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Nutritional Potential of Aqueous Soaked Fermented *Pentaclethra macrophylla* (Benth) Seeds

Ugbogu Amadike Eziuche, Emmanuel Okezie*

Department of Biochemistry, Abia State University, Uturu, Abia State, Nigeria
*Corresponding author: emmanuelokezie7@gmail.com

Abstract The effect of fermentation on nutritional composition of *Pentaclethra macrophylla* (Benth) seeds was evaluated. *P. macrophylla* seeds were procured and divided into four portions. The seeds were hydrothermally treated to extract the cotyledons which were allowed to ferment for 24 h, 48 h and 72 h, while 0 h served as the control. The samples were differently oven dried and milled at the end of each fermentation period. There was significant difference ($p< 0.05$) observed in all the assayed proximate parameters when compared to the control sample at 0 h fermentation. However, moisture content, ash content, lipid and crude proteins significantly increased, while crude fibre and carbohydrate contents significantly reduced as fermentation period increases. The quantified minerals include; calcium (Ca^{2+}), magnesium (Mg^{2+}), phosphorous (P), sodium (Na^{+}), zinc (Zn^{2+}), manganese (Mn^{2+}), iron (Fe^{2+}) and potassium (K^{+}). Although, there was statistical difference ($p< 0.05$) observed in all the tested minerals, but all significantly increased in values with the exceptions of Mg^{2+} (24 h fermentation), Zn^{2+} (24 h and 72 h fermentation periods) and K^{+} when compared to the control. The results obtained from the antinutritional estimation revealed a significant decrease in values of phytate, tannins, hydrogen cyanide and oxalate with increased fermentation period. The present study has therefore shown that increase in fermentation period increases nutritional composition and decreases antinutrients in *P. macrophylla* seeds.

Keywords: *antinutrients, fermented food, Pentaclethra macrophylla, nutritional composition*

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1. Introduction

Fermentation is a metabolic process in which microorganism converts carbohydrate, such as monosaccharide (glucose, galactose, fructose), disaccharide (lactose, maltose, sucrose), oligosaccharides (stachyose, raffinose) or polysaccharides (starch) into an alcohol or an acid. It involves a dynamic process in which several catabolic and anabolic reactions proceed simultaneously which depends on several conditions, including substrate, micro flora, and environmental factors [1]. This metabolic conversion in food is owed greatly to microbial enzymic reactions [2].

Fermented foods play crucial roles in human diet especially in developing countries where numerous fermented foods (like cereal based and legume-based) are integral part of their daily food intake. These countries as well as Nigeria depend on these foods to satisfy their nutritional needs [1]. Over 80 % of the inhabitants of Nigeria consume various fermented foods and beverages, which include: staple foods like "garri", "fufu" (akpu), "elubo", "abacha", "akara-akpu", "amala", "ogi" (akamu), "agidi", "tuwo", "fura" ; beverages like "mmanya ngwo" (raffia palm wine), "mmanya nkwu" (oil palm wine), "ogogoro" (distilled palm wine), "burukutu"; milk products like "gindiri" (fermented milk), "warakasi" (local cheese) and condiments like "dawadawa", "ugba", "ogiri" and "eketeke" [3].

The growing worries of malnutrition and the need to improve the nutritional benefits of food especially in the time of hardship have also prompted different areas of the world in the development of fermented foods whose nutritional importance and shelf lives are of inestimable values when compared to their unfermented counterpart. This process has been practiced by man for centuries in various part of the world especially in Africa [3]. Not only does the process of fermentation preserve foods, it also improves digestibility by breaking down proteins within foods and have been known to enrich nutrients such as vitamins, amino acids and fatty acids [4]. Many foods pack a big nutritional wallop into a small package, like the odd-sounding African oil bean seed.

The African oil been seed, popularly known by its scientific name, *Pentaclethra macrophylla*, is a native to tropical regions of Africa especially Cameroon, Cote d'Ivoire, Democratic Republic of Congo, Ghana, Niger Republic, Nigeria and Togo [5]. This nutritional plant belongs to the family Leguminosae (sub-family Mimosoideae) in the order Fabales. The trees are predominantly found in the Eastern and Southern parts of Nigeria [6]. The local names include "Congo acacia" in Congo, "Duala Kombola" in Cameroon and "Ugba", Ukpaka" in South Eastern part of Nigeria [7]. The leaves are small and reddish when young, but gradually turn to dark green [6]. *P. macrophylla* plays an economic role in

agro-forestry supplying wood and farming materials (stakes and mulch). The tree yields forest products for making wooden household utensils, while its trunk provides timber used for structural work [8].

Ugbogu *et al*. [9] has shown that the fermented aqueous extract of the *P. macrophylla* could be employed in ethnomedicine for the treatment of peptic ulcer using Wistar rat as an experimental animal. It has also been reported that the plant is rich in essential amino acids as well as fatty acids and minerals (calcium, phosphorus, magnesium) in nutritive animal feed when fortified [10], and also serves as a cheap source of protein thus increasing the hematological parameters (red blood cells, platelets and white blood cells) [11]. Studies have also shown that *Pentaclethra macrophylla* seed extract has a spectrum of efficacy and does not damage both the internal and external environments of a living system. However, it is regarded as a new age antibiotic, antifungal, antinoceptive and anti-inflammatory [12].

The fermentation of the sliced oil bean seed is known to produce nutritionally better products than the raw seeds [6,13]. Therefore, the present study is aimed at investigating the effect of fermentation on the nutritional composition of *P. macrophylla* (Benth) seeds.

2. Materials and Methods

2.1. Collection and Identification of Plant Sample

P. macrophylla seeds were purchased from Eke Okigwe, Okigwe Local Government Area of Imo State, Nigeria. Eke Okigwe lies between latitude $5^{o}50^{1}0.9^{II}N$ and $5^{o}49^{1}55^{II}N$ and longitude $7^{o}21^{1}32.6^{II}E$ and $7^{o}23^{1}17^{II}E$. The samples were transported to the Department of Biochemistry, Abia State University, Uturu and were identified in the Department of Plant Science and Biotechnology of the same institution.

2.2. Preparation of the Plant Extract

The raw *P. macrophylla* seeds were divided into four portions of 400 g each. They were hydrothermally treated to soften the seed coat. The softened seed coats were manually removed to extract the cotyledons. The beakers were thereafter labeled 0 h, 24 h, 48 h and 72 h. The contents of these beakers were subjected to traditional fermentation in water [14].

The 0 h sample was taken as the control sample. The 24 h sample was removed from water after 24 hours and oven dried at 100 °C for 24 hours. The oven dried samples were milled to pass through a 20 mm sieve using a mechanical homogenizer. The remaining interval evaluations of 48 and 72 h samples were treated the same way. The milled extracts obtained were stored in air-tight containers and properly labeled until required for analyses.

2.3. Proximate Analysis of Fermented *P. macrophylla*

The proximate compositions of fermented 0, 24, 48 and 72 h samples namely; moisture, ash crude lipid, nitrogen content, crude fibre, and carbohydrate were determined according to the recommended methods of the Association of Official Analytical Chemists [15].

2.4. Determination of Mineral Composition of Fermented *P. macrophylla*

Exactly 1 g of the sample was weighed and digested for a short period of time in 69 % HNO_3 and 30 % H_2O_2 (v/v: 10 mL) and later heated at 120 ^{0}C. The digested solutions were filtered using Whatman n^{o}1 filter paper and diluted to 50 mL with deionized water. The concentrations of the micronutrients in the digested solutions were determined using Atomic Absorption Spectrophotometer [16].

Antinutritional Composition of Fermented *P. macrophylla*

The antinutritional compositions of fermented 0, 24, 48 and 72 h samples namely phytate, tannins, hydrogen cyanides and oxalate were quantified.

Tannin was spectrophotometrically estimated by methods of Folin-Denis [17], while hydrogen cyanide was determined by methods described by [18].

Phytate Content Determination: This was determined by the method of wheeler and Ferrel [19]. Exactly 100 mL of the sample was extracted with 3 % trichloroacetic acid. The extract was treated with $FeCl_3$ solution and the iron content of the precipitate was determined using Atomic Absorption spectrophotometer (CyeUnicam 2900). A 4:6 Fe/P atomic ratio was used to calculate the phytic acid content [20].

Oxalate Determination: The titration method as described by Day and Underwood, 1986 was followed. Exactly 1 g of the sample was weighed into 100 mL conical flask and 75 mL 3 M H_2SO_4 was added and stirred for 1hr with a magnetic stirrer. This was filtered using a Whatman N^{o} 1 filter paper. 25 mL of the filtrate was then taken and titrated while hot against 0.05M $KMnO_4$ solution until a faint pink colour persisted for at least 30 sec. The oxalate content was then calculated by taking 1mL of 0.05 M $KMnO_4$ as equivalent to 2.2 mg oxalate [21].

2.5. Statistical Analysis

One-way analysis of variance (ANOVA) with the R^{TM} Statistic software package, version 3.0.3 and excel package were used for statistical analysis. The normal distribution of the data and the homogeneity of variance were tested by Bartlett homogeneity test. One-way ANOVA with a Tukey test post-hoc was used to identify statistical differences among groups. A p-value of ≤ 0.05 was considered statistically significant.

3. Results

The effect of fermentation on proximate composition of *P. macrophylla* seed expressed in percentage (%) was shown in Table 1. There was significant difference ($p<$ 0.05) in all the assayed proximate parameters when compared to the control sample at 0 h fermentation. However, moisture content, ash content, lipid and crude protein significantly increased, while crude fibre and

carbohydrate contents significantly reduced with increased fermentation period in relation to the control samples.

Table 2 showed the effect of fermentation on the mineral composition of *Pentaclethra macrophylla* Seed expressed in mg/100 g. The assayed minerals include; calcium (Ca^{2+}), magnesium (Mg^{2+}), phosphorous (P), sodium (Na^{+}), zinc (Zn^{2+}), manganese (Mn^{2+}), iron (Fe^{2+}) and potassium (K^{+}). Although, there was statistical difference ($p< 0.05$) observed in all the tested minerals, but all increased except Mg^{2+} (24 h fermentation), Zn^{2+} (24 h and 72 h fermentation periods) and K^{+}.

The results obtained from the effect of fermentation on anti-nutrient composition of *Pentaclethra macrophylla* Seeds expressed in mg/100 g are presented Table 3. From the result, there was a significant decrease ($p< 0.05$) in the values obtained as fermentation period increases.

Table 1. Effect of fermentation on the proximate composition of *Pentaclethra macrophylla* Seed

	Fermentation period (hr)			
Parameter	0	24	48	72
Moisture content (%)	30.42 ± 3.10^{a}	32.51 ± 1.73^{a}	35.53 ± 0.94^{b}	39.97 ± 0.45^{c}
Ash content (%)	0.67 ± 0.08^{a}	1.09 ± 0.02^{ab}	1.46 ± 0.19^{b}	2.15 ± 0.23^{c}
Lipid content (%)	19.73 ± 1.24^{a}	25.00 ± 1.00^{d}	23.54 ± 1.00^{c}	21.85 ± 1.63^{b}
Crude protein (%)	22.17 ± 1.18^{a}	24.00 ± 1.00^{b}	28.14 ± 1.20^{c}	28.38 ± 0.63^{c}
Crude fibre (%)	4.59 ± 0.97^{c}	3.03 ± 0.06^{b}	2.76 ± 0.19^{ab}	1.65 ± 0.21^{a}
Carbohydrate (%)	20.94 ± 0.97^{c}	$14.00\ 1.00^{b}$	8.94 ± 0.98^{a}	7.48 ± 1.10^{a}

Values are mean of triplicate determination ± SD. Values in the same row bearing the same letter of the alphabet are not statistically significant at ($P< 0.05$).

Table 2. Effect of fermentation on the mineral composition of *Pentaclethra macrophylla* Seed

	Fermentation period (hr)			
Parameter	0	24	48	72
Ca^{2+} (mg/100g)	0.20 ± 0.10^{a}	0.82 ± 0.21^{b}	2.08 ± 0.21^{c}	5.57 ± 0.15^{d}
Mg^{2+} (mg/100g)	0.46 ± 0.27^{a}	0.41 ± 0.09^{a}	0.63 ± 0.06^{b}	0.83 ± 0.06^{c}
P (mg/100 g)	50.43 ± 1.69^{a}	55.00 ± 1.00^{b}	60.47 ± 0.50^{c}	63.13 ± 0.81^{d}
Na^{+} (mg/100 g)	58.07 ± 2.11^{b}	57.00 ± 2.65^{b}	61.14 ± 1.79^{c}	32.78 ± 2.34^{a}
Zn^{2+} (mg/100g)	10.49 ± 1.11^{bc}	9.14 ± 1.21^{b}	11.47 ± 1.36^{c}	7.67 ± 0.58^{a}
Mn^{2+} (mg/100g)	14.23 ± 1.37^{a}	14.50 ± 1.80^{a}	16.17 ± 1.04^{b}	17.20 ± 1.06^{b}
Fe^{2+} (mg/100g	9.46 ± 0.67^{a}	9.83 ± 1.61^{a}	12.17 ± 1.76^{b}	11.20 ± 0.72^{b}
K^{+} (mg/100g)	11.47 ± 0.55^{b}	8.67 ± 0.58^{a}	9.67 ± 1.53^{a}	11.00 ± 1.00^{b}

Values are mean of triplicate determination ± SD. Values in the same row bearing the same letter of the alphabet are not statistically significant at ($P< 0.05$).

Table 3. Effect of fermentation on the anti-nutrient composition of *Pentaclethra macrophylla* Seed

	Fermentation period (hr)			
Parameter	0	24	48	72
Phytate (mg/100g)	2.00 ± 0.17^{b}	1.32 ± 0.11^{b}	0.66 ± 0.14^{a}	0.28 ± 0.09^{a}
Tannins (mg/100g)	1.16 ± 0.07^{b}	0.37 ± 0.06^{a}	0.28 ± 0.02^{a}	0.14 ± 0.06^{a}
Hydrogen cyanide (mg/100g)	1.13 ± 0.06^{c}	0.67 ± 0.12^{b}	0.30 ± 0.10^{b}	0.06 ± 0.01^{a}
Oxalate (mg/100g)	1.35 ± 0.05^{c}	0.94 ± 0.05^{b}	0.15 ± 0.06^{a}	0.10 ± 0.01^{a}

Values are mean of triplicate determination ± SD. Values in the same row bearing the same letter of the alphabet are not statistically significant at ($P< 0.05$).

4. Discussion

The nutritive value of food defines the available bioactive components, its impact on the body and the roles of nutrients in food [21,22]. The present study investigated the effect of fermentation on the nutritional composition of *Pentaclethra macrophylla*.

The effect of fermentation on proximate composition of *P. macrophylla* seed expressed in percentage (%) was shown in Table 1. There was significant difference ($p< 0.05$) in all the assayed proximate parameters when compared to the control sample at 0 h fermentation. Although, moisture content, ash content, lipid and crude protein significantly increased, while crude fibre and carbohydrate content significantly reduced with increased fermentation period in relation to the control samples. It has been shown that high moisture content aids in stabilizing the protoplasmic contents of the cell although, high moisture contents have been implicated in food spoilage due to its growth thriving attributes exhibited in microorganisms [23]. The ash content of a plant is directly proportional to the mineral elements present in such plant [22,24]. This implies the higher the ash content, the more nutritional the plant would be. From the study, ash content increased with increase in the fermentation period. Studies have shown that increase in ash content in fermented products could be attributed to the increased metabolic state of the fermenting microbes especially the fermentation originating from *Bacillus* species. This microbial species are capable of synthesizing divalent metals which are major mineral constituents [25,26].

The result of the proximate analysis (Table 1) also showed a significant increase in the values of crude lipid and crude protein as fermentation increases. Lipids are group of heterogenous organic compounds that are insoluble in water, but soluble in organic solvents. The abnormal increase in lipids especially the low density lipoproteins have been implicated in the occurrence of diseases associated with hyperlipidemia like coronary artery disease, myocardial infarction, cerebrovascular accident and hypertension [27], although nutritional lipids helps in the production of energy in a living system [28]. The present study also showed a significant increase in protein contents as fermentation increases. Reports have shown that the African oil bean seed is a good source of edible protein and high energy calories. It contains twenty essential amino acids and essential fatty acids [29]. However, protein is important for growth in young ones, formation of enzymes, hormones, repair of worn out tissues, egg and milk production [22,28]. This observable increase in protein as fermentation period increases could be attributed to the action of extracellular enzymic reactions and the production activity elicited by the fermenting microorganism. Fogarty and Griffin [30] have reported the biological role of *Bacillus* species in the fermentation of *P. macrophylla* and are important producers of enzymes that cleave proteins into short peptide chains and amino acids. This enzyme is called proteases. This cleavage activity exhibited by protease can increase the total nitrogen content of the plant.

In the study, crude fibre and carbohydrate contents significantly reduced with increased fermentation period. Ofuga and Nwajiba [31] reported an over 35% loss of cellulose, a crucial component of crude fibre, on the study of solid state fermentation of cassava peel with *Rhizopus sp*. and *Aspergillus niger* grown on rice straw. The observed decrease in the values of crude fibre may be due to the action of cellulolytic microorganism present in the fermenting substrate. Despite the decrease in the values recorded in crude fibre and carbohydrate when the fermented samples were compared to the control, they still have major roles to play when absorbed in the body. For example; dietary fibre helps in the maintenance of bowel movement and can prevent diverticulosis by aiding the absorption of trace elements in the guts [32], while carbohydrate in our diet improves the following deficiencies; poor mental function, fatigue, endurance and lack of stamina [22,33].

Table 2 showed effect of fermentation on the mineral composition of *Pentaclethra macrophylla* Seed expressed in mg/100g. Nutritionally, minerals are very important in human system for diverse metabolic activities and are required for basic body functions such as heart beat, muscle contractions, movement, growth and regulatory processes [34, 22]. The assayed minerals in the present study include; calcium (Ca^{2+}), magnesium (Mg^{2+}), phosphorous (P), sodium (Na^{+}), zinc (Zn^{2+}), manganese (Mn^{2+}), iron (Fe^{2+}) and potassium (K^{+}). Although, there was statistical difference ($p< 0.05$) observed in all the tested minerals, but all significantly increased in values with the exceptions of Mg^{2+} (24 h fermentation), Zn^{2+} (24 h and 72 h fermentation periods) and K^{+} when compared to the control. The tested minerals are nutritionally sound and play some unique roles in a living system. Calcium and phosphorous have been implicated in growth and maintenance of bones, teeth and muscles [35]. Potassium helps in the regulation of heart beat and muscle functioning [36]. Magnesium is known to prevent cardiomyopathy, muscle degeneration, growth retardation, alopecia, dermatitis, immunologic dysfunction, gonadal atrophy, impaired spermatogenesis, congenital malfunctions and bleeding disorders [37]. Iron plays numerous biochemical roles in the body including oxygen binding in hemoglobin and acting as an important catalytic centre in many enzymes such as the cytochrome oxidase [38]. Zinc is required for collagen formation and the synthesis of protein. It is an essential micronutrient for human growth and immune functions and also functions as part of enzymes and as co-factors. Its deficiency results in a decreased sense of smell and taste, thin brittle nails, hair loss, high cholesterol, increased susceptibility to illness and infection, recurring colds and slow healing [39]. All these minerals could as well elicit and trigger the above functions when African oil bean is consumed within the tested fermented periods.

Antinutritional factors are chemical substances produced by plants that have the potentials of affecting the availability of nutrients by interfering with metabolic processes [40]. They are generally reported to possess growth retarding capacity, lowering of digestibility and absorption of important dietary nutrients [29]. Fermentation of the African oil bean seed helps to remove the anti-nutritional factors as well as improve its nutrient bioavailability and digestibility [29]. The results obtained from the effect of fermentation on anti-nutrient composition

of *Pentaclethra macrophylla* Seeds expressed in mg/100 g are presented Table 3. From the result, there was significant decrease ($p< 0.05$) in the values obtained as fermentation period increases. It has been reported that phytate and oxalates have the ability to form chelates with di-and trivalent metallic ions such as Cd, Mg, Zn and Fe to form poorly soluble compounds that are not readily absorbed from the gastrointestinal tract thus decreasing their bioavailability [22,41]. The decrease in the value of oxalate in the fermented *P. macrophylla* may not create renal disorder which is associated with consumption of diet high in oxalate [42]. Also, tannins to some extent, bind to proteins, thereby making them unavailable to the body [29]. Toxicity of cyanogenic glycoside shows symptoms of diarrhea, vomiting, heart failure and could cause metabolic acidosis due to its inhibitory action on electron transport chain pathway in human system. It has been reported that during food processing such as soaking, boiling or frying, hydrogen cyanide content with other antinutrients are reduced as seen in fermented cassava [43]. However, the decrease in the values of the assessed antinutrients (phytate, tannins, hydrogen cyanide and oxalate) as seen in our findings confirms the acclaimed assertion that hydrothermal treatment, soaking and fermentation would significantly reduce the levels of the antinutritional factors found in plants.

5. Conclusion

The present study has therefore shown that increase in fermentation period increases nutritional composition and decreases antinutritrional factors in *P. macrophylla* seeds.

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