The Effects of Processing on the Anti-Nutritional Properties of 'Oze' *(Bosqueia angolensis)* Seeds

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Abstract: 'Oze' (*Bosqueia angolensis*) is found in the tropical rain forest and grows in thick humid forest of undisturbed land. It belongs to the family *Moracea.* Wholesome 'oze' (*Bosqueia angolensis*) seeds were given different treatments, which included blanching, cooking, roasting and malting. The samples obtained from these treatments were analyzed for anti-nutritional properties. The 'oze' seeds had up to eleven anti-nutrients with alkaloids (2730 mg/100 g) and Total phenols (2500 mg/100 g) predominating. Except for Total phenols and Trypsin inhibitors (37.3 TIU/100 g) all the other anti-nutrients were found more in the hulls than the edible cotyledons. Also all anti-nutrients except phytates and oxalates were eliminated by malting. [Journal of American Science. 2011;7(1):1-6]. (ISSN: 1545-1003).

Key words: anti-nutritional factors, malting, blanching,

1. Introduction

Oze (*Bosqueia angolensis*) referred to, as the "hospitality tree" in the cultural Igbo Community is a member of the botanical family, *Moracea*. It is a tropical rain forest tree and grows in the thick, humid forest of undisturbed land (Keay, 1989). The tree grows up to 30 – 40 meters high as it competes with other hard wood for sunlight. Its green glossy leaves resemble those of 'Ogbono' (*Irvingia gabonensis*); but it is readily distinguished by the remarkably abundant latex flow observed immediately at a slash of its node. This plant called "Oze" in the Igbo speaking states of South Eastern zone of Nigeria is called "koko eran" in the Yoruba speaking states of South Western states of Nigeria.

In most developing tropical countries the food situation is worsening owing to increasing population; shortage of fertile land, high prices of available staples and restrictions on the importation of food (Sadik, 1991; Weaver, 1994). This has resulted in a high incidence of hunger and malnutrition, a situation in which children and women, especially pregnant and lactating women, are most vulnerable (Coulter *et al.*, 1988; Pelletier, 1994). Predictions of future rates of population increase and food production emphasize the seriousness of this problem (FAO, 1990). There seems to be no immediate single solution to the problem of food sufficiency, thus interdisciplinary approach is necessary (Avery, 1991). All information on new sources of food will be of value in dealing with the food problem.

While every measure is being taken to boost food production by conventional agriculture, a lot of interest is currently being focused on the possibilities of exploiting the vast numbers of less familiar food plant resources existing in the wild (RAO, 1994). Many such plants have been identified, but the lack of data on their chemical composition has limited the prospects for their broad utilization (Vijayakumari *et al.*, 1994; Viano *et al*., 1995). Most reports on some lesser-known and unconventional crops indicate that they could be good sources of nutrients and many have the potentials of broadening the present narrow food base for human (Janick and Simon, 1990).

The aroma of roasted Oze seed is reminiscence (resembles) that of its family member, African breadfruit; but its usual traditional dehulling process is more laborious (drudgery) than that of African breadfruit seeds. This factor has limited the traditional processing of 'Oze' to mere hot-ash roasting and a limited consequent utilization as snacking kernels, just as roasted cashew nuts. Thus 'Oze' though aromatically and morphologically more like African breadfruit is utilized mainly as indigenous snacking nuts just like cashew nuts.

Usually, the consumption of hot ash roasted Oze seed results in high gasing phenomenon, suggesting the presence of some anti-nutritional factors. Also the gas has a smell reminiscence of hydrogen sulphide suggesting the presence of sulfur containing amino acids which are among the essential amino acids needed in our daily diet.

Application of different processing methods to 'Oze' seed will give some information, which may increase the utilization of Oze seeds and enhance its potential in food formulations. It is envisaged that a more preferred process for the elimination or reduction of any detected anti-nutritional factor may be found for the production of safer 'Oze' product.

The objective of this study therefore is to investigate the effects of given treatments on the anti-nutritional compounds identified in 'oze' seeds.

2. Materials and Methods

2.1 Materials Collection and Preparation

The 'oze' seeds with intact pulp were obtained from abandoned shrine spots at Ubomiri in Mbaitoli Local Government Area and Umuchima in Ideato South L.G.A both in Imo State. The pulp was washed off with water by rubbing with the hands. The seeds were then dried in the oven at 50°C – 55°C for 24 hours. The cleaned dry seeds were then given different treatments, which included blanching, cooking, roasting and malting after which they were dehulled. Blanching was carried out for 4,6, and 8, min respectively; while cooking was carried out at boiling temperatures for 20, 40, and 60 min respectively. Roasting was carried out in the oven at a temperature of 150°C for 45 minutes. Malting was done by steeping the 'oze' seeds for 24 hours in water at a ratio of 1:2; (seed to water) then germinating the seeds at room temperature for 3 weeks before drying. All samples were dehulled and then milled using the manual grinder (Corona model), sieved to obtain fine powder, which were packaged in airtight plastic containers until needed for analysis.

3. Determination of Anti-Nutritional Factors in 'Oze' Seed Flour

Anti-nutritional factors were determined in the 'oze' seed flour as follows:

3.1 Tannins

Ten grams of each sample was weighed into a 100ml conical flask and 50ml of methanol was added. The flask was stoppered, shaken and left for 24 hours. The contents of the flask were shaken after extraction and the solid particles were allowed to settle. After filtration, the volume of the extract was measured. To 1ml portion of the extract, 5ml of fresh vanillin – HCl was added and the solution was left to develop colour in 20 minutes. The absorbance was measured at 500nm against a reagent blank using corning 253 spectro-photometer.

3.2 Phytate

The phytic acid in the samples was precipitated with excess $FeCl_3$ after extraction of 10g of each sample with 100ml 0.5N HCl. The precipitate was converted to sodium phytate using 2ml of 2% NaOH before digestion with an acid mixture containing equal portions (1ml) of conc. H_2SO_4 and 65% $HCl0_4$. The liberated phosphorus was measured colorimetrically at 620nm after colour development with molybdate solution.

3.3 Hydrogen cyanide determination (AOAC, 1984)

Two grams of the sample was weighed into a flask and 100ml of distilled water added to it and allowed to hydrolyse for 1hr. 10ml of 2.5% NaOH was measured and carefully poured into the sample holder. The soxhlet apparatus was set up and was distilled into the sample holder containing the 2.5% NaOH until about 70ml was collected. It was carefully transferred to a 100ml volumetric flask and the sample holder rinsed with distilled water successively and also poured into volumetric flask. It was made up to the mark. Twenty-five milliliters (25ml) of the distillate was pipetted into a conical flask, 2ml of 6M NH_4OH was added and 0.5ml of 10% KI soln, it was titrated with 0.02M$AgN0_3$ to a first turbid colour

NB: 1ml of 0.02 M $AgN0_3$ = 1.08mg cyanide.

3.4 Trypsin inhibitor

This was done using the spectrophotometric method, described by Amtfield *et al*., (1985).

A measured weight (10g) of the test sample was dispersed in 50ml of 0.5 M Nacl solution and stirred for 30min at room temperature. It was centrifuged and the supernatant filtered through Whatman No 42 filter paper. The filtrate was used for the assay.

Standard trypsin was prepared and used to treat the substrate solution (N-α - benzoyl - Dl – arginine – p – anilide; BAPA). The extent of inhibition was used as a standard for measuring the trypsin inhibitory activity of the test sample extract. Into a test tube containing 2ml of extract and 10ml of the substrate (BAPA) 2ml of the standard trypsin solution was added. Also 2ml of the standard trypsin solution was added in another test tube containing only 10ml of the substrate. The latter served as the blank.

The content of the tubes were allowed to stand for 30min and then the absorbances of the solution measured spectrophotometrically at 410nm wavelength. One trypsin activity unit inhibited is given by an increase of 0.01 absorbance unit at 410nm.

$$\text{Trypsin unit inhibited} = \frac{Au}{As} \times 0.01 \times F$$

Where Au = Absorbance of test sample

As = Absorbance of standard (uninhibited) sample

F = Experimental factor given as

$$\frac{Vf}{Va} \times \frac{1}{W}$$

Vf = Total volume of extract

Va = Volume of extract analysed

W = Weight of sample analysed

3.5 Alkaloids (AOAC, 1984)

Five grams of the sample was dispersed in 10% acetic acid solution in ethanol to form a 1:10 w/v dispersion. The sample was stirred for every 30min for 4hr. The mixture was filtered using Whatman filter

paper. The filtrate was concentrated by evaporation over a water bath until it remained ¼ of the original volume.

Concentrated ammonia solution was added in drops and the alkaloid in the filtrate was precipitated. It was filtered using a pre-weighed filter paper (Whatman). The filter paper and the precipitate were dried in the oven at the temperature of 60^{o}C, cooled in a desicator and reweighed. The difference between the mass of filter paper plus the precipitate and the mass of filter paper alone gave the mass of the alkaloid.

$$\% \text{ Alkaloid} = \frac{\text{Mass of the alkaloid}}{\text{Mass of sample}} \times \frac{100}{1}$$

3.6 Determination of Saponins (AOAC, 1984)

One gram metric method employing the use of soxhlet extractor and two different organic solvents was easily used. The first solvent extracted lipids and interfering pigments while the second solvent extracted saponins proper.

Five grams of the ground sample was weighed into a thimble and transferred into the soxhlet extractor chamber fitted with a condenser and flask. Some quantity of petroleum spirit (boiling point 40 – 60^{o}C) enough to cause a reflux was put into the flask. Extraction continued for 3hr, which extracted the lipids and interfering pigments. The defatted material in the thimble was then used for the second extraction for saponins.

A fresh preweighed flask was fitted into the soxhlet apparatus (bearing the thimble containing the defatted sample) and methanol was put in the flask. The quantity of methanol should be enough to reflux and flush for 3 hours. The saponin was exhaustively extracted by heating the flask on a heating mantle. After the thimble and its content was removed and the methanol recovered leaving the saponin and little quantity of methanol in the flask. It was then taken to an oven and kept at slanting position at a temperature of 70^{o}C to evaporate the residual methanol. The flask and content was weighed and the difference between the flask plus saponin and flask alone was the mass of saponin extracted.

$$\text{Calculation \% Saponin} = \frac{\text{Mass of saponin in g}}{\text{Mass of sample}} \times \frac{100}{1}$$

3.7 Total Steroids

The total steroids was determined colorimetrically with reference to the saponin content. The saponic crystals were dissolved in a 50 ml formaldehyde- conc. H_2SO_4 mixture and the absorption was measured at 470nm. The steroid content was calculated as follows:

$$\% \text{ Steroids} = \frac{\text{Abs.}}{\text{wt of sample}} \times \frac{100}{1}$$

3.8 Oxalate determination

Five grams of the sample was weighed into a 100ml beaker, 20ml of 0.30N HCl was added and warmed to (40 – 50^{o}C) using magnetic hot plate and stirred for one hour. It was extracted three times with 20ml of 0.30N Hcl and filtered into a 100ml volumetric flask. The combined extract was diluted to 100ml mark of the volumetric flask.

The oxalate was estimated by pipetting 5ml of the extract into a conical flask and made alkaline with 1.0ml of 5N ammonium hydroxide. A little indicator paper was placed in the conical flask to enable us know the alkaline regions. It was also made acid to phenolphtalein (2 or 3 drops of this indicator added, excess acid decolourizes solution) by dropwise addition of glacial acetic acid. 1.0ml of 5% $CaCl_2$ was then added and the mixture allowed to stand for 3hrs after which it was then centrifuged at 3000rpm for 15min. The supernatants were discarded and the precipitates washed 3 times with hot water with thorough mixing and centrifuging each time. Two milliliters of 3N H_2S04 was added to each tube and the precipitate dissolved by warming in a water bath (70 – 80^{o}C). The content of all the tubes was carefully poured into a clean conical flask and tiltrated with freshly prepared 0.01N KMnO4 at room temperature until the first pink color appeared throughout the solution. It was allowed to stand until the solution became colourless. The solution was then warned to 70 – 80^{o}C and titrated until a permanent pink colour that persisted for at least 30 seconds was attained.

3.9 Total Phenols

Two grams of sample was weighed into a separator containing 30ml of chloroform plus 10ml of water and 1ml of diluted sulphuric acid in the ratio 1:1 (acid to water) and was vigorously shaken. The chloroform layer was drained off into 100ml volumetric flask. The extraction was repeated thrice with 20ml portions of chloroform. The three extracts were combined and made up to 100ml with chloroform and mixed. The chloroform extract solution (20ml) containing about 400mg diethyl-stilbestrol was transferred to small Erlenmeyer flask and evaporated to dryness on steam bath. The extract was cooled in a vacuum dessicator for about 10min and 20ml of alcohol was added, and residue dissolved by swirling. After 15min, the solution was mixed with 10 drops of diluted sulphuric acid and potassium at a ratio of (1:1) and the mixture cooled. Five drops of 10% sodium nitrate ($NaNO_3$) solution was added and the preparation was allowed to stand for 45mins with occasionally mixing. Some quantity was washed into 25ml volumetric flask with about 20ml alcoholic ammonium hydroxide (NH_40H) solution (equal volume of alcohol + diluted NH_40H) (4+6). Next the sample was cooled in ice bath

and allowed to stand at room temp for 1hr. Then was diluted to volume with dilute NH_40H solution and mixed. A white precipitate formed, was filtered through a dry filter paper. The clear yellow alkaline solution obtained was analysis at 420nm wavelength using spectronic 200 spectrophotometer against alcohol (1:2) as blank.

The percentage total phenols was calculated as follows:

$$\% \text{ Total Phenol} = \frac{\text{Sample Abs.}}{\text{Sample wt}} \times \frac{100}{1}$$

3.10 Oligosaccharides (Starchyose and Raffinose)

The method of Balagopalan *et al.*, (1988) was used. One gram of sample was boiled in 10mls of M HCl solution until it was negative to iodine starch test. It was centrifuged and the hydrolysate (Supernatant) used for the analysis. 2mls of the hydrolysate was mixed with 4mls of anthrone reagent in a test tube and boiled for 10min in a water bath while covering the test tubes. After boiling, the mixture was filtered and diluted with distilled water. Similarly, a standard sugar solution (glucose) was prepared and treated as described above and the absorbances of both the samples and sugar solutions were read spectrophotometrically at 625nm using Genway spectrophotometer against a blank reagent at zero.

The Oligosaccharides content was calculated as:

$$\text{Percent sugar} = Au/As \times C \times F$$

Au = Absorbance of test sample
As = Absorbance of Std sugar solution
C = Conc. of sugar solution
F = Experimental factor given by

$$\frac{Vf}{Va} \times D \times \frac{100}{w}$$

Vf = Total filtrate volume
Va = Volume of aliquot analysed
D = Dilution factor
W = wt of sample used

4. Results and Discussions

4.1 The Anti-nutritional Components in "Oze" Seed Flour Samples

Eleven anti-nutritional components at different levels were found in raw 'oze' seed flour. There were trypsin inhibitors, tannins, phytates, oxalates, saponins, hydrogen cyanide, alkaloids, stachyose, raffinose, phenols and steroids (Table 1). With regards to levels observed, the most prominent among them were the alkaloids, (2730mg/100g), phenols (2500mg/100g), saponins (840mg/100g) and hydrogen cyanide (280mg/100g), while the oxalates (2.80mg/100g) and tannins (3.11mg/100g) were the least.

Interestingly, in all except phenols, the anti-nutritional factors identified were more in the raw hulls than in the raw edible cotyledons, and even there, the alkaloids (7640mg/100g), saponins (1920mg/100g), hydrogen cyanide (400mg/100g), phenols (400mg/100g) and steroids (300mg/100g) were more prominent. Heat treatment and malting affected all the nutritional stress factors and all decreased with increased period of heating, though not at the same rate of reduction. For instance, at 6min blanching period, a reduction of 92.86% (840mg/100g – 60mg/100g), was achieved in saponins, and reduction of 94.87% (2730 – 140mg/100g), 99.6% (2500 – 10mg/100g), 63.71% (15.68 – 5.69mg/100g) and 46.62% (3.11 – 1.66mg/100g) were achieved in alkaloids, phenols, phytates and tannins respectively, while complete elimination was achieved in hydrogen cyanide, oxalates, stachyose and raffinose. These results were in agreement for other similar seed as reported by Uzogara *et al*., (1990); Jood, *et al*., (1989) and Osagie, (1998). With the exception of tannins and phytates which had 27% and 22% respectively of their raw material values retained at 20min cooking period, all other factors were completely eliminated or degraded at 20min cooking period. All factors were destroyed after 40min cooking. Roasting (dry heat treatment) for 45min destroyed all factors with the exception of tannins and phytates. Specifically, samples roasted for 45mins had 19.94% and 17.35% of their raw material of tannins and phytates (respectively) retained. Also the malting operation destroyed all factors with the exception of phytates and oxalates. Malted samples retained 2.55% of its original level of phytates and 72.14% of its original level of oxalates. These findings indicated that generally moist heat application was more effective in eliminating the anti-nutritional factors in oze seeds than dry-heat application. It was interesting to observe that with exception of phenols where the raw cotyledon level (2500mg/100g) was 6.3 times higher than the level (400mg/100g) found in the hulls, but for all other anti-nutritional components studied, the hulls had levels ranging from 1.4 – 7.9 times higher than those observed in the raw cotyledons. For instance, the tannin level (20.12mg/100g) in hulls is 6.5 times of its level (3.11mg/100g) in cotyledon and hydrogen cyanide level (400mg/100g) in hulls is 1.4 times of its level (280mg/100g) in the cotyledons. There were significant differences ($P<0.05$) between the levels of each factor in the hulls and the raw cotyledons. There were also significant differences ($P<0.05$) between the levels of each factor in the raw sample and samples given moist heat for up to 6min.

The raw cotyledon had 37.3 TI units/100g sample and like other anti-nutritional components, this decreased as heat treatment progressed. Specifically, a reduction of 60.1% (from 37.3 – 0.74 TI units/100g) was achieved at 6min blanching period, reaching total trypsin inhibitor elimination after 8min of heat treatment. As observed for total phenols, the hulls had

lower level (9.70 TI units/100g) of trypsin inhibitor than the raw edible cotyledons. Malting, roasting (45min) and cooking (≥ 8min), completely eliminated trypsin inhibitory effect.

Tannin affect the digestive tract and their metabolites are toxic (Ene-obong, 1992). The precise toxic amount of tannin to cause depression in humans is not known yet. The values obtained in 'oze' seed flour were quite lower than the values of Ezeh (1992) for legumes.

For hydrogen cyanide the fatal dose in food is 50mg/100g which is higher than what was obtained in 'oze' seed cotyledons (2.8mg/100g). Such illnesses arising from its excesses like gasping, staggering, paralysis convulsion could be avoided.

Phytate, a chelator of cations and found in all seeds causes reduction in protein availability (Macrae and Joslyn 1993) but was reduced by germination (Oksana and Bills 1984).

As saponins haemolyse red blood cells, its elimination by heat makes it safe for human consumption. Alkaloids which causes depressed growth was eliminated through heating.

Hydrolysis of oligosaccharides with formation of simple diasaccharides and monosaccharides or other compounds, decreases its levels. Dehulling also helped to decrease them thereby reducing flatulence.

Table 1. Mean values of the anti-nutritional components in 'Oze' seed flour sample

Samples	Trypsin Inhibitors T.IU/100g	Tannins mg/100g	Phytates mg/100g	Oxalates mg/100g	Saponins mg/100g	HCN mg/100g	Alkaloids mg/100g	Stachyose mg/100g	Raffinose mg/100g	Total Phemols mg/100g	Total steroids mg/100g
Raw	37.3^{e}	3.11^{d}	15.68^{d}	2.80^{b}	840^{b}	2.80^{b}	2.730^{cd}	38^{ab}	15^{ab}	2500^{c}	38.0^{c}
4 min blanch	14.90^{d}	2.28^{c}	6.66^{c}	0.20^{a}	100^{b}	0.00^{a}	270^{c}	3^{a}	2^{a}	50^{b}	8.0^{b}
6 min blanch	0.74^{b}	1.66^{c}	5.69^{c}	0.00^{a}	60^{b}	0.00^{a}	140^{b}	0.00^{a}	0.00^{a}	10.0^{a}	4.0^{ab}
8 min blanch	0.00^{a}	2.24^{b}	4.62^{c}	0.00^{a}	20^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
20 min cook	0.00^{a}	0.83^{b}	3.39^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
40 min cook	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
60 min cook	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Roasted (45 min)	0.00^{a}	0.62^{b}	2.72^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Malted	0.00^{a}	0.00^{a}	0.40^{a}	2.02^{b}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
Hulls	9.70^{c}	20.12	35.98^{e}	7.80^{c}	19.20^{d}	400^{c}	7640^{e}	58.0^{ab}	52.0^{a}	400^{d}	300^{c}
LSD	12.21	5.189	9.52	2.14	0.53	0.12	2.08	0.18	0.14	6.65	0.08

Note: Means with different superscripts along the column have significant difference at $P<0.05$.

Conclusion

The results obtained from the project have shown that *Bosqueia angolensis* popularly known as 'oze' in Igbo speaking community yields flour which contains some naturally occurring toxins /antinutrients, the level at which they occur coupled with the natural detoxification methods and loss of these toxins during processing make their presence to be of little concern.

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