International Journal of Basic Science and Technology
March, Volume 8, Number 1, Pages 31 - 41

Different Packing Materials and their Effects on The Quality of Fermented African Oil Bean Seeds

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Article Information

Article # 08005
Received: 9th March 2022
1st revision: 4th April 2022.
2nd revision: 9th April 2022.
Acceptance:11th April 2022
Available on line:15th April 2022

Key Words
African oil bean Proximate, Mineral, Vitamin, Shelf-Life, Microbiological Quality

Abstract

Different packing materials and their effects on the quality of fermented African oil bean (*Pentaclethra macrophylla*, *Benth*) seeds was studied. Five (5) samples were generated by wrapping the sliced ugba seeds in *Newbouldia laevis*, *Mallotus oppositifolius Mull*, cocoyam (*Xanthosoma sagittifolium*), plantain (*Musa paradisiaca*) and *Alchornea lavifora*, *Benth* leaves. The samples were fermented for five days. Evaluation of the proximate, mineral, vitamin composition, microbial count and organoleptic attributes of the samples were carried out using; AOAC standard, Gen-Way spectrophotometer at 360nm, nutrient and macconkey agar and a multiple comparison test in a 9 hedonic scale. The proximate analysis revealed moisture (50.19% - 51.85%), protein (9.13% - 10.37%), fat (11.06% - 12.76%), ash (2.15% - 2.60%), fibre (2.16% - 2.32%) and carbohydrate (20.31% - 25.28%). The minerals included magnesium (120.17mg/100g – 147.11 mg/100), iron (33.26mg/100g -39.61mg/100g), potassium (83.74mg/100g – 85.09mg/100g), calcium (117.2mg/100g – 118.0mg/100g), and phosphorus (103.69mg/100g – 104.05mg/100g). Significant ($p<0.05$) differences were observed in the mineral contents of the samples. The vitamins analyzed showed values in the range of 0.05mg/100g – 0.06mg/100g (vitamin B1), 0.25mg/100g – 0.33mg/100g (vitamin B2) and 0.25mg/100g – 0.32mg/100g (vitamin B3). There is no significant ($p>0.05$) difference in the vitamin concentrations of the samples. The microbial analysis revealed the presence of bacterial isolates such as *Bacillus* species, *Streptococcus* species, *Staphylococcus* species and *Pseudomonas* species, attributed to the leaves. Except *staphylococcus* species, the total viable counts of these bacteria were found to increase with increase in fermentation period but not above the acceptable limit (7.0×10^6). The *P. macrophylla, Benth* sample wrapped in *Mallotus oppositifolius Mull* leaf was most organoleptically preferred by the judges. Generally, other leaves are good for packaging

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Introduction

African oil bean seed (*Pentaclethra macrophylla, Benth*) is one of the many tropical underutilized crops that are recently attracting worldwide attention because of its high nutritive value. It is a native plant in the tropical region of Africa and grows abundantly in rain forests. The African oil bean is a popular traditional food condiment among the South Eastern tribes (Akpabio *et al*., 2012). The oil bean seed is found in the fruit of the bean tree. The fruit is black, hard, and with a woody pod. When it is mature, it splits open, revealing up to 8 flat brown and shiny seeds. (Mohapatia *et al*., 2010). African oil bean seed is an excellent source of energy, protein, amino acids, phosphorus, magnesium, iron, vitamins, calcium, manganese and copper. It is also an excellent source of phytonutrients such as tannins, alkaloids, sterols, glycosides and saponins. (Ogbule, 2018). It is said to be high in potassium, zinc, and nicotinic acid but low in trypsin inhibitor compared to other edible materials (Anyanwu *et al*., 2016). It can be processed through fermentation and wrapped with different leaves which may include cocoyam leaves, plantain leaves, banana leaves etc. Ugba is the Igbo name for the fermented African oil bean seed. It is called Ukanna by the Efiks in Southen Nigeria. It is consumed by an estimated 15 million people in eastern Nigeria,the majority of who are Ibo. It is a traditional food generally prepared in homes as a small family business. The method of production varies from one producer to another resulting in a non-uniform product (Ogueke *et al.*, 2010). The fermented (three days plus) are taken as a delicacy together with other food combinations. It is an important cheap source of protein. Studies on African fermented foods have focused on the isolation and identification of desirable micro-organisms involved in the fermentation process but not their food quality. Ugba has a short shelf-life arising from

ISSN 2488-8648
http://www.ijbst.fuotuoke.edu.ng/

uncontrollable fermentation which occurs after the normal production period. Thus, it's vulnerable to contamination by pathogenic microorganisms during production and storage. The traditional leaves so far used to wrap ugba do not offer prolonged preservation which deteriorates after a short run alongside the oil bean, leading to high post-harvest loss that discourages large-scale production (Ayodele, 2013). Exportation of ugba is extremely hampered by the short shelf-life. Also, the preservative/protective effect of many other leaves like cocoyam, plantain, ororompo, ogirishi and okpopia leaves on ugba is not widely known. Only one leaf is popularly known as ugba wrapping leaf and is going extinct or scarce due to excess usage. This contributes to the increased cost. Also, the uncontrolled and rapid fermentation in the package leads to postharvest losses if the product is not consumed within a short period. So using different packaging materials will help us discover one in which fermentation can take place, the rate of the fermentation, and quality changes if any in the products. Thus, this study aims to focus on the effects of various packaging materials on the food quality of African oil bean seeds.

Materials and Methods

Whole and viable African oil bean seeds were sourced from Eke – ukwu market, Owerri, while the five (5) different packaging leaves; Plantain leaves, Cocoyam Leaves, *Newbouldia laevis*, *Alchornealaxiflora*, *Mallotusoppositifolius Mull* were collected and identified at Imo Agricultural Development Program (Imo ADP). The African oil bean seeds were produced using the traditional methods according to Njoku and Okemadu (1989). The raw African oil bean seeds were washed with clean water and then put in a pot covered with water and boiled for 45 minutes. After boiling, the seeds were removed and dehulled while hot. After dehulling, a local metal (nkwoo) designed to slice ugba was used to slice the seeds. The sliced seeds were cut into desired sizes with a sterilized sharp kitchen knife. Then the slices were poured into a covered pot of boiling water for 30 minutes. The boiled slices were poured into a sterile stainless sieve to drain off the hot liquor and cool. The slices were wrapped using five (5) different traditional packaging leaves and allowed to ferment. The fermented samples were subjected to comparable indices of taste, texture, aroma, appearance, microbial identification, and nutrients.

AFRICAN OIL BEAN SEED

↓
Sorting
↓
Washing
↓
First boiling (45min)
↓
Dehulling
↓
Slicing
↓
Second boiling (30min)
↓
Washing and Draining
↓
Soaking (24h)
↓
Warm conditioning (5h)
↓
Wrapping
↓
Perforation
↓
Fermentation
↓
Packaging
↓
UGBA

Proximate Analyses: The proximate analyses were carried out according to the standard procedures of AOAC (2010).

Determination of mineral compositions; Five grams (5g) of each ugba sample was heated gently over a Bunsen burner flame until most of the organic matter is destroyed. This is further heated strongly in a muffle furnace for several hours until white-grey ash is obtained. The ash material is cooled. About 20ml of distilled water and 10ml of hydrochloric acid was added to the ashed material. This mixture was boiled, filtered into a 250ml volume trick flask, cooled and made up to volume. The mineral content of each sample was analyzed using the spectrophotometric method. Samples were analyzed for potassium (K), calcium (Ca), iron (Fe), magnesium (Mg), and phosphorus (P). The concentration of each mineral was calculated as follows

$$\frac{\text{The Absorbance of sample} \times \text{Concentration of standard solution} \times \text{Dilution factor}}{\text{The Absorbance of standard solution} \times \text{Sample Volume}}$$

Determination of Vitamins; Determination of vitamin B1 (Thiamine); Thiamin content was determined using the spectrophotometric method described by AOAC (2010). A weighed portion of each ugba sample (2g) was extracted by homogenizing it in 50ml molar ethanol sodium hydroxide solution. The homogenate is filtered to obtain the extract in 100ml flask. Ten (10) milliliters of the filtrate is pipetted and the colour is developed by the addition of 10ml of potassium dichromate and the wavelength is read at 360nm with a Gen-Way spectrophotometer at 360nm. A standard solution of thiamine was prepared and treated the same way as the sample. The thiamine content was calculated as shown;

$$\text{Thiamine (mg/100g)} = \frac{100}{W} \times \frac{AU}{AS} \times C$$

Where; W = Weight of ugba sample analyzed, AU =Absorbance of the sample, AS = Absorbance of the standard, and C = Concentration of the standard.

Determination of riboflavin (vitaminB_2);Riboflavin content of ugba was determined by the method described by AOAC (2010). Two (2) grams of ugba sample were extracted with 100ml of 50% ethanol solution and shaken for one hour. The suspension is filtered into 100ml flask. A measured volume (10ml) of 30% hydrogen peroxide was added and allowed to stand for about 30 minutes. A 2ml of 41% sodium sulphide solution was added which will result in the formation of a yellowish pale colour. The absorbance was measured in a Gen-Way spectrophotometer at 500nm wavelength. Riboflavin (Vit.B_2) was calculated in mg/100g using the formula below.

$$\text{Riboflavin (mg/100)} = \frac{AV}{AS} \times \frac{C}{1} \times \frac{100}{1}$$

Where; AV = Absorbance of ugba sample, AS = Absorbance of standard and C = Concentration of Standard vitamin.

Determination of niacin (VitaminB3); The method described by AOAC (2010) was used to determine vitaminB3content of the ugba samples. Five (5) grams of the ugba sample were treated with 50ml of 1N sulphuric acid and shaken for 30minutes. Three (3) drops of ammonia solution were added to the sample and filtered. Ten (10) milliliters of the suspension were pipetted into a 50ml volumetric flask and 5ml of $0.02NH_2SO_4$ and absorbance was measured in the spectrophotometer at 47nm wavelength. This is used to plot the calibration curve. The thiamine riboflavin and niacin content can be calculated thus;

$$\text{\% thiamin, riboflavin or niacin} = \frac{AU}{AS} \times C \; \frac{100}{1} \times \frac{V_f}{V_a}$$

Where; AU = Absorbance of test sample, AS = Absorbance of the standard, C = Concentration of the standard, Vf = Volume of total extract, W = Weight of sample, and Va = Volume of extract analyzed.

Microbiological Analysis; One millilitre of each sampled ugba was put in 9ml of sterile distilled water in sterile test tubes, shaken and then serially diluted. From the appropriate dilution, 0.1ml was inoculated separately onto Nutrient agar and MacConkey agar plates and spread evenly using a sterile bent glass rod. Each experiment was carried out in triplicates to get a mean standard value of the colony-forming units (cfu/ml) on the plates. The inoculated Nutrient agar and MacConkey agar plates were incubated at 30ºC and 35ºC for 24 hours. After the period of incubation, the colonies on the plates were counted and recorded as colony-forming units per millilitre (cfu/ml) and coliform respectively. Each of the bacteria colonies on the agar plates was sub-cultured and the pure culture was obtained. Isolates were identified by carrying out tests which include Gram staining, spore staining and biochemical tests such as catalase, coagulase, and oxidase, citrate utilization, indole, methyl red, urease, Voges Proskauer and sugar fermentation.

Sensory evaluation of the ugba samples; sensory evaluation was carried out using multiple comparison tests. The samples of ugba were served to 30 semi-trained panellists made up of staff and students of Imo State University, Owerri who are familiar with the sensory attributes (taste, aroma, flavour, color, mouth feel, etc.). They were respectively provided with water to rinse their mouth after testing each sample and were instructed to evaluate; appearance, taste, aroma, mouth feels and general acceptability using a 9 hedonic scale. Where:1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely.

Statistical Analysis; Data generated in this research was subjected to analysis of variance (ANOVA)). Tukey's test was used to separate means and the differences between the means were considered to be significant at P < 0.05.

Results and Discussion

TABLE 1: Proximate composition of fermented african oil bean seed rapped with different leaves

PARAMETERS SAMPLES	Moisture Content	Protein	Fat (%)	Ash	Fibre	CHO
A	$51.85^{a} \pm 0.05$	$10.37^{a} \pm 0.04$	$12.76^{a} \pm 0.03$	$2.55^{a} \pm 0.05$	$2.16^{b} \pm 0.06$	$20.31^{d} \pm 0.19$
B	$50.75^{b} \pm 0.07$	$9.97^{c} \pm 0.03$	$12.34^{b} \pm 0.04$	$2.34^{b} \pm 0.02$	$2.28^{a} \pm 0.02$	$22.32^{c} \pm 0.02$
C	$50.19^{c} \pm 0.09$	$9.13^{e} \pm 0.03$	$11.06^{e} \pm 0.04$	$2.15^{c} \pm 0.06$	$2.16^{b} \pm 0.05$	$25.28^{a} \pm 0.10$
D	$50.26^{c} \pm 0.06$	$9.55^{d} \pm 0.03$	$12.14^{c} \pm 0.05$	$2.48^{ab} \pm 0.03$	$2.30^{a} \pm 0.03$	$23.17^{b} \pm 0.05$
E	$50.73^{a} \pm 0.07$	$10.23^{b} \pm 0.04$	$11.68^{d} \pm 0.03$	$2.60^{a} \pm 0.03$	$2.32^{a} \pm 0.06$	$22.44^{c} \pm 0.02$
LSD	0.09	0.08	0.15	0.15	0.10	0.30

Means in the same column with the superscripts are not significantly different (p>0.05) and those with different superscripts are significantly different (p>0.05) from each other. SAMPLE: A = Ugba wrapped in Ogirishi leaves, B = Ugba wrapped in Ororompo leaves, C = Ugba wrapped in cocoyam leaves, D = Ugba wrapped in plantain leaves, E = Ugba wrapped in Okpopia leaves.

Proximate Composition of Fermented African Oil Bean Seed

Moisture content; The moisture content of the ugba samples as depicted in Table 1 ranges from 50.19 to 51.85%. Sample A (ugba wrapped in ogirishi leaves) has the highest moisture content (51.85±0.05%) followed by samples B (50.75%), E (50.73%), D (50.26%) while sample C (50.19%) had the least. There were significant (p<0.05) differences between sample A and other samples moisture-wise. Kabuo *et al.* (2015) stated that different food wrapping materials have different capacities for absorbing water. The lowest mean value of 50.19% recorded by sample wrapped in cocoyam leaves (sample C) could therefore mean that cocoyam leave can absorb moisture better than the other leaves used as wrapping material. This lowest mean value of 50.19% obtained by sample C (ugba wrapped in cocoyam leaves) shows the certainty of prolonging shelf life as it is known that moisture and water activities of any food product determine greatly the keeping quality of such food product.

The Protein content; Values of the protein content of the fermented African oil bean seeds (ugba) were 10.37±0.04%, 9.97±0.03%, 9.13±0.03% and 10.23±0.04% for sample A, B, C, D and E respectively. The protein content of these samples exhibited significant (p<0.05) differences from each other. Ugba sample wrapped in ogirishi leaves (N. *laevis*) has the highest mean value (10.37±0.04%) followed by the sample wrapped in okpopia leaves (*Alchornaelaxi flora*) with 10.32% while the sample wrapped in cocoyam leaves (*Colocasia spp*) has the lowest mean value of 9.13±0.03% of protein content. The percentage of protein content obtained from this research work was found to be higher than 6.77% to 8.59% protein content reported by Kabuo *et al*. (2015). This means that the leaves did not adversely affect the protein content of the samples. Proteins perform a vast array of functions within organizations including catalyzing metabolic reactions, DNA replication, responding to stimuli, providing structure to cells and organisms, and transporting molecules from one location to another (Wikipedia, 2021).

The Fat content; Percentage fat content of the ugba samples wrapped in different packaging materials ranges from 11.06±0.04% to 12.76±0.06%. Sample A (ugba wrapped in ogirishi leaves) has the highest value (12.76%), followed by the sample wrapped in ororompo (sample B) with 12.34% of fat while the lowest mean value (11.96%) of fat was obtained in sample wrapped in cocoyam leaves (sample C). All the values obtained for samples A, B, C, D and E had significant (p<0.05) differences from each other. The value (12%) reported for ugba wrapped in cocoyam and plantain leaves by Kabuo *et al*. (2015) closely corresponds wi*th values obtained for fat in the present work. Fat plays a significant role in the shelf life of food products. This is because fat can promote rancidity in foods leading to the development of unpleasant and odourous compounds (Mbata and Orji, 2007). Thus, sample A (ugba wrapped in ogirishi leaves) with the highest fat and moisture content could be more prone to deterioration in comparison to other samples.

Ash content; Percentage ash content of the ugba samples ranged from 2.15+0.06 to 2.60+0.03%. The cocoyam leaf-wrapped sample (sample C) has the lowest mean value (2.15%) and was found significantly ($p<0.05$) different from other samples. Sample A (2.55%), D(2.48%), E(2.60%), sample B (2.34%) and sample D (2.48%) did not show significant differences ($p>0.05$) in their ash content. Okereke and Onukwo, (2014) reported ash content within the range of 0.25 – 0.65% and were found very much lower than values obtained from this research work, while Kabuo *et al*. (2015) reported values (13 - 33%) higher than the ones obtained from this study. The low mean value for ash recorded in this research work could be attributed to fermentation processes because Tho B. (2004) explained that fermentation significantly reduced the ash content of African oil bean seeds. Ojewumi (2016) reported that during fermentation, some essential salts are being utilized by microorganisms for their metabolic activities which may result in reduction in ash content.

Fibre content; Fibre content values range from 2.16+0.05% to 2.23+0.06% with the ugba sample wrapped with okpopia leaves (sample E) having the highest value while the sample wrapped with ogirishi and cocoyam (samples A and C) has the lowest mean value. There was no significant ($p<0.05$) difference between samples A (2.16%) and samples B (2.28%), D (2.30%) and E (2.32%). Nwachukwu *et al*. (2018) reported a mean value of 3.22% for unfermented ugba and within the ranges of 2.62% to 3.05% for fermented ugba samples, which implies that fermentation reduces the crude fibre content of African oil bean seeds (Ugba). Too much reduction in fibre should not be encouraged because the fecal bulking action of insoluble fibre makes it useful in the treatment of constipation and diverticular disease (Mohammed *et al*., 2020). Fibre also helps in fighting against cancer and related diseases.

Carbohydrate content of ugba samples; mean values from the carbohydrate content analysis of ugba samples packaged with different materials were presented in Table 1 as 20.31+0.19, 22.32+0.02%, 25.28+0.10%, 23.17+0.05% and 22.44+0.02% for samples A, B, C, D, and E respectively. The samples have a least significant difference of 0.30 which revealed that there was no significant difference ($p<0.05$) between samples B and E. However the results show that there were significant ($p<0.05$) differences between samples A, C and D. Kabuo *et al*. (2015) recorded carbohydrate content in the range of 35.23% to 44.57% for ugba wrapped in different leaf-type and their mean values were higher than those obtained from this present research work. The results imply that ugba could be a good source of calories if consumed.

Table 2: Mineral composition of fermented oil bean seeds wrapped with different leaves

Parameters samples	Magnesium	Iron	Potassium (mg/100g)	Calcium	Phosphorus
A	$144.06^{b} \pm 0.03$	$33.29^{e} \pm 0.06$	$83.74^{d} \pm 0.02$	$117.21^{d} \pm 0.03$	$103.75^{c} \pm 0.04$
B	$138.28^{c} \pm 0.03$	$35.45^{c} \pm 0.08$	$84.47^{c} \pm 0.07$	$117.48^{b} \pm 0.02$	$103.88^{b} \pm 0.04$
C	$120.17^{e} \pm 0.07$	$39.61^{a} \pm 0.02$	$84.92^{b} \pm 0.01$	$117.52^{b} \pm 0.04$	$104.05^{c} \pm 0.03$
D	$133.45^{d} \pm 0.03$	$36.16^{c} \pm 0.07$	$84.42^{c} \pm 0.04$	$117.35^{c} \pm 0.02$	$103.91^{b} \pm 0.03$
E	$147.11^{d} \pm 0.02$	$33.86^{c} \pm 0.07$	$85.09^{a} \pm 0.02$	$118.01^{a} \pm 0.02$	$103.69^{c} \pm 0.04$
LSD	0.09	0.14	0.11	0.05	0.12

Values are means of a triplicate determination ± SD. Mean values along the same column with same superscripts are insignificantly different ($p>0.05$) from one another.

Magnesium content: The value of the magnesium contained in the ugba samples wrapped with ogirishi leaves (Sample A), ororompo leaves (sample B), cocoyam leaves (sample C) plantain leaves (sample D) and okpopia leaves(sample E) were as follows; 144.06±0.03, 138.28±0.03, 120.17±0.07, 133.45±0.02, and 147.11±0.07Mg/100g respectively. The results revealed that the magnesium content of all the samples were significantly ($p<0.05$) different from one another. From the results presented in table 4.2, it was observed that sample wrapped in okpopia leaves (also known as akwukwo ugba) has the highest magnesium content which could be attributed to the high magnesium content of the leaf. The magnesium content of 292.90mg/100g and 186.14 to 235.14mg/100g were reported by Baja *et al*.,(2017) and Nwachukwu *et al*. (2018) respectively. The values were found to be higher than those obtained from this research work. Magnesium is needed for making protein, muscle contraction, nerve transmission and maintenance ofa healthy immune system (Healthwise, 2020).

Iron content

The values presented in Table 2 for iron were within 33.29 ± 0.06mg. 100g to 39.61±0.02mg/100g. The iron content of the fermented *P. macrophylla* seeds varied significantly ($p<0.005$) from each other. It was observed that samples wrapped with *colocasia spp* leaves (sample C) had the highest (39.61mg/100g) value for iron, followed by sample wrapped with M. *paradisiaca* leaves (sample D) with 36.16mg/100g iron content while sample wrapped in N. *laevis* leaves (sample A) had the lowest mean value (33.29mg/100g). Nwachukwu *et al*. (2018) reported the iron content of unfermented *P.macrophylla* seeds (ugba) as 11.40mg/100g, perhaps the higher iron contained in these ugba samples could be attributed to the fermentation process as well as the iron deposited into ugba during fermentation by the various leaves used as packaging materials. The values recorded for iron in this present study were found to be higher than United State Recommended Daily Allowance (10-15 mg/100g) for iron (Okechukwu *et al*, 2011). When foods with iron are eaten, it is absorbed into the bloodstream where it helps to carry and release oxygen throughout the body. An iron deficiency called “anaemia” is very common around the world, especially for women and children in developing nations. Symptoms of iron deficiency take years to develop and include fatigue, weakness and shortness of breath (Robert, 2018). The values of iron discovered in this study suggest that African oil bean seed could be recommended for anaemic patients.

Potassium content

The potassium content of the fermented P. *macrophylla, Benth* (ugba) seed ranged from 83.74±0.02mg/100g to 85.09±0.02mg/100g. The potassium content of the ugba samples has a least significant difference of 0.11, hence the value obtained for all the samples varied significantly ($p < 0.05$) from one another with the exception to samples wrapped in ororompo leaves (*Musa paradisiacal)* that exhibited no significant difference ($p > 0.05$) from each other. The potassium content of unfermented and fermented ugba reported by Okechukwu *et al*. (2011) was 20.40mg/100g and 49.60mg/100g respectively. The above values by Okechukwu *et al*. (2011) were found to be lower than the values obtained from this research work. It was revealed that fermentation increased the potassium content of fermented African oil bean seeds. Thus, the high value obtained for potassium in this study could be attributed to the fermentation process as well as extra potassium deposited into the ugba samples during fermentation by the various leaves used to wrap the samples. Potassium is required in the body for the maintenance of acid-base balance, body water balance and nerve function. Its absence may result in muscular weakness and paralysis (Akpabio *et al*., 2012).

Calcium content: The values of calcium content range from 117.21±0.03mg/100g to 118.±0.02mg/100g. There were significant differences ($p<0.05$) between samples A, B, C, D and E while samples B and C had no significant variation ($p<0.05$) from one another. Okechukwu *et al*. (2011) and Nwachukwu *et al*. (2018) reported calcium content of 0.10mg/100g and 0.07mg/100g and 86.35mg/100g and 92.11mg/100g for unfermented and fermented *P.macrophylla Benth* (ugba) seeds respectively. The results of these researches were found to be lower than those obtained from this study. Calcium plays an important role in building and maintenance of strong bones, teeth and large parts of human blood and cellular fluids. Calcium is also necessary for normal function of cardiac muscles, blood coagulation, clotting and regulation of cell permeability. Calcium deficiency causes rickets, back pain, osteoporosis, indigestion, irritability, premenstrual tension and uterus cramping (Soetan *et al*., 2010).

Phosphorus content

The mean values of phosphorus for the ugba samples packaged with different materials as presented in Table (4.2) were 103.75±0.04mg/100g, 103.88±0.04mg/100g,104.05±0.03mg/100g,103.91±0 .03mg/100g and 103.69±0.0mg/100g for samples A, B, C, D and E respectively. These values have a least significant difference of 0.12 from which it was deduced that sample C (sample wrapped in cocoyam leaves) was significantly different (p<0.05) from the other samples. Samples A and E and samples B and D did not show any significant (p>0.05) difference from each other. The higher phosphorous content of samples wrapped with cocoyam leaves (sample C) could be an indication that cocoyam is a rich source of phosphorous. The values obtained for phosphorus in this present study were found to be within 102.48mg/100g to 117.23mg/100g reported by Nwachukwu *et al*. (2018) for unfermented and fermented African oil bean seeds. Phosphorus is essential in the body because it helps to keep the bones strong and healthy. Phosphorus also helps to remove waste and repair damaged tissues (Fletcher, 2019).

Table 3: Vitamin composition of fermented African oil bean seeds (ugba) wrapped with different leaves

Parameters Samples	Thiamine (Vit. B1)	Riboflavin (Vit. B2) mg/100g	Niacin (Vit. B3)
A	$0.06^{a} \pm 0.01$	$0.25^{b} \pm 0.01$	$0.25^{c} \pm 0.04$
B	$0.06^{a} \pm 0.00$	$0.25^{ab} \pm 0.01$	$0.27^{bc} \pm 0.03$
C	$0.05^{a} \pm 0.01$	$0.33^{a} \pm 0.05$	$0.30^{ab} \pm 0.01$
D	$0.06^{a} \pm 0.02$	$0.29^{ab} \pm 0.03$	$0.28^{abc} \pm 0.03$
E	$0.06^{a} \pm 0.00$	$0.28^{ab} \pm 0.01$	$0.32^{a} \pm 0.03$
LSD	0.03	0.06	0.05

Mean values in the same column with the same superscripts are not significantly different (p>0.05) from each other.

Vitamin B1 content : The vitamin B1 content of the fermented *Pentaclethra macrophylla, Benth* (ugba) seeds were ranged from 0.05±0.01mg/100g to 0.06±0.00mg/100g. From the results presented in Table 4.3, it was observed that ugba samples packaged with ogirishi, ororompo, plantain and okpopia leaves (samples A, B, D and E) had the same value of thiamine (0.06mg/100g) while sample packaged in cocoyam leaves (sample C) had the lowest mean value of 0.05mg/100g. However, from the least significant difference of 0.03 obtained, it showed that there were no significant differences (p>0.05) in the vitamin B1 content of all the samples. Dasofujo *et al.,* (2013) reported a vitamin B1 content of 0.07mg/100g for both unfermented and fermented ugba and their values are closely related to those obtained from this study. Based on the similarity of values obtained for these samples, it is obvious that the different leaves used as packaging materials did not cause any change in the vitamin B1 content of the ugba samples.

Vitamin B2 content: The riboflavin content of the fermented *P. macrophylla* (ugba) seeds were found to be within 0.25mg/100g to 0.33mg/100g. Statistically, The values have a least significant difference of 0.06 from which it was revealed that A, B, D and E as well as samples B, C, D and E were insignificantly (p>0.05) different from one another. The trend of these results also revealed that the packaging materials did not affect any significant change in the vitamin B2 content of the ugba samples. Obum-Nnadi *et al*. (2018) reported 0.32mg/100g and 0.30mg/100g for unfermented and fermented ugba samples respectively. This revealed that the riboflavin content of ugba as reported by Obum-Nnadi *et al*. (2018) decreased during fermentation. Thus, the irregularity in the values obtained for vitamin B2 could be attributed to the fermentation process and not the packaging materials.

Vitamin B3 content:The vitamin content of the ugba samples were shown in Table 4.5 as ; 0.25±0.04mg/100g,0.27±0.03mg/100g,0.30±0.01mg/100g,0.28±0.03mg/100g, 0.32±0.03mg/100g for samples wrapped in ogirishi leaves (sample A), ororompo leaves (B), cocoyam leaves (sample C), plantain leaves (sample D) and okpopai leaves (sample E) respectively. This mean value has a least significant difference of 0.05 which revealed that sample A, B and D; B, C and D and C, D and E respectively were significantly different (p<0.05) from each other. Again Danielli *et al*., (2018) reported a value of 0.90mg/100g and 0.30mg/100g for unfermented and fermented ugba sample relates to values obtained from the present research work. The variations in the values of the vitamin B3, just like in vitamin B1 and B2 could be attributed to the fermentation process and not the packaging materials used.

Table 4: Microbial Count Of Fermented African Oil Seeds (Ugba) Wrapped With Different Leaves

Parameter samples	*Bacillus* Species	*Streptococcus* Species (CFu/g)	*Staphylococcus* Species	*E-coli*	*Pseudomonas* Species
DAY 1					
A	7.28 x 10^3	3.7 x 10^3	2.28 x 10^3	-	1.24 x 10^3
B	7.11 x 10^3	3.66 x 10^3	2.41 x 10^3	-	2.13 x 10^3
C	7.30 x 10^3	3.82 x 10^3	2.54 x 10^3	-	2.42 x 10^3
D	7.31 x 10^3	3.54 x 10^3	2.15 x 10^3	-	2.86 x 10^3
E	7.25 x 10^3	3.64 x 10^3	2.40 x 10^3	-	2.24 x 10^3
DAY 3					
A	9.04 x 10^3	6.54 x 10^3	2.41 x 10^3	-	2.14 x 10^3
B	9.04 x 10^3	6.27 x 10^3	2.23 x 10^3	-	4.04 x 10^3
C	9.25 x 10^3	6.67 x 10^3	2.37 x 10^3	-	4.28 x 10^3
D	9.42 x 10^3	6.22 x 10^3	2.18 x 10^3	-	4.29 x 10^3
E	9.23 x 10^3	6.52 x 10^3	2.21 x 10^3	-	4.25 x 10^3
DAY 5					
A	10.07 x 10^3	7.71 x 10^3	0.99 x 10^3	-	5.37 x 10^3
B	10.13 x 10^3	7.46 x 10^3	0.65 x 10^3	-	5.14 x 10^3
C	10.02 x 10^3	7.53 x 10^3	0.56 x 10^3	-	5.44 x 10^3
D	10.14 x 10^3	7.45 x 10^3	0.51 x 10^3	-	5.55 x 10^3
E	10.01 x 10^3	7.44 x 10^3	0.31 x 10^3	-	5.16 x 10^3

Samples; A = Ugba wrapped in Ogirishi leaves, B= Ugba wrapped in Ororompo leaves, C = Ugba wrapped in Cocoyam leaves, D = Ugba wrapped in Plantain leaves, E = Ugba wrapped in Okpopia leaves

Microbial Count of Fermented African Oil Bean Seeds Wrapped With Different Leaves

Analysis of the fermented African oil bean (ugba) sample with different materials revealed the association of bacterial isolates. There were four species of bacteria namely- *bacillus* species, *streptococcus* species, *staphylococcus* species and *pseudomonas* species. E. coli was not detected in the samples.

***Bacillus* species**: The total viable count of Bacillus species as depicted in the table (4.4) shows a range of 7.11 x 10^3 (cfu/g) to 7.31 x 10^3 (cfu/g) within 24 hours of fermentation time. As the fermentation progresses (72 hours), it increased to a range of 9.04 x 10^3 (cfu/g) - 9.42 x 10^3 (cfu/g) and then 10.01 x 10^3 (cfu/g) - 10.14 x 10^3 (cfu/g) (120 hours). The ugba sample wrapped with plantain leaves (sample D) was found to have the highest viable count while samples wrapped in ororompo leaves (sample B) had the lowest viable count within 24 hours and 72 hours of fermentation. Sample wrapped in okpopia leaves (sample E) has the lowest viable count at the end of the fermentation period (120 hours). From the results obtained, it was found that B. species were the highest occurring bacteria from this study. Bacillus has severally been reported as a major microorganism that is responsible for the physiochemical and organoleptic attributes of ugba and it is an active fermentation agent (Anyanwu *et al*., 2016).

***Streptococcus* species:** The total viable count of *streptococcus* species in the fermented "ugba" samples were found to be in the range of 3.54x 10^3 (cfu/g) to 3.82 x 10^3 (cfu/g); 6.22 x 10^3 (cfu/g) to 6.67 x 10^3(cfu/g) and 7.44 x 10^3(cfu/g) to 7.71 x 10^3(cfu/g) for fermentation periods of 24h, 72h and 120 hours respectively. Ugba samples wrapped in cocoyam leaves (sample C) had the highest viable count of streptococcus during 24h and 72 hours fermentation period while samples wrapped in ogirishi leaves (sample A) had the highest viable count at the end of the fermentation period (120 hours). Ugba wrapped with plantain leaves (sample D) had the least viable count in the 24 and 72 hours fermentation period while the sample wrapped in okpopia leaves (sample E) had the least in 120 hours of fermentation time. *S.* species are enteric bacteria and their presence in the study shows that the samples could have been contaminated. This assertion is so because Mohammed *et al*. (2020) reported that the discovery of enteric bacteria such as *streptococcus* suggests contamination of the product. The trend of the results shows that the contaminants might have been obtained from the different leaves used to wrap the samples as well as water and utensils used in their preparation.

***Staphylococcus* species** : The total viable count of *staphylococcus* species in the fermented ugba samples were found to be in the range of 2.15 x 10^3 (cfu/g) to 2.54 x 10^3 (cfu/g), 2.18 x 10^3 (cfu/g) to 2.41 x 10^3 (cfu/g) and 0.31 x 10^3 (cfu/g) to 0.99 x 10^3 (cfu/g) for 24h, 72h and 120 hours of fermentation period respectively. Ugba sample wrapped in cocoyam leaves (sample C) had the highest viable count during the first 24 hours of fermentation while sample A (ugba wrapped in ogirishi leaves) had the highest count of staphylococcus during the second (72 hours) and last stage (120 hours) of the fermentation process. From the result (Table 4.4) it was observed that the number of viable counts observed within the period of the study corresponds to the findings of Ogueke *et al.* (2010). The report has it that these microbes are found in the skin and nasal cavity of humans and pose a high risk of enterotoxin (Anyanwu *et al.*, 2016). Since the African oil beans were boiled for hours before fermentation, both the fermentation agents and other microorganisms (*Staphylococcus sp*.) isolated could not have originated from the beans. These bacteria were probably introduced through the air, water, utensils, and leaves used in wrapping or by handling during the preparatory stages. But it is worthy to note that the *S. species* are far below the limit (7 x 10^6 cfu/g) as asserted by Ogueke *et al.* (2020). So the samples could be considered safe for consumption.

***Pseudomonas* species:** The total viable count of *Pseudomonas* in *Macrophylla, Benth* seeds ranges from 1.24 x 10^3 (cfu/g) to 2.86 x 10^3 (cfu/g); 2.14 x 10^3 to 4.29 x 10^3(cfu/g) and 5.14 x 10^3 (cfu/g) to 5.55 x 10^3 (cfu/g) for 24, 72 and 120 hours of fermentation respectively. The results obtained revealed that samples wrapped in plantain leaves had the highest viable count throughout the fermentation (24,72 and 120 hours) period. Mohammed *et al*. (2020) isolated P. sp. In ugba sold in Minna market and attributed its presence to water and utensils utilized in the preparation of ugba sample. Nevertheless, from the trend of the results obtained, it could be said that the different leaves used as wrappers introduced the bacteria into the product. The values obtained also revealed that extension of the fermentation process could have caused a reduction in the viable count of the P. species isolated from these samples because the growth rate was not geometric.

E. coli

E. coli were not detected in any of the fermented samples in this study. This did not concur with the report of Anyanwu *et al*. (2016) on the microbial count of fermented ugba sold in Mbaise. The presence of *E. coli* suggests contamination by excreta or faeces. So its absence reflects the hygienic standard of both the processor and the utensils used in the preparation of the ugba samples.

Table 5: Sensory evaluation of fermented African oil bean (ugba) wrapped with different leaves

Parameters Samples	Taste	Aroma	Appearance	Mouthfeel	General acceptability
A	6.22^{b}	5.78^{b}	6.33^{c}	6.10^{b}	6.50^{c}
B	7.10^{a}	6.92^{a}	7.10^{a}	7.33^{a}	7.42^{a}
C	6.66^{ab}	6.66^{a}	7.00^{ab}	7.14^{a}	7.33^{a}
D	6.48^{b}	6.72^{a}	6.79^{b}	7.20^{a}	7.10^{ab}
E	6.40^{b}	6.88^{a}	7.22^{a}	6.39^{b}	6.82^{bc}
LSD	0.45	0.33	0.28	0.39	0.40

Mean values in the same column with different superscripts are significantly different at ($p<0.05$).

Sensory Evaluation of Fermented African Oil Bean

Values for taste of fermented ugba sample wrapped in Ogirishi (sample A), Ororompo (sample B), Cocoyam (sample C), plantain (sample D) and Okpopia (sample E) leaves were found to be 6:22, 7.66, 6.58 and 6.40 respectively. The taste of sample B had the highest mean score of 7.10 (i.elikert moderately), but was found to be insignificantly different ($p<0.05$) from sample C which had the second- highest mean score of 6.66 (i.e liked slighted, but approximately like moderately). Although the taste sample wrapped in Ororompo leaves was more preferred by the judges, they also found the taste of other samples to be quite good. The aroma of the fermented ugba samples was valued by the judges as followed: 5.78, 6.92. 6.66, 6.72 and 6.88 For samples A, B, C, D and E respectively. The aroma of these samples was slightly liked by the judges except samples wrapped in Ogirishi (sample wrapped in Ogirishi (sample A) leaves that were neither liked nor disliked by the judges. Sample B, C, D and E were found to be significant difference ($p>0.05$). for each other but exhibited significant difference ($p<0,05$) with sample A. The close rating of samples B,C,D and E on aroma could therefore mean that their wrapping materials did not impact flavour on them unlike in sample wrapped with Ogirishi (sample A) leaves. The appearance of the sample was scored by the judges as followed: 6.33, 7.10, 7.00, 6.79 and 7.22. For samples A, B, C, D and E respectively. The appearance of ugba sample wrapped in Okpopia leaves (sample E) had the highest mean score of 7.22 (liked moderately) while the sample wrapped in Ogirishi (sample A) has the least mean score of 6.33 for appearance and exhibit significant difference ($p<0.05$) with other samples appearance-wise. The appearance of all the ugba samples was all liked by the panelists. The values for mouth feel of fermented *PentaclethraMacrophyllaBenth* (Ugba) samples were depicted in the table as follows: 6.10, 7.33, 7.14, 7.20 and 6.39 for samples A, B, C, D and E respectively. These samples has a least significant difference of 0.39 which shows that a significant difference ($p<0.05$) existed between samples B,C and D that were all liked moderately and samples A and D that were both liked slightly by the panel of judges. The overall acceptability of all the samples were rated by the judges as 6.50, 7.42, 7.33, 7.10 and 6.32 for samples A, B, C, D and E respectively. Samples wrapped in ororompo (sample B), cocoyam (sample C) and Plantain (sample D) leaves were all liked moderately by the judges while samples wrapped in ogirishi (sample A and okpopia leaves (sample E) were both liked slightly. Samples A, E, D and E respectively were found to be insignificantly different ($p>0.05$) from each other. Despite of the variations amongst all samples organoleptically evaluated, the overall acceptability means score revealed that none of the sensory parameters was disliked by the judges.

Conclusion and Recommendation

The proximate analysis of the fermented African oil bean seeds wrapped in different packaging leaves indicated that samples wrapped in Ogirishi leaves irrespective of its high moisture content, also obtained the highest percentage for protein, fat and its ash content did not exhibit any significant difference with sample wrapped in Okpopia leaves with the highest ash content. From the values obtained for minerals, it could be deducted that Ogirishi leaf is a good source of magnesium, potassium and calcium. And Okpopia (Akwukwo ugba) leaves is a good source of iron and phosphorus as samples wrapped in these leaves obtained the highest content of the aforementioned minerals respectively. There were no significant variations in the vitamin B1, B2 and B3 content of all the ugba samples wrapped in Ogirishi, Ororompo, cocoyam, plantain and Okpopia leaves. Enteric bacteria like *streptococcus sp., staphylococcus sp*., and *pseudomonas sp.* were isolated from the “Ugba” samples. These bacteria are capable of causing food infections/poisoning and their presence in these samples are believed to be from these leaves used as wrappers. Based on the scores obtained by ugba sample wrapped in ororompo leaves within the period of this study, it is concluded that sample B is organoleptically better than other samples.

References

A.O.A.C. (2010). Official methods of Analysis, Association of Official Analytical Chemists.Akpabio, U., Akpakpan, A., Udo, U. and Essien,U. (2012). Physiochemical characterization of exudates from raffia palm (*Raphiahookeri*).*Adv.Appl. Sci. Res*.,3(2):838-843.

Anyanwu,N., Okonkwo, O., Iheanacho, N. and Ajide, B. (2016). Microbiological and nutritionalqualities of fermented ugba (*Pentaclethramacrophylla, Bent*) sold in Mbaise, Imo State, Nigeria.*Ann.Res.Rev.Bio.*, 9(4):1-8.

Ayodele, S., (2013).The effects of herbal remedies. Paper presented at the 12th Annual Conference of the Botanical Society of Nigeria (BOSON). University of Lagos. August 8th – 12th.

Bada,S., Oyetayo, A., Adaramola- Ajibola,K. and Giwa, E. (2017).A preliminary study of the effect of *Alchornealaxiflora* onhaematologicalparameters and organ weight in albino rats. Int.Blood Res.Rev., 7(3): 1-6.

Bosha,J., Anaga, A. and Asuzu, I.(2018). Chemical composition of *Newbouldialaevis* (P.Bauv) used in the treatment of diabetes mellitus in Nigeria. *J. Nutr. Obe*. 1 (1): 1-5.

Danielli, D., Gontard, N., Spyropoulos, D., Zondervan- Van den Beuken, E. and Tobback, P. (2018). Active and intelligent food packaging: Legal aspects and safety concerns. *Trends Food Sci.Technol.*, 19(1): 103- 112.

Dasofunjo,K., Nwodo,O., Johnson.J., Ukanukpong,R., Ugwu, M. and Odinaka E.(2013). Haematopovetic effect of ethanolic leaf extract of piliostigmathonmingii on male albino rat.*J.Nat. Prod. Plant Res.*, 3(2): 1-4.

Fletcher, J. (2019).What are the health benefits of phosphorus? Retrieved from https;//www.medicalnewstoday.com on 02-09-2021. Healthwise (2020). Minerals and their Functions.

Kabuo, N., Asoegwu, S., Nwosu, J. N., Onuegbu, N., Akajiaku, L. and Nwaimo, J.(2015). Assessment of leaf types and number of leaves used in wrapping on the quality of "Ugba" (fermented *Pentaclethramacrophylla, Bent* seed). *Euro.J. Food Sci.Technol.*, 3(1): 11- 23.

Mbata, T. and Orji, M. (2007). Process optimization in the production and preservation of 'Ugba', A Nigerian fermented food. *Internet J. Micobio.*, 3(2): 1-5.

Mohammed, A., Mohammed, M. and Sulaiman, M. (2020). Bacteriology and proximate composition of ugba from Minna market after prolonged fermentation.Bina *J. Sci. Technology*. 4(1): 189-200.

Mohapatra, D., Mishra, S. and Sutar, N. (2010). Plantain and its by-product utilization: An over view. *J. Sci. Ind.Res.*, 69:323-329.

Nwachukwu, C., Enyoh,E., Enyoh, C.andAmaobi, C. (2008). Effects of fermentation time on the proximate and mineral composition of fermented African oil bean seeds (Ugba). Sustainable Food Production.2:13-20.

Obum-Nnadi, C., Okey-Ndeche, N., Nnagbo,P. and Ohabughiro, B. (2020).Phytochemical and antibacteria activities of *Newbouldialaevis* leaves (ogirishi) on two drug resistant bacteria. *Int. J. Res. Rev*. 7(5): 59-66.

Ogbulie, J., Njoku, H., Uwaezuoke,J. and Onyicha, R.(2018). Evaluation of packaging materials for the preservation of African oil bean (*Pentaclethramacrophylla*, Benth) for the production of Ugba. *J. Innov.Life Sci.*, 3:110-118.

Ogueke, C., Nwosu, J. N, Owuamanam,C. and Iwouno, J. (2010).Ugba the fermented African oil bean Seeds., It's production Chemical Composition, preservation, safety and health benefits. *Pak.J. Biol. Sci*., 13:489-496.

Ojewunu, M. (2016). Optimizing the conditions and processes for the production of protein from *Paekiabiglobosa* seeds.Dep. Che. Eng. Coll. Cove. Uni. Ota. p.91.

Okechukwu,R., Mgbemena,I., Egboka,T., Azuwuike,C., Adjeroh,A. and Opara, F.(2011).Microorganisms with Fermented and non-Fermented African oil bean "ugba"(*PentaclethramacrophyllaBenth*). *Curr.Trends Microbiological*.,7:88-92.

Okereke, A. and Onunkwo, D. (2014). Effects of processing methods on proximate composition and sensory properties of "Ugba" (PentaclethramacrophyllaBenth) an African Oil bean product. *J. Environ. Sci. Toxicol*. 8(11): 32-38.

Okorie,C.P. and Olasupo,N.A. (2013).Controlled fermentation and preservation of ugba - an indigenous Nigerian fermented food.Springer plus.2:470.

Okwu,D. and Ekeke, O.(2003). Phytochemical screening and mirord composition of chewing sticks in South Eastern Nigeria. Global J. Pure Appl. Sci.,9:235-238.

Olasupo, N., Okorie, C. and Oguntoyinbo,F.(2016).The Biotechnology of ugba,a Nigerian traditional fermented food condiment.Fro.Microbio.,7:1-10.

Oluwabukola,R.,Ibukun, O.and Olubunmi, O.(2020). Preservative effects of *Newbouldialaevis* (Boundary Tree) leaf extract on shelf -life of fresh chicken meat under tropical conditions. *J.zoo. Res.*,2(1): 8-13.

Robert, K. (2018).Consumer and their brands: developing relationship theory in consumer research. *J. Consumer Res*., 24(4): 43-73.

Soetan,K.,Olaiya, C.and Oyewole, O.(2010).The importance of mineral elements for humans, domestic animals and plants: A Review. *Afr.J. Food Sci.*, 4(5): 200-222.

Tho, B. (2004). Chefs find banana leaves appealing: Natural food wrapper, an authentic way to showcase tropical ethnic foods. Nations Restaurant News. pp. 18 – 49.