoh *et al,* (2012) recorded the presence of glycoside, saponin, alkaloid, phytate and oxalate in beniseed.

Some of these phytochemicals as have been detected in the oil bean seed in this work have been shown to possess antioxidant properties. In some plants, saponin may serve as antifeedant, and to protect the plant against microbes and fungi. Saponins and tannins are also often bitter in taste, and so can reduce the seed palatability (Khan *et al*; 2012). It is therefore possible that their presence in the fermented oil bean seed account for the bitter taste often reported by the local processors of the oil bean seed into ugba in their product. Saponins, tannins and cynogenic glycosides have been shown to possess cytotoxic effect (Oakenfull *et al*, 1979). Saponins have been shown to possess both beneficial (cholesterol – lowering) and deleterious (permeabilization of the intestine) properties (Price, et al 1987; Oakenful *et al*, 1989). Their presence in the fermented product must therefore be controlled to fall below the toxic level.

Soaking of the oil bean seed in hot water overnight before fermentation had a significant reduction in the detected phytochemical contents of the seed. Apparently, no work has earlier been done on the effect of processing (soaking) before fermentation on the phytochemical contents of oil bean seed to compare this result with. However, Momoh *et al*, (2012) reported that boiling and soaking had a significant reduction in the phytochemical content of beniseed. Ruiz-Teran and Owens (1999) reported that such substances are leached out during soaking in soya bean tempeh production. Oladele *et al*, (2009) also observed a significant reduction in tannin, alkaloid and phytate contents of tigernut after soaking it in water. Akindahunse, (2004) observed that salting and soaking before fermentation did not affect the level of tannin in his samples. His result is in disagreement with what have been observed in this work where tannins were significantly reduced by soaking in water (from 12.58mg/100g- 3.65mg/100g). Soaking the seed in water before fermentation as has been shown in this work is therefore one critical step the processor must observe to ensure that these toxic substances are reduced to a non toxic level in their final product. The processor must also ensure that the soaking period is long enough to allow for significant leaching out of these phytochemicals before commencing fermentation. It is possible that the bitter taste of ugba sometimes observed by the local processors could have resulted from shorter soaking period which did not allow for enough leaching out of these phytochemicals. Some of these phytochemicals have been shown to possess cytotoxic properties at certain levels. It is therefore necessary for the processor to ensure that enough soaking period is observed during their production process to ensure that their levels in the final products are reduced to non toxic points. This is especially necessary as there have been occasional oral reports of serious health challenges after the consumption of this product.

The result of the work on the effect of fermentation on the phytochemical content of oil bean seed shows that fermentation had a reductive effect on the contents of these phytochemicals in the final product. Similar result was recorded by Ruiz-Teran and Owens (1999) during soya bean tempeh production. Momoh *et al*, (2012) in their study of the effects of different treatments on the phytochemicals, proximate and mineral contents of beniseed also observed that fermentation among all the factors they investigated had the highest reducing effect on the phytochemical content of their product. Fermentation has been reported to be the most effective way of reducing phytate in food substances (Fagbemi *et al*, 2005).

Result obtained in this work also revealed that the phytochemicals as detected in the oil bean seed had a great effect on the fermentation of the seed into ugba. Soaking the seed in water before fermentation significantly reduced the phytochemical content of the seed. Soaked and un-soaked samples of the seed were left to ferment for 72hrs. Result obtained showed that the soaked samples fermented into ugba with all its characteristic features while the un-soaked samples could not be fermented. Some of the phytochemicals detected in the oil bean seed have been shown to have prominent antimicrobial properties. Saponins and alkaloids have been shown to have prominent antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus*

cereus and *Klebsiella pneumonia* (Khan *et al*; 2012). Sterols have also been found to exhibit antimicrobial activity against *Staphylococcus aureus*. The most prominent group of organisms involved in the fermentation of oil bean seed into ugba have been shown to be *Bacillus* species, particularly *Bacillus subtilis* (Odunfa, 1981; Antai and Ibrahim,1986; Odunfa and Oyewole, 1986; Diawara *et al.*, 1992). It is therefore reasoned that the level of these phytochemicals in the un-soaked samples was so high that they did not permit the proliferation of this group of organisms in the samples. This explained why the un-soaked samples could not be fermented. This fact again lends credence to earlier assertion that soaking the raw seed in water before fermentation is a very critical step the processor must observed to ensure good quality product.

CONCLUTION:

The raw African oil bean seed does contains some anti-nutritional factors/phytochemicals. Some of these phytochemicals have been shown to possess cytotoxic effects outside being anti-nutritional when present at above a certain level in a food substance. Fortunately however, the content of most of these substances has been shown by this study to be significantly reduced by processing method (soaking in water) and fermentation. The processors of the oil bean seed into ugba are therefore advised to avail themselves with the findings of this study in order to reduce the incidence of deleterious effect occasionally reported after the consumption of this product. This will also help to ensure a consistent high quality product.

REFERENCES:

Achinewhu, S. C. (1983). Saponin content of some Nigerian oil seeds.

Qual. Plant, Plant Foods Hum. Nutr., **33**, 3

Akindahunse, A. A. (2004). Phytochemical studies on African oil bean

(*Pentaclethra macrophylla*) seed. *Journal of Food, Agric. And Environ*., **2:**

14-17

Anhwange, B. A., Ajibola, V. O. and Okibe, F. G. (2006). Nutritive value and

Anti-nutritional factors in *Hibiscus sabdariffa. Journal of Forensics*

International, **24**: 75-76.

Antai, S. P. and Ibrahim M. H. (1986). Microorganisms associated with African locust bean (*Parkia filicoidea welw*) fermentation for Dawadawa production. *Journal of Applied Bacteriology* **61**, 145-148.

A. O. A. C. (2010). Association of Official Analytical Chemists. Official *Method of Analysis, 19th Edition, Washington, D. C.*

Chinedu, P. A., Ijeoma, E., Olusola, A. and Ayobami, O. A. (2011). Polyphenolic Content and antioxidant activity of *Hibiscus sabdariffa* calyx. *Research Journal of Medicinal Plant*, **10**: 1-10

Diawara, B.; Sawadogo, L.; Amoa Awua, W. K. A. and Jaobsen, M. (1998). *HACCP system for Traditional African Fermented Foods*: Soumbala. Taastrup; ISBN 87-90737-00-8 WAITRO Danish Technological Institute.

Duke, J. A. (1981). Handbook of legumes of World Economic Importance, *Plenum Press, New York*, 185.

Elegbede, J. A. (1998). Legumes. In: Nutritional Quality of Plant Foods (A. U. Osagie and O. U. Eka, eds.). Ambik Press, Benin City Nigeria. 53- 83.

Fagbemi, T. N., Oshodi, A. A. and Ipinmoroti, K. O. (2005). Processing effects on some anti-nutritional factors and *in vitro* multi-enzyme protein digestibility (IVPD) of three tropical seeds: Breadnut (*Artocarpus altilis*), Cashewnut (*Anacardium occidentallis*) and Fluted pumpkin (*Telfaria occidentallis*). *Pakistan Journal of Nutrition*, **4**(4): 250-256.

Foerster, H. (2006). Metacyc pathway: Saponin biosynthesis.

Francis, G., Zohan Kerem, H. P.S. and Klaus, B. (2002). The biological action of Saponins in animal systems: a review; *British Journal of Nutrition* **88** (6): 587-605.

Griffiths, D. W. (1979). The inhibition of enzymes by extract of field beans, *Viviz feba. Journal of Agric. Food Chem*. **28**:459-461

Harborne, J. B. (1973). Phenolic compounds. In: Phytochemical methods. *A Guide to modern techniques of plant analysis*. Chapman and Hall Ltd, London, England. 33-38.

Khan, H., Khan, M. A. and Dullah, A. (2012). Antibacterial, antioxidant and Cytotoxic studies of total saponins, alkaloids and sterols contents of decoction of Joshanda: identification through thin layer chromatography. *Toxicology and Industrial Health*. On line publication.

Momoh, A. O., Adebolu, T. T. and Ogundare, A. O. (2012). The effects of different treatments on the phytochemicals, proximate and mineral contents of beniseeds (*Sesamum indicum linn*). *Global Advanced Research Journal of Biotechnology*, **1**(1): 008-011

Nancy, J. and Wendt, T. (2003). AOAC.official method 934.01. In: W. Horwitz, Editor (17th ed; revision 2). AOAC International Vol. 1, AOAC International, Gaithersburg, USA.

Oakenfull, D. G., Fenwick, D. E. Hood, R. L., Topping, D. L., Illman, R. L. and Storev, G. B. (1979). Effects of saponins on bile acids and plasma lipids In the rat, *Br. J. Nutr.*, **42**, 209

Odunfa, S. A. (1981a). Microorganisms associated with fermentation of African locust bean (*Parkia filicoidea*) during Iru preparation. *Journal of Plant*

Foods, **3**: 245-250

Odunfa, S. A. and Oyewole, O. B.(1986). Identification of *Bacillus species* from iru, fermented African locust bean product. *Journal of Basic Microbiology* **26**, 101-108

Okasha, M. A.M., Abubakar, M. S. and Bako, I. G. (2008). Study of the effect of aqueous *Hibiscus sabdariffa* linn seed extract on serum prolactin level of lactating female albino rats. *European Journal of Scientific Research*, **22**(4): 575-583.

Okorie, P. C. and Olasupo, N. A. (2013). Growth and extracellular enzyme production by microorganisms isolated from UGBA- an indigenous Nigerian fermented food. *African Journal of Biotechnology* **12** (26): 4158-4167.

Oladele, A. K., Osundahunsi, O. F. and Adebowale, Y. A. (2009). Effect Of processing techniques on the nutrients and anti-nutrients contents Of tiger nut (*Cyperus esculentus* I.). *Nigerian Food Journal*, **27**(2): 210-218.

Onwuliri, V. A., Attah, I.,and Nwankwo, J. O. (2004). Anti-nutritional factors, essential and non-essential fatty acids composition of ugba (*Pentaclethra macrophylla*) seed at different stages of processing and fermentation. *Journal of Biological Science*, **4**: 671-675.

Ruiz-Teran, F. and Owens, J. D. (1999). Fate of oligosaccharides during

production of soya bean temph. Journal of Science, Food and Agric., **79**: 249-252

Thompson, D. and Erdman, J. (1982). Phytic acid determination in soybeans. Journal of Food Science, **47**, 234-239

Table 1: Preliminary assay for anti-nutritional factors and phytochemicals in African oil bean Seed.

Phytochemical	Processing method		Fermentation period (hr)		
	Unsoaked	Soaked	24	48	72
Tannin	+++	+	-	-	-
Saponin	+++	++	+	+	+
Flavonoid	+++	+	+	+	+
Alkaloid	++	-	-	-	-
Steroid	++	+	+	+	+
Glycoside	+++	+	++	+	+

Key:

+ = positive, - = negative

Table 2: Effect of soaking on the anti-nutritional factors/phytochemical contents of African oil bean seed

Phytochemical (mg/100g)	Soaking period (hr)				
	0	6	12	18	24
Tannin	12.58	10.26	7.02	4.63	3.65
Saponin	52.00	49.56	40.23	34.29	22.00
Flavonoid	4.66	4.02	3.46	2.96	2.49
Alkaloid	2.52	1.94	1.03	0.76	0.14
Steroid	26.48	12.06	8.68	6.97	5.43
Glycoside	34.76	30.54	22.09	17.78	11.33
Phytate	25.63	22.06	18.34	15.69	14.47

Table 3: Effect of fermentation period on the anti-nutritional factor/phytochemical content of African oil bean seed.

Phytochemical (mg/100g)	Fermentation period (hr)			
	0	24	48	72
Tannin	3.65	1.79	0.46	0.00
Saponin	22.00	16.06	8.00	2.00
Flavonoid	2.49	1.96	1.10	0.43
Alkaloid	0.14	0.06	0.00	0.00
Steroid	5.43	3.68	2.96	2.07
Glycoside	11.33	8.64	5.71	0.78
Phytate	14.47	8.67	1.26	0.15

Table 4: Effect of phytochemical contents of African oil bean seed on its fermentation into Ugba

Key indicator of Fermentation	Unsoaked sample Fermentation period (hr)				Soaked sample Fermentation period (hr)			
	0	24	48	72	0	24	48	72
pH	6.2	6.3	6.1	6.2	6.3	6.9	7.2	7.6
Moisture (%)	52	54	53	54	56	64	71	73
Texture	H	H	H	H	H	S	SS*	SS*
Typical aroma Of ugba	-	-	-	-	-	+	++	+++
Tempt. (oC)	30.8	30.9	30.6	30.8	30.8	32.6	34.5	36.5

Key:

H = Hard

S = Soft

SS* = Soft and Slimy

+ = Present

- = Absent