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PROTEIN AND MINERAL PROFILE IN FERMENTED AFRICAN OIL BEAN (*PANTACLETHRA MACROPHYLLA BENTH*) SEED

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Abstract: The study aimed at evaluating the protein and mineral profile in fermented African oil bean (*Pantaclethra macrophylla Benth*) seed. Total protein, free amino acid profile, protein fractions and total mineral content were monitored in the fermenting seed using standard analytical procedures. The total protein content decreased as the fermentation progressed from 45.33 ± 9.24 to 13.30 ± 4.62 mg/100g while free amino acid profile increased from 5.00 ± 3.46 to 22.6 ± 2.31 mg/100g. Glutellin levels increased as the fermentation progressed from 50.6 ± 18.48 to 106.6 ± 4.62 mg/100g while decrease in levels of albumin, globulin and prolamin were observed. The mineral and amino acid content were higher than the recommended daily allowance. This study showed that fermented *Panthaclethra macropylla Benth* seed may be a good source of amino acid and minerals.

Keywords*: Fermentation, Free amino acids, Mineral, Total protein, Seeds*

1. INTRODUCTION

Fermented African Oil bean seed (*Panthaclethra macrophylla benth*) known as Ugba is an indigenous fermented food commonly consumed in the eastern part of Nigeria. It is rich in protein and minerals and can be processed by solid-state fermentation. African oil bean seed is native to tropical regions of Africa. This seed plant which is also serves as shade tree has been cultivated as a commercial crop as early as 1937 in the eastern part of Nigeria. There are about an average of eight seeds in its flattened pod and this explodes as it becomes ripen as such dispersing the dispersing the seeds. The unfermented seeds taste bitter and contain toxic alkaloids and saponins. Fermentation eliminates the bitter taste. *Panthaclethra macrophylla benth* commonly serves as food, salt substitute, and source of edible oil, seed craft, dye, fencing and paling, charcoal and craving bowls. It contains up to 44% protein, which comprise of at least 17 of the 20 amino acids, amongst which are all the essential amino acids. It also contains minerals such as Mg, Fe, P, Ca, Mg, Zn, Na and K.

The natural fermentation of the seed which at present is still done at household level renders it nutritious and palatable. This enhances better nutrient availability and digestibility with significant softening of the cotyledons. Ugba serves as a popular delicacy among the Igbo's and some other ethnic group in Nigeria. It is served as snacks, side-dish and condiment in soup and local porridges.

Several works have been done on fermented African bean seed, such as the use of starter culture (*Bacillus* spp) and improved packaging technique to produce Ugba. This resulted in reduced fermentation time and extended shell life. Thermal processing technique increase the nutrient bioavailability, digestibility and functionality African oil bean seeds. However, the present study was carried out to determine the changes in the free amino acid profile, total protein and some protein fractions of fermenting Ugba seed as the fermentation progressed for the period of five days. Total mineral content and individual amino acid profile were also estimated.

2. Materials and Methods

2.1 Raw materials: About 1.5kg of brown edible African oil bean seed was obtained from Oyingbo market in Lagos, Lagos State, Nigeria.

2.2 Preparation of Ugba: The seeds used in this study were boiled for 3 hours, cooled and dehulled. The cooled seeds were then sliced (0.5 – 1 mm thickness) and boiled again for another 2 hours, drained, rinsed thrice in water and then steeped in cool water for 4 hours so as to eliminate the bitter taste. The sliced beans were then wrapped with banana leaves (*Musa sapientum linn*) and packed in clean container to ferment at room temperature for 5 days.

2.3 Chemical Analysis

Protein profile: 2g of the fermented bean seed sample was assayed for total free amino acids using the Ninhydrin method as described by Rosen and serine as the standard, the absorbance was taken at 420nm. This was measured as the fermentation progressed from day 0 – 5. 2g of the ground sample was assayed for total protein using the new improved Biuret reagent method as described by Schück. The total Protein was finally estimated using albumin as the standard at an absorbance of 545 nm. This was also measured as the fermentation progressed from days 0 – 5 daily.

2.4 Protein fractions

0.5g of the defatted sample was used to determine the protein fractions (albumin, prolamin, globulin and glutellin) according to method described by Aguirre *et al.*

2.5 Amino acids

Individual amino acids were determined according to the method described by Spackman *et al*. The fermented sample were dried at 40ºC, defatted, hydrolysed and evaporated in a rotary evaporated and then loaded into Technicon Sequential Multi Sample amino acid analyzer (TSM). To the TSM analyzer, 10 µl was loaded for 72 minutes. The net height of each peak produced by the chart record of the TSM was measured. The approximate area of each peak was then obtained by multiplying the height with the width of half-height.

2.6 Mineral content: The mineral content of the fermenting African Oil bean seed was determined at the end of the fermentation period using the procedure described by Association of Official Analytical Chemists (A.O.A.C) 0.5g of the finely ground sample was assayed for K, Ca, Mg, Mn, Na, Fe, Pb and Zn.

3. Results and Discussion

The fermentation process of the *Panthaclethra macrophylla benth* seed shows the variation in free amino acids and total protein of the sample as the fermentation progressed are presented in Figure 1. The free amino acids increased significantly ($p<0.05$). The total protein level decreased although were not significantly ($p>0.05$) different. This indicates that there is an inverse relationship between free amino acid and total protein content with increase in fermentation time. The increase in percentage of free amino acid content may be due to the hydrolysis of the seed protein (decreased protein) by microbial enzymes from the fermenting organism. However, decrease in protein content may in part result from leaching of soluble proteins into the processing medium during ugba production. Nutrient availability as well as digestibility of several foods and food products may be triggered by fermentation.

The protein fractions albumin and globulin significantly decreased ($p<0.05$) during the first three days; Prolamin progressively decreased as the fermentation continued for five days whereas Glutellin significantly ($p<0.05$) increased on day 3 although an insignificant ($p<0.05$) increase was observed from day 3 to day 5 (Table 1). The protein fractions albumin and globulin decreased progressively from day 0-3 and then from day 4-5, an increase in both fractions were observed. This does not agree with the study of Giami, who reported an initial increase in these fractions (albumin and globulin), reaching their maximum levels on the 5th day, but declined thereafter. This may be attributed to the effect of protease activity. The levels of prolamin decreased as the fermentation progressed whereas glutellin level rise progressively (Table 2), indicating increased hydrolysis of protein due to an increase in protease activity.

The amino acid profile of the fermented "*Ugba*" seed in this study shows that glutamate, aspartate and leucine are the three most abundant amino acids in the seed. This observation closely agrees with that reported for egusi melon seed by Olaofe *et al*. Olaofe and Akintayo, Adeyeye, Aremu *et al*., and Godwin, except that in this study, in place of arginine that was reported by these authors, leucine was predominant and also agrees with Onuekwusi *et al*., [24] for *ackee* seed. In addition, this result shows that ugba seeds contain essential amino acids such as leucine, isoleucine, lysine, phenylalanine, valine, arginine and threonine. Among the essential amino acid obtained in this study, leucine was present in the highest amount in the fermented ugba seed (6.19 g/100 g protein). This value obtained for leucine compared very well with the values reported for *ackee* seed (6.94 g/100 g protein), apple, water melon, guava, and paprika seeds which are in the range of (5.61 – 6.72 $g100g^{-1}$), but higher than that of apricot and orange seeds in the range (3.45 – 3.94 g/100 g protein). Leucine has a function of protecting muscles as well enhancing bone, skin, and muscle tissue healing and are recommended for those recovering from surgery. Sulphur containing amino acids have been reported to be the limiting amino acids in legumes. Fermented ugba seed contained moderate amounts of methionine (0.77) which is lower in comparison with that of cashew nut (1.7 g/100 g protein) and also lower than the reported value for papaya, apple, water melon, guava, orange, apricot and paprika in the range (0.92 – 4.09 g/100 g protein) but higher than that of prickly pear. Cystine content (1.68 g/100 g protein) was higher than the values reported by Samia El-Safy *et al*., [25] for the different seeds in the range (1.01 – 1.44 g/100 g protein) but lower than that of guava and paprika seeds.

The amino acid contents showed glutamic acid to be highest (16.73mg/100g) while methionine had the least concentration (0.77mg/100g) (Table 3). Glutamic acid is important in the metabolism of sugars and fats as well as assist in transporting potassium through the blood-brain barrier. The brain can utilize glutamic acid as fuel. It can as well detoxify ammonia by picking up nitrogen atoms, in this wise the process creating another amino acid, glutamine. The conversion of glutamic acid into glutamine is one of the major way of ammonia detoxification in the brain. Glutamic acid also plays a vital role in the treatment of certain childhood behavioural disorders.

The nutritive value of a protein depends primarily on the capacity to satisfy the needs for nitrogen and essential amino acids. The total essential amino acids (with histidine) of the fermented ugba seed protein (27.19g/100 g protein) shown in Table 5, compares favourably with that reported for papaya, apple, water melon, guava, prickly pear and apricot seeds in the range of (26.17 – 29.49

g/100 g protein) and is greater than that of orange (18.3 g/100 g protein) but lower than that of paprika (34.14 g/100 g protein). The total acidic amino acid (TAAA) of the fermented ugba seed protein (32.43 g/100 protein) was greater than the total basic amino acid (TBAA) (13.28 g/100 g protein), implying that the fermented ugba seed protein is probably acidic in nature.

The total sulphur amino acid (TSAA) of the fermented ugba seed protein (2.45 g/100 g protein), which is about two-third the value (5.8 g/100 g protein) recommended for infants. The aromatic amino acid (ArAA) for the current report (Table 4) was within the range suggested for ideal infant protein (6.8-11.8 g/100 g protein) indicating that fermented ugba seed could be used to prepare gruel as weaning food. The percentage ratio of EAA to TAA in the fermented ugba seed was 31.61. This value is below the 39% considered to be adequate for ideal protein food for infants, but above the 26% for children and 11% for adults. Hence, the amino acid profile of the studied plant seed suggests that its protein has moderate nutritive value.

The result obtained for the mineral composition of fermenting ugba seed as presented in Table 2 shows calcium as the most predominant mineral in the seed while the lowest was manganese. This contrast with the observation of Olaofe and Sanni who on different studies reported potassium as the most abundant mineral in Nigerian agricultural products. However, potassium is fourth in concentration after magnesium and sodium in the fermenting ugba seed in this study. The Na^+ / K^+ ratio of the fermenting ugba seed is more than one. Thus, on the basis of the recommendation of Nieman *et al.* (1992), this suggests that the fermenting ugba seed would not be suitable for reducing high blood pressure. Calcium is always found in association with phosphorus in the body, both contributing to the formation of blood and supportive structure of the body. The mineral content especially calcium obtained for the fermented ugba seeds imply that consumption of the seed could help to ameliorate metabolic bone disease.

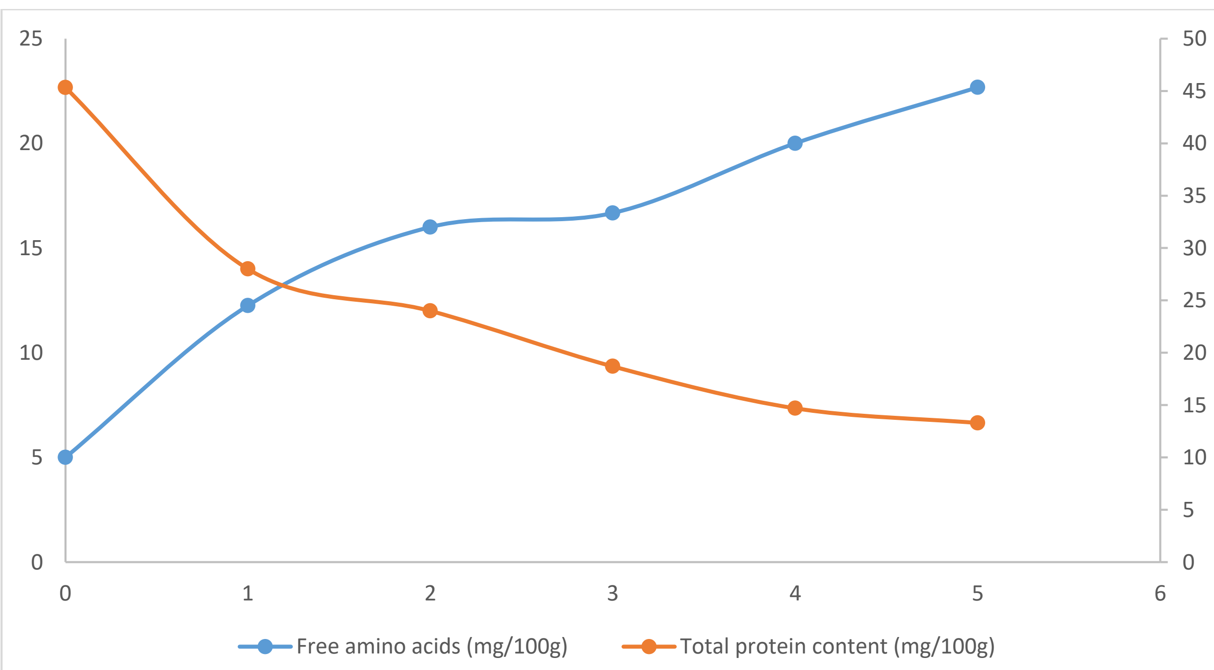

Figure 1: Variation of Free Amino Acids and Total Protein in Fermenting African Oil Bean

Table 1: Variation of Protein Fractions in Fermenting African Oil Bean Seed

Days	Albumins (mg/100g)	Globulins (mg/100g)	Prolamins (mg/100g)	Glutellins (mg/100g)
0	45. 33 ± 9.24^{c}	33.33 ± 6.11^{c}	34.67 ± 6.11^{c}	50.67 ± 18.48^{a}
1	26.67 ± 3.33^{b}	20.00 ± 4.00^{b}	25.33 ± 4.62^{b}	38.00 ± 2.31^{a}
2	20.00 ± 4.00^{a}	17.31 ± 2.31^{b}	24.00 ± 4.00^{ab}	68.00 ± 4.00^{b}
3	17.33 ± 6.93^{a}	10.67 ± 2.31^{a}	21.33 ± 4.62^{a}	102.67 ± 2.31^{c}
4	20.00 ± 6.93^{a}	22.67 ± 4.62^{bc}	20.00 ± 4.00^{a}	105.33 ± 6.11^{c}
5	25.33 ± 8.33^{a}	29.33 ± 8.33^{c}	17.33 ± 4.62^{a}	106.67 ± 4.62^{c}

Each value is a mean ± SD of three replications. Values with different alphabet(s) along a column are significantly ($p<0.05$) different.

Table 2: Mineral Content of Fermented African Oil Bean Seed at the End of Fermentation

Mineral Element	Concentration (mg/100g)
Fe	6.90
Mn	0.90
Zn	1.32
Pb	1.00
Ca	450.00
Mg	230.00
Na	190.00
K	160.00

Table 3: Amino Acid Content of Two Day Fermenting African Oil Bean Seed (Defatted)

Amino acid	Concentration (g/100g)
Lysine	6.02
Histidine	1.58
Arginine	5.68
Aspartic acid	15.70
Threonine	2.38
Serine	4.02
Glutamic acid	16.73
Proline	4.04
Glycine	3.13
Alanine	4.96
Cystine	1.68
Valine	4.02
Methionine	0.77
Isoleucine	2.53
Leucine	6.19
Tyrosine	2.90
Phenylalanine	3.70

Table 4: Classification of amino acid composition (g/100g crude protein) of fermented Ugba Seeds

Classification	Fermented *Panthaclethra macrophylla* seed	Fermented *Panthaclethra macrophylla* seed (%)
TAA	86.03	---
TEAA: TNEAA	0.46	---
TSAA: TNSAA	0.03	---
TArAA: TNArAA	0.08	---
TEAA without Histidine	25.61	29.77
TEAA with Histidine	27.19	31.61
TNEAA	58.84	68.39
TSAA	2.45	2.85
TNSAA	83.58	97.15
TArAA	6.60	7.67
TNArAA	79.43	92.33
TAAA	32.43	37.70
TBAA	13.28	15.44

TAA – total amino acid, TEAA – total essential amino acid,
TNEAA – total non-essential amino acid, TSAA – total sulphur amino acid
TNSAA – total non-sulphur amino acid, TArAA – total aromatic amino acid
TNArAA – total non-aromatic amino acid, TBAA – total basic amino acid
TAAA – total acidic amino acid

4. CONCLUSION

Therefore, fermented African bean seed (Ugba) can serve as a cheap alternative source of amino acids and mineral for food supplements, flavour development, pet feed as well as nutriceuticals.

Conflict of Interest

No Conflict of interest exist between the authors

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