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Original article

Evaluation of the nutritional potentials of fermented oil bean seed *Pentaclethra macrophylla* benth

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ARTICLE INFO

Article history,

Received 28 June 2014

Accepted 19 July 2014

Available online 28 July 2014

Keywords,

Nutritional composition and anti-nutritional factors

Fermented seeds

Pentaclethra macrophylla benth

ABSTRACT

The seeds of fermented African oil bean seed, *Pentaclethra macrophylla* Benth were analyzed for mineral elements, nutritional and anti-nutritional contents. The concentrations (mg/100g) of fermented African oil bean seed for 4days, 7days and 14days with regards to calcium, magnesium, potassium, sodium and phosphorus were 28.2mg/100g, 26.12mg/100g, 28.04mg/100g; 126.14mg/100g, 160.1mg/100g, 180.2mg/100g; 210.40mg/100g, 216.60mg/100g, 218.40mg/100g; 12.42mg/100g, 14.16mg/100g, 16.12mg/100g and 210.04mg/100g, 216.10mg/100g; 216.12mg/100g respectively. The percentage composition of protein, lipids, carbohydrates, Ash and fibre of the graded levels of fermented African oil bean seed (*Pentaclethra macrophylla* Benth) after 4days, 7days and 14days duration of fermentation were 31.95%, 38.38%, 39.04%; 35.00%, 38.20%, 42.40%; 28.31%, 20.75%, 15.97%; 1.08%, 1.19%, 1.33% and 3.66%, 1.48%, 1.26% respectively. The concentration of fermented oil bean seed for 4days, 7days and 14days with regards to anti-nutritional factors, phytate, tannins, hydrogencyanide (HCN) and oxalate were 0.04mg/100g, 0.02mg/100g, 0.01mg/100g; 0.042mg/100g, 0.004mg/100g, 0.002mg/100g; 0.02mg/100g, 0.04mg/100g, 0.008mg/100g and 0.12mg/100g, 0.11mg/100g, 0.10mg/100g respectively. The African oil bean seeds could serve as supplementary source of essential nutrients for man and his livestock

provided the anti-nutrients inherent in the seeds are adequately detoxified to the barest minimum levels.

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1. Introduction

The African oil bean, *Pentaclethra macrophylla* Benth is a tropical tree crop which exists in abundance in the Southern and Middle belt zones of Nigeria and in other rain forest areas of West and Central Africa. It belongs to the Leguminosae family and the sub-family of Mimosoideae with no known varietal characterization (Keay, 1989 & ICRAF, 2004). The tree is recognized by peasant farmers in these parts of the country for its soil improvement properties and as a component of an agro-forestry system (Okafor and Fernandez, 1987). It is cultivated in forest areas (Ogbo, 2007). The African oil bean plant, *Pentaclethra macrophylla* Benth has been recognized as a food tree species for outlying farms in the forest zone. According to Asoegwu et al. (2006) they grow either wild or semi-wild with no organized cultivation in plantations or orchards in Nigeria. Okafor et al. (1991) reported suitable techniques for the vegetative propagation of the plant with buds and stem cuttings resulting in drastically reduced fruiting age of 2-4 years and fruiting at breast height. The edible seeds of the African oil bean need rigorous but careful processing and fermentation prior to their consumption as food supplement (Asoegwu et al., 2006; Ogbo, 2007; Enujiugha and Akanbi, 2008). Some parts of the plant have medicinal values, in Cameroon, the seeds are used to treat infertility while the pods are used to treat convulsion. In Nigeria comparative study of cancer risks and cancer levels have been carried out between the Easterners who ate fermented oil bean and those who did not. An improvement index was also measured between cancer patients who ate ugba as a meal supplement and those who did not, the result indicated that the fermented form of the African oil bean seed as a food supplement has greatly reduced the risks of cancer and some tobacco related diseases. It was discovered that cancer patients who regularly ate fermented oil bean seed as food supplement showed marked improvements in regaining quality health (Chidozie, 2006). In Ghana the smoke from burnt leaf is used to treat convulsion, the leaf/stem bark are used to treat diarrhea, the bark as ointment is used to treat itching, the bark decoction is used to treat lactogenicity, the bark as lotion is used in the treatment of wounds. The seed, when crushed and eaten with red ants, can induce abortion (Tico, 2005). The seed is also used to poison fish and arrows. Ashes of pods are used as cooking salt. The seeds are edible after roasting or boiling for at least 12hours. The seeds yield oil (30-36%) which is rich in protein and suitable for making candles and soap, flour from the seed can be used for bread making. The wood from the plant is used for fuel. Even though it makes good firewood, it gives unpleasant smell. It is also used for charcoal

According to Tico (2005) the anthelmintic bark when pounded is used for treatment of leprosy sores. In the Agro-forestry industry they supply wood and farming materials (stakes and mulch). Its trunk provides timber used for structural work. The oil bean seed tree yields forest products for making wooden household utensils (Okafor, 1987). The persistent flat glossy brownish flattened pod explodes at maturity and disperses the seeds. The number of seeds in the pod depends on the length of the pod and size of the seeds, the pods normally contain about 8 flat glossy brown seeds up to 7cm long. The mature dispersed seeds are harvested by gathering them manually from around the tree. The kernel (a dicotyledon), which is gray in colour, is embedded in a glossy brownish seed coat. The seeds may be said to be irregular and oval and lies flat in its natural position.

Pentaclethra macrophylla Benth was identified as a minor food supplement by Okafor (1987), while other workers have investigated and found that this oil seed contains 23-28% protein. It also contains the twenty (20) essential amino acids and essential fatty acids that make up over 10% of the fatty acids in the oil (Enujiugha and Agbede, 2000; Ogbo, 2007). The oil content and carbohydrate contained in raw African oil bean seeds are $53.98 \pm 0.99\%$ and $19.16 \pm 0.76\%$ dry wt, respectively (Enujiugha and Ayodele-Oni, 2003; Enujiugha and Akanbi, 2005). The seed when boiled, processed and fermented for about 3-4 days (72 hours) is called Ugba ukpkala (in Igbo language of Eastern Nigeria). The fermented seeds are used traditionally as condiments and seasonings to add flavour to food (Enujiugha and Akanbi, 2002; Ogbo, 2007; Enujiugha and Akanbi, 2008) and used for the preparation of many delicious African delicacies and snacks including, African salad, soups and sausages for eating with different staples. It is rich in vitamins and mineral and in high demand for both local consumption and for export. It is a low acid food which could be prepared into flour and explored in food fortification and confectionaries. The seed is a

source of edible oil and used for candle making, cooking and soap. The seed shells are decorative and are often used to make beads which are worn as necklace, rosaries and sometimes as local dancing apparels. The seeds of African oil bean which are rich in nutrients (protein, fatty acids and minerals) have been found to be a highly nutritive animal feed when fortified (Egonu and Njoku, 2006).

The incidence of protein deficiency in Nigeria is high because many people cannot afford meat, fish and other proteinous animal products due to their astronomic costs. African oil bean seed is consumed by many people especially the Igbos without knowing the nutritive and anti-nutritive contents. Thus, this study titled "evaluation of the nutritional potentials of fermented oil bean seed (*Pentaclethra macrophylla*)" was conducted with the aim of determining the nutritive contents of fermented oil bean seed, determining the anti-nutritional contents in the fermented oil bean seed and creating awareness on the best level of fermentation that would be useful for human consumption. The result of this study will therefore, serve as a guide to those who cannot afford conventional sources of animal protein like meat, fish, eggs and so on to go for the cheaper alternative plant source of protein (oil bean seed) using the best identified level of fermentation with invariably maximum level of nutrients and least levels of anti-nutrients.

2. Materials and methods

2.1. Sample collection/identification

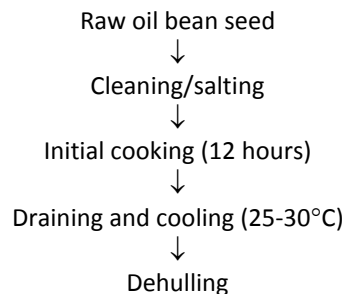
The seeds of *Pentaclethra macrophylla* Benth were bought from a local market in Sabon Gari, Zaria, Kaduna State, Nigeria. The samples were deposited at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, and Zaria for identification.

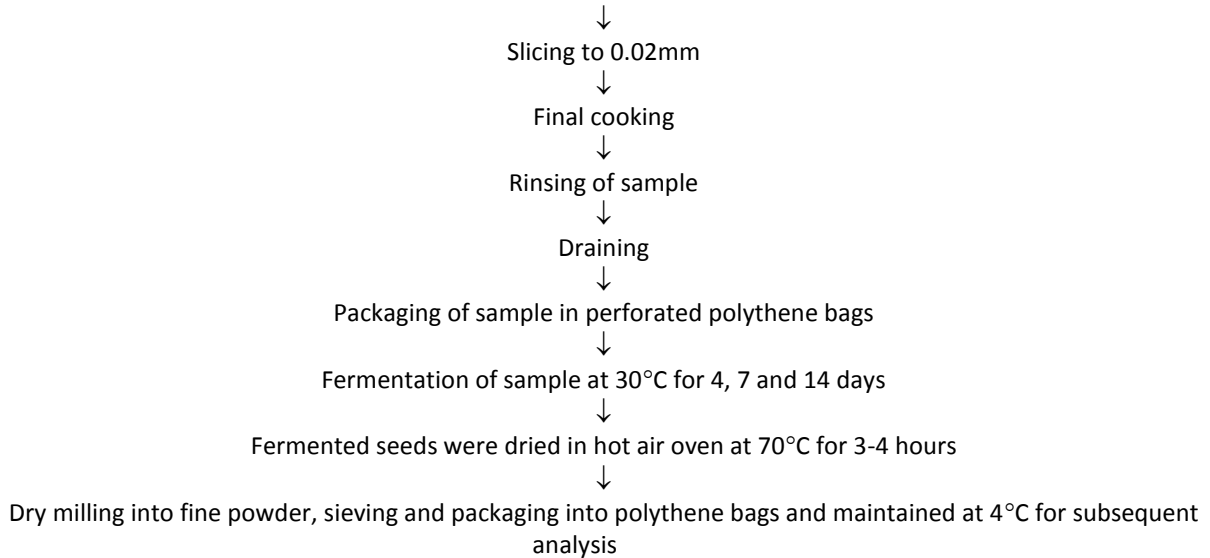
2.2. Sample preparation

The seeds were sorted out to remove dirt, stones and other forms of impurities. Two hundred grams (200g) of the seeds were weighed and then the following procedures were carried out:

- Initial cooking: Whole oil bean seeds, *Pentaclethra mycrophylla* Benth were cooked for 12hours (when the seeds were cooked, they normally split open when pressed manually). After opening, it is only the decorticated/dehulled seeds that were used.
- Slicing: The whole seed samples were sliced into smaller pieces (0.02mm) systematically to aid in fast fermentation.
- Final cooking: After slicing into smaller pieces the seeds were then cooked for the second time (24 hours).
- Washing of the sample: After the second cooking the seed samples were thoroughly washed to remove the bitter taste. Subsequently the water was drained off using a sieve and the seeds were packed into polythene bags for fermentation. The fermentation process was monitored on daily basis.
- Duration of fermentation: The seeds were fermented at a temperature of 30°C. The fermentation lasted for 14days. The first phase of the experiment lasted for 4days, the second phase (7days) and the last phase (14days).
- Fermented samples were oven dried at 70°C for 3-4 hours, milled to fine powder and thereafter packed in polythene bags.

2.3. Flow chart for production of fermented oil bean seed





2.4. Biochemical analyses

The samples were taken to the Central laboratory of the National Research Institute for Chemical Technology (NARICT), Basawa, Zaria, Nigeria, for analyses which included proximate, anti-nutritional factors and mineral analyses.

2.5. Determination of nutritional content

The samples were analyzed for proximate composition (crude protein, lipids, soluble carbohydrate, ash and crude fibre). The protein was determined by micro Kjeldahl method (AOAC, 1980), the crude protein = $6.25 \times \%N$; the oil was also extracted by adoption of AOAC, 1980 method using petroleum ether 40-60°C by means of soxhlet extractor in a continuous extraction process whereby non polar components of the sample were easily extracted into the organic ether. The soluble carbohydrate as Nitrogen Free Extract (NFE) was calculated by difference as: $NFE = 100 - (\% \text{ crude- protein} + \% \text{ crude lipid} + \% \text{ Ash} + \% \text{ moisture} + \% \text{ crude fibre})$. The Ash content of the sample which was determined by AOAC 1980 was determined from the loss in weight that occurs during igniting the sample at 55°C in muffle furnace which was enough to permit all the organic matter to burn off without allowing any appreciable decomposition of the ash constituents.

The AOAC (1980) method was adopted to determine the crude fibre content of the sample, this involved sequential digestion of the sample with dilute acid and alkali solutions. The insoluble residue obtained was ignited at 550°C in a muffle furnace for 2hours, cooled in a desicator and weighed to obtain crude fibre.

2.6. Determination of anti-nutritional factors

2.6.1. Hydrogen cyanide determination

Hydrogen cyanide was determined by the alkaline titration procedure (AOAC, 1995). Ten gram (10g) of ground sample was soaked in a mixture of 200cm³ of the distilled water and 10cm³ of orthophosphoric acid. The mixture was left overnight to release all bounded hydrocyanic acid. The mixture was then distilled until 150cm³ of the distillate was collected, 20cm³ of distillate was taken into a conical flask containing 40cm³ of distilled water then 8cm³ of 6mol dm³ aqueous ammonia and 2cm³ of 5% potassium iodide solutions were added. The mixture was titrated with 0.02 mol dm³ silver nitrate to a faint end point with permanent turbidity.

1ml 0.02N AgNO₃ = 1.08mg HCN

2.6.2. Determination of tannin

Tannin was determined using the standard method described by AOAC (1980). The method was however, slightly modified. About 2g of the ground sample was defatted for 2 hours using soxhlet extraction apparatus. The

residue was dried in an oven for 12 hours at 100°C, boiled with 300ml of distilled water, diluted to 500ml in standard volumetric flask and filtered through non-absorbent cotton wool.

A volume of 25ml of the infusion was measured into 2 litre porcelain dish and titrated with 0.1N potassium permanganate (0.1N potassium permanganate was standardized against 0.1N oxalic acid) until the blue solution changed green, then few drops of 0.1N potassium permanganate was added. The difference between the two titration was multiplied by 0.006235 to obtain the amount of tannin in the sample since 0.1N oxalic acid = 0.006235g tannin.

2.6.3. Determination of percentage phytate (Reddy et al., 1982)

A known weight of each ground sample was soaked into 100ml of 2% HCl for 5 hours and filtered. Twenty-five cubic centimetres (25cm³) of the filtrate was taken into a conical flask and five cubic centimetres (5cm³) of 0.3% ammonium thiocyanate solution was added. The mixture was titrated with a standard solution of FeCl₃ until a brownish – yellow colour persisted for 5 minutes.

The concentration of the FeCl₃ was 1.04% W/V

Calculation mole ratio of Fe to phytate = 1:1

Concentration of phytate phosphorous

= Titre value x 0.064

1000 x Weight of sample

Phytic acid content was calculated on assumption that it contains 28.20% phosphorus by weight.

2.6.4. Determination of total oxalate

The total oxalic acid of the powdered samples was determined by the modified method of Abeza et al. (1968). About 2g aliquot of the plant materials was weighed into a 250ml flask, 190ml, distilled water and 10ml of 6M hydrochloric acid were added. The mixture was digested for 1 hour on boiling water bath, cooled, transferred into a 250ml volumetric flask, diluted to volume and filtered. Four drops of methyl red indicator were added followed by concentrated ammonia until the solution turned faint yellow. It was then heated to 100°C, allowed to cool and filtered to remove precipitate containing ferrous ions. The filtrate was boiled, 10ml of 5% calcium chloride added with constant stirring and was allowed to stand overnight. The mixture was filtered through Whatman No. 40 filter paper. The precipitate was washed several times with distilled water. The precipitate was transferred quantitatively to a beaker and 5ml of 25% sulphuric acid was added to dissolve the precipitate. The resultant solution was maintained at 80°C and titrated against 0.5% potassium permanganate until the pink colour persisted for approximately one minute. A blank was also run for the test sample. From the amount of potassium permanganate used the oxalate content of the unknown sample was calculated thus

1ml of potassium permanganate = 2.24mg oxalate

2.7. Elemental analysis

The ground sample was ashed and pellets of 0.2983g and 19mm diameter were made by applying a pressure of 10 tons (204081.60Nm²) on a hydraulic press using three drops of an organic binder (10% solution of styopore in toluene) (Anhwange et al., 2005). The pellets were introduced into the X-ray fluorescence (SRF) generator (model SL12170) and analyzed (Funtua, 1999). Measurements were taken using an annular 25 mCi 109Cd as the excitation source, that emit Ag-K X-rays (22.1 keV) and 55Fe that emits Mn-K X-rays (5.89 keV), in which case all elements with lower characteristic excitation energies were accessible for detection in the samples. The spectra for the samples were collected for 3000s with the 109Cd source and the spectra were then evaluated using the AXIL-QXAS program (Bernasconi, 1996). Sodium and magnesium were analyzed by Atomic Absorption Spectrometer (Unicam 969 AAS), while sulphur and phosphorus were analyzed by standard colorimetric procedures (Allen et al., 1974).

3. Results and discussion

The proximate analysis of fermented oil bean seed in table 1 shows that protein increased from 31.95% in 4days to 38.38% (7days) and 39.04% (14days) while the lipid content increased from 35.00% in 4days to 38.20% (7days) and 42.40% (14days). Therefore, this result proved that the longer the duration of fermentation the higher the percentage protein and lipid content. This finding is in concordance with the findings of Olaniyi et al. (2009b)

and Tamburawa (2010) who worked on Parkia (Locust bean seed). Fermentation is associated with microbial activities which stimulates better nutrient availability (particularly protein) and digestibility with significant softening of the cotyledons (Enujiugha and Akanbi, 2002). Fermented African oil bean seed has been established to produce better nutritional quality products compared to the raw seeds (Enujiugha and Agbede, 2005). The microbial enzymes from the fermenting organism aid in hydrolysis of the seed macromolecules (Enujiugba, 2003). The result of this finding is also in concordance with the findings of Ogbo (2007) which reported that due to the successive processing steps during the fermentation process there was progressive softening of the cotyledons with increased palatability of fermented African oil bean (*Pentaclethra macrophylla* Benth) seeds. The crude fibre content did not however follow the same pattern as the protein and fat in this study, it reduced with increased duration of fermentation. It reduced from 3.66% in 4days to 1.48% (7days) and 1.26% (14days) respectively. This result agrees with the findings of Tamburawa (2010) which revealed that crude fibre values decreased in soaked and fermented locust bean seed with increased duration of soaking and fermentation due to breaking/softening of the fibre. The NFE followed the same trend as the CF content.

The result of this finding agrees with that of Ogbo (2007) which reported a slight increase in Ash contents of fermented African oil bean (*Pentaclethra macrophylla* Benth). Result (Table 3) shows that the mineral elements of oil bean seed which includes calcium, magnesium, potassium, sodium and phosphorus increased with longer duration of fermentation.

The anti-nutritional factors of oil bean seed which includes phytate, cyanide, and oxalate reduced with increased duration of fermentation as indicated in table 2. Therefore, the longer the duration of fermentation the lesser the quantity of anti-nutritional factors in the fermented seed. This finding is in accord with the findings of Tamburawa (2010) which indicated that increased duration of fermentation progressively reduced levels of anti-nutrients in Parkia seed. Anti-nutrients have been established to interfere with digestive processes by binding with nutrients and making them unavailable for efficient body utilization. (Makkar and Becker, 1997; Francis et al, 2001a; Francis, 2002). The presence of anti-nutrients like Tannin interferes with digestive processes by binding the feed proteins, vitamins, minerals and digestive enzymes. The presence of cyanide and oxalate could lead to poisoning. Phytate reduces the availability of dietary phosphorus, it also forms complexes with dietary protein and reduces their availability.

Conclusively, the results indicated that the longer the duration of fermentation the higher the protein, lipid, and mineral element contents in the oil bean seeds. Fermentation at longer periods has also been shown to reduce the level of the anti-nutritional composition of the seeds.

Table 1

Proximate composition of fermented African oil bean seed (*Pentaclethra macrophylla* Benth) at different day intervals.

Proximate composition	Fermentation period		
	4 days	7 days	14 days
Dry matter	95.00	97.13	97.12
Ash	1.08	1.19	1.33
Crude protein	31.95	38.38	39.04
Crude lipid	35.00	38.20	42.40
Crude fibre	3.66	1.48	1.26
Nitrogen free extract	28.31	20.75	15.97

Table 2

Anti-nutritional composition (mg/100g) of fermented African oil bean seed (*Pentaclethra macrophylla* Benth) at different day intervals.

Anti-nutritional composition	Fermentation period		
	4 days	7 days	14 days
Phytate	0.04 mg/100g	0.02 mg/100g	0.01 mg/100g
Tannins	0.042 mg/100g	0.004 mg/100g	0.002 mg/100g
Hydrogen cyanide	0.02 mg/100g	0.04 mg/100g	0.008 mg/100g
Oxalate	0.12 mg/100g	0.11 mg/100g	0.10 mg/100g

Table 3

The mineral composition of fermented African oil bean seed (*Pentaclethra macrophylla* Benth) at different day intervals.

Mineral composition	Fermentation period		
	4 days	7 days	14 days
Calcium	28.2 mg/100g	26.12 mg/100g	28.04 mg/100g
Magnesium	126.14 mg/100g	160.10 mg/100g	180.20 mg/100g
Potassium	210.40 mg/100g	216.60 mg/100g	218.40 mg/100g
Sodium	12.42 mg/100g	14.16 mg/100g	16.12 mg/100g
Phosphorus	210.04 mg/100g	216.10 mg/100g	216.12 mg/100g

4. Conclusion

Based on the results of this study, it has been discovered that graded levels of fermented oil bean seed (*Pentaclethra macrophylla* Benth) in terms of nutrients is best at 14 days level of fermentation which has highest amount of protein 53.12% and lipids 42.40% (source of energy) along with minimum anti-nutrient levels. With this protein level it will go a long way to alleviating protein deficiency among the poor and less privileged people in the study area and Nigeria at large particularly with the aim of meeting up with the FAO protein requirement recommended levels for man. However, efforts should be made to ensure that fermented Ugba product should not extend beyond 2 weeks (14 days) because it has been established to have a high rate of deterioration and high susceptibility to microbial spoilage within 2 weeks of production.

Recommendations

- Food nutritionist should endeavour to explore avenues of extending the shelf life of fermented *Pentaclethra macrophylla* Benth (Ugba) without impairing its nutritional quality, freshness (flavour, colour and appearance) and consumer acceptance.
- Processing techniques like hydro-thermal boiling and roasting could be evaluated to ensure that anti-nutrient inherent in the seeds are reduced to the barest minimum for better efficient utilization of nutrients.

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