IJMAAS International Journal of Microbiology and Applied Sciences
ISSN 1115-4004 Volume 3 issue 1 Jan, 2024 Research Article

Proximate Composition and Microorganisms Associated with Fermented African Oil Bean Seed (*Pentaclethra macrophyla Benth*) Using Different Storage Methods

Didia, Confidence*, Wemedo, S. A., and Nrior, R. R
Department of Microbiology, Rivers State University,
Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria.
***Corresponding Author:** *didiaconfidence67@gmail.com*

ABSTRACT

Fermented African oil bean (*Pentaclethra macrophyla Benth*) known as 'Ugba' in Ibo is a traditional food condiment generally produced by natural fermentation in homes as a small family business. It is an important and cheap source of protein in foods and is also eaten as a delicacy. This study evaluated proximate composition and microorganisms involved in the fermentation of African oil bean at different stages of storage using standard procedures. Bulk samples of leaf-wrapped and polythene-wrapped unfermented 'Ugba' were collected from Mile 3 market, Port Harcourt. Samples (25g) were weighed from the leaf-wrapped and polythene-wrapped Ugba and separately packaged aseptically into leaves and polythene bags and stored at room temperature for fermentation for a period of twenty-one (21) days. Results of mean microbial counts of leaf-wrapped and polythene-wrapped samples were $9.0\pm0.1x10^7$CFU/g and $6.1\pm0.1x10^7$CFU/g respectively. Day 1 had the lowest count in both samples followed by Day 7. Counts increased further in Day 14 and were highest in Day 21. Bacteria isolated were *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Bacillus licheniformis, Klebsiella* sp and *Micrococcus luteus*. Fungi isolates were *Penicillium sp, Aspergillus niger* and *Saccharomyces* sp. *Klebsiella* sp, and *Micrococcus luteus* were isolated at day14 and day 21. Fermentation of Ugba involved a mixture of bacteria and fungi that help in enhancing colour darkening and softening of the oil bean in which *Bacillus* spp were the predominant and most actively involved organisms and was present throughout the fermentation period. *Aspergillus niger, Penicillium* sp and *Saccharomyces* sp were isolated in low amount in both leaf and polythene packed samples. This study showed that fermentation improved the nutritional quality of African oil bean as increasing fermentation period resulted in increased moisture, fibre and protein content and decreased ash, fat, and carbohydrate contents.

Keywords: African oil bean, *Pentaclethra macrophyla*, 'Ugba', fermentation, proximate composition, *B. licheniformis*

Introduction

The African oil bean, (*Pentaclethra macrophylla*) is a tropical tree crop that belongs to the leguminoseae family and the mimosoideae sub-family. Different Nigerian tribes have different names for the African oil bean for example; it is known as Ukpaka or Ugba in Igbo, the Yorubas call it Apara while the Efik call it Ukana (Odunfa, 2019). African oil bean seed is a nutrient-packed natural food that has remained untapped and underused thus calling for immediate attention to be widely exploited. Although there are several staple foods such as rice, maize and beans, yet there is still a great need to supplement these food products with certain unpopular foods that are equally packed with loads of essential nutrients and vitamins needed for the healthy functioning of the body Ogueke and Aririatu, 2004).

African oil bean seed is one of such unpopular yet essential food. UGBA seeds are utilized by the Ibos and other ethnic groups in southern Nigeria as a delicacy and food flavouring (Ikenebomeh *et al*., 2014). The oil bean seed is mainly composed of proteins (42%), lipids (43%) and carbohydrates (15%) (Odunfa and Oyeyiola, 2004; Ogueke and Aririatu, 2004).

Studies on the microbiology of the fermentation of African oil bean seeds have identified *Bacillus* spp as the main microorganism responsible for its fermentation. The predominant species is *Bacillus subtilis* but other species like *B. pumilus, B. megaterium, B. lichenformis* have also been found (Odunfa, 2019; Antai and Ibrahim 2019; Odunfa and Oyewole, 2014).

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The same group of organisms has been implicated in the fermentation of other fermented food condiments like Iru, Dawadawa, Soumbala, Afiyo and Ogiri. (Odunfa, 2019). The seeds are 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, flavonoids, sterols, glycosides and saponins. Notwithstanding the high nutritional content of the African oil bean seeds, studies reveal that the fermentation process which they undergo before consumption usually eradicates most of these minerals and vitamins such as phosphorus (Akindahunsi, 2004). Thus, the present study evaluated the proximate composition and microorganisms associated with the fermented African oil bean seed stored in polythene and leaves.

Materials and Methods

Study Area

The study area was carried out in Port Harcourt city. Already processed unfermented African oil bean samples were purchased from local processors who packaged the samples in plantain (*Musa X paradisiaca*) leaves and in polythene bags from Mile 3 market. Bulk samples of leaf-wrapped and polythene-wrapped unfermented 'Ugba' were collected and immediately transported in a sterile cool box to the Department of Microbiology laboratory of Rivers State University, Port Harcourt for microbiological and proximate analysis samples.

Study Design

Twenty-five grams (25g) of unfermented oil bean (Ugba) were weighed from the leaf-wrapped and polythene-wrapped samples and separately packaged aseptically into leaves and polythene bags. Six replicate samples were wrapped in leaves and polythene bags and stored at room temperature to fermentation for a period of twenty-one (21) days and analyzed for microbial load and proximate composition at Day 1, Day 7, Day 14 and Day 21

Enumeration and Isolation of Bacteria and Fungi

Aliquot (0.1ml) of appropriate dilution of the sample was transferred into sterile nutrient agar. McConkey agar and Sabouraud dextrose agar plates in duplicates. The plates were uniformly spread with sterile bent glass rod (spread plate method). The plates were incubated in inverted position at 37°C for 24 hours. The SDA plates were incubated for a period of five (5) days. After incubation, the plates were observed and the colonies that developed were counted and recorded (Robinson *et al.*, 2020).

Characterization and Identification of bacterial and fungal Isolates

The bacterial isolates were identified based on their phenotypic characteristics such as gram staining, sugar fermentation tests, citrate, methyl red, voges Proskauer, oxidase and starch hydrolysis test. The response was inputted in the ABIS online system for identification of bacterial isolates at https://www.tgw1916.net/bacteria_logare.html. The fungal isolates were identified based on their macroscopic and microscopic characteristics and referenced with the fungal atlas of Sarah *et al.* (2016).

Proximate Analysis

The methods described by Association of Official analytical chemists (AOAC) were used to determine proximate content of the samples during the period of fermentation process for 21 days. In these analyses, the moisture, ash, crude protein, crude fibre, crude fat and carbohydrate contents were analysed (Helvich, 1990).

Moisture content

The moisture content of the samples was determined using AOAC (1990) procedure 14.006. Aluminum moisture cans were dried in a hot air oven (DHG-9140A), transferred to a desiccator, cooled for 20 minutes and weighed.

Five gram (5g) of each test sample was placed into the cans and the weight noted; the can and its content were heated at 105^0C for 4hours. The cans at the end of heating was cooled in the desiccator and weighed.

The moisture content was calculated using the formula.

$$\text{Moisture (\%)} = \frac{weight\,loss}{weight\,of\ sample} \times \frac{100}{1}$$

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Ash content

The ash content was determined using the method of AOAC (1990) procedure (77.062). Two gram (2g) of sample was weighed into preciously ignited and cooled porcelain crucible. The crucible and samples were heated on a heating mantle (Gerhardt, Germany) until smoking ceased. The crucible and the content were transferred into a muffle furnace. (SXL) and was ashed for 3hours at 550^0C. The crucible and the ash were removed from the furnace and cooled in a desicator, and weighed again. The ash was calculated as follows:

$$\text{Ash content (\%)} = \frac{weight of\ ash}{weight of\ sample} \times \frac{100}{1}$$

Crude fat

The crude fat was determined using a micro-extraction fat unit. Half a gram of the sample was weighed, wrapped in a whatman number 1 filter paper and placed in a thimble. The thimble was placed inside the extraction flask and forty millilitres (40ml) of hexane poured into the fifty ml extraction flask. The flask and its content were placed on malfunction shaker and oscillated for 3hours. At the end of extraction process, the thimble was removed and solvent evaporated, and the flask was dried in an air oven (DHG- 9140A) for 30 minutes at 105^0C. cooled and weighed. The difference in weight in the extraction flask before and after the extraction was recorded as the amount of fat or ether extract and was calculated as follows:

$$\text{Crude fat (\%)} = \frac{weight\ fat}{weight of\ sample} \times \frac{100}{1}$$

Crude Protein

Determinations of crude protein content follow the method of the AOAC (1990) procedure 2.057. Half of a gram of the sample was weighed into a two hundred and fifty-ml digestion flask. To this was added 2 tablet Kjedahl catalyst and twelve ml of sulphuric acid. The content of the flask was placed on a digest furnace (Tecator Digestor 8), set at 420°C and digested for 1hour. The digest was allowed to cool and made up to one hundred ml using distilled water. Twenty ml of the digest was introduced into hundred and fifty ml kjedahl distillation flask and to which was added 20ml of 40% sodium hydroxide.

The flask was then placed on kjedahl distillation unit (Foss, 2100) and the ammonia liberated distilled into ten ml boric acid indicator. The distillate was titrated against 0.1HNCL solution to pink end point. A blank determination was carried out and was subtracted from the sample reading and the %N and % crude protein was calculated as follows:

$$\text{N (\%)} = \frac{(Sample\ titre - Blank)\ \text{x}\ Normality\ of\ Acid\ \text{x}1.4}{Weight\ of\ Sample}$$

Crude protein (%) = % N x 6.25

Determination of crude fibre

Half gram of the sample was weighed and place in a one hundred ml beaker, twenty-five ml 0f 1.25% W/V sulphuric acid was added and covered with a watch glass. The content of the beaker was heated gently on a hot plate (Gehardt, Germany) for 10 minutes (acid hydrolysis). The content of the beaker was filtered through a Buchner funnel with filter paper (Whatman No.1 and washed with distilled water until the washing was no longer acid to litmus. The residue was then washed back into the beaker with twenty-five ml of 1.25% sodium hydroxide. This was heated for 10 minutes' watch glass (alkaline hydrosis). The resulting insoluble material was transferred to a dried pre-weighed ash less filter paper (Whatman No 42) and washed thoroughly with hot distilled water until the washings was no longer alkaline to litmus. The filter paper with insoluble material was dried at 105^0C to constant weight for one hour. The dried filter paper and its content was incinerated to an ash at 500^0C for 1 hour, cooled and weighed.

The fibre was calculated thus,

Weigth of insoluble material- weight of ash

Fibre = weight of insoluble material – weight of ash

$$\text{Crude fibre} = \frac{weight of\ fibre}{weight of\ sample} \times \frac{100}{1}$$

Fibre = weight of insoluble material – weight of ash

Carbohydrate (By Difference)

The carbohydrate content was determined difference i.e 100 - (% moisture content + % Ash % + % Fat +% crude protein + % crude fibre).

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Data Analysis

Statistical analysis was carried out on the bacterial counts from the food sample obtained from the study. Analysis of variance (ANOVA) and Ducan Multiple Range Test (DMRT) was used to test for the significance and and means. This was done using a computer-based programme –SPSS version 25.

Results

Results of the proximate composition of unfermented African bean seed (Ugba) samples are presented in Table 1 while changes in proximate composition during fermentation are presented in Table 2. The mean range of the moisture, fat, ash, crude fibre, crude protein and carbohydrate was 41.79±0.0-52.98±0.0, 5.98±0.0-7.53±0.0, 2.58±0.0-2.90±0.0, 20.03±0.0-20.60±0.0, 10.50±0.0-11.37±0.0 and 3.34±0.0-8.02±0.0 %, respectively. The moisture, fat and carbohydrate content were less in the unfermented sample while the fermented bean stored with leaf had highest moisture, fat and ash contents.

The carbohydrate and protein contents were higher in the fermented African bean seed stored in polythene (waterproof).

Results of the changes in the counts of total heterotrophic bacterial, total coliform and fungi of the different samples are presented in Figures 1, 2 and 3, respectively. Results showed that the bacterial load in the fermented African bean seed stored in the leaf was lower in Day 1 (8.8×10^7), higher on Day 7 (7.0×10^7), Day 14 (2.23×10^8) and Day 21 (2.72×10^8). While low bacterial load was recorded on Day 7 (2.8×10^5), Day 14 (5.5×10^7) and Day 21 (1.17×10^8) in the samples stored with waterproof.

Thus, the results showed an increase in bacterial load in samples stored with leaf and waterproof at the end of the storage (day 21). For the coliform counts, the results showed that the coliform counts increased from Day 1 to Day 14 and dropped significantly ($P<0.05$) on Day 21 for both waterproof and leaf storage. Similar observations were noted for the fungal counts (Fig 3). Day 1 of fermentation had the highest fungal counts but declined significantly ($P<0.05$) as the fermentation continued.

A total of five bacterial isolates belonging such as *Bacillus lichenformis, Bacillus cereus, Micrococcus leutus, Staphylococcus aureus* and *Bacillus subtilis* were isolated during the fermentation of the African oil bean seed (Ugba) while the fungi isolated were *Aspergillus niger*, *Saccharomyces* sp and *Penicillium* sp.

Table 1: Proximate composition of unfermented African bean seed (Ugba) samples

	Proximate composition (%) of African oil bean seeds					
Samples	**Moisture**	**Fat**	**Ash**	**Crude fibre**	**Protein**	**Carbohydrate**
Unfermented	41.79±0.0	5.98±0.0	2.79±0.0	20.60±0.0	10.50±0.0	3.34±0.0
Leaf wrapped	52.98±0.0	7.53±0.0	2.90±0.0	20.03±0.0	10.50±0.0	6.01±0.0
Polythene wrapped	51.79±0.0	6.16±0.0	2.58±0.0	20.07±0.0	11.37±0.0	8.02±0.0

Table 2: Changes in the proximate composition of African oil bean seeds during fermentation

Components of oil been seed	**Leaf wrapped**			**Polythene wrapped**		
	Day 1	**Day 7**	**Day 21**	**Day 1**	**Day 7**	**Day 21**
Moisture (%)	52.98±0.0	51.00±0.0	54.16±0.0	51.79±0.0	53.29±0.0	54.49±0.0
Fat (%)	7.53±0.0	6.16±0.0	8.21±0.0	6.16±0.0	8.36±0.0	8.21±0.0
Ash (%)	2.90±0.0	2.58±0.0	2.99±0.0	2.58±0.0	2.99±0.0	2.69±0.0
Crude (%)	20.03±0.0	20.07±0.0	21.07±0.0	20.07±0.0	21.82±0.0	17.36±0.0
Protein (%)	10.50±0.0	11.37±0.0	11.37±0.0	11.37±0.0	11.37±0.0	10.50±0.0
Carbohydrate (%)	6.01±0.0	9.89±0.0	3.20±0.0	8.02±0.0	2.17±0.0	6.35±0.0

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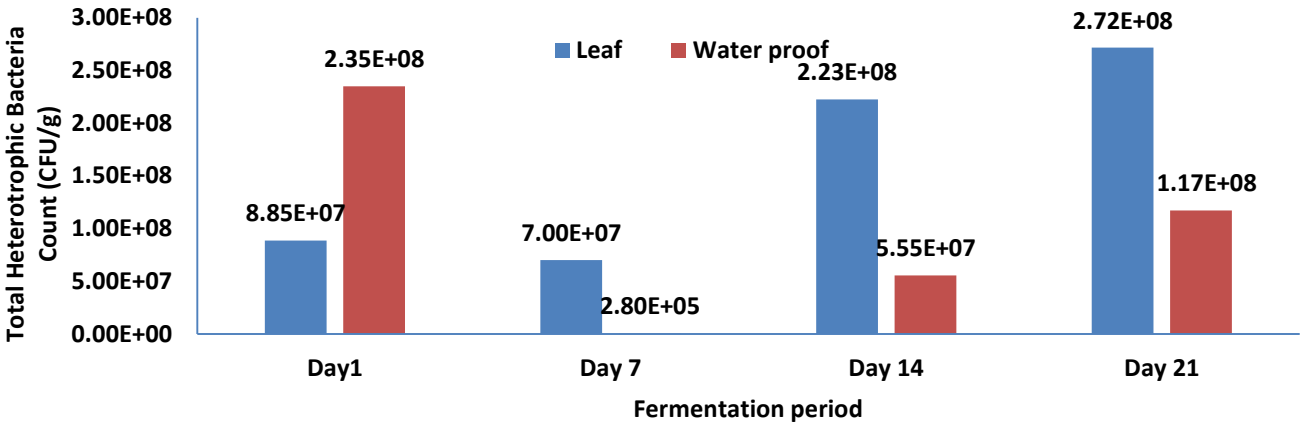

Fig. 1: Change in total heterotrophic bacterial count during the fermentation of African oil bean seeds

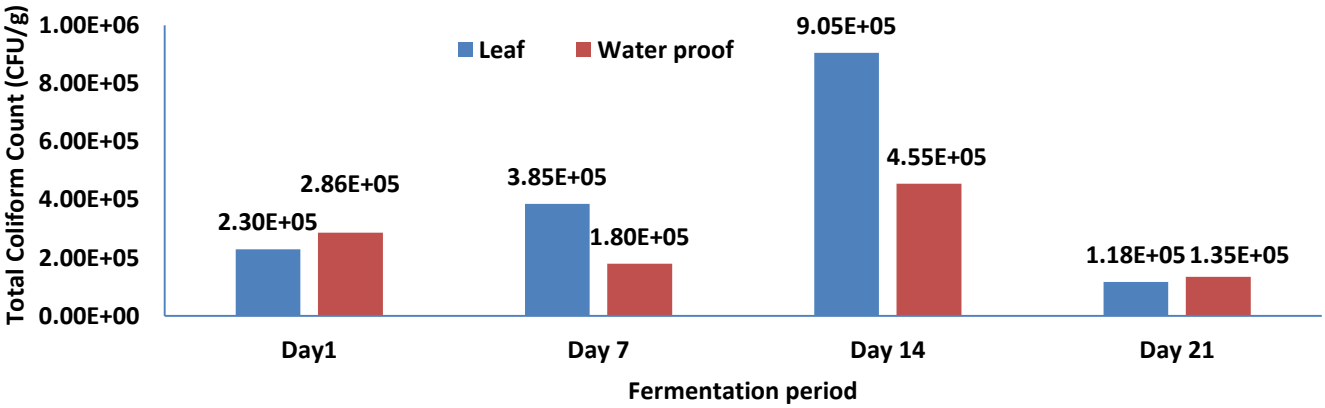

Fig. 2: Change in total coliform count (TCC) during fermentation of African oil bean seeds

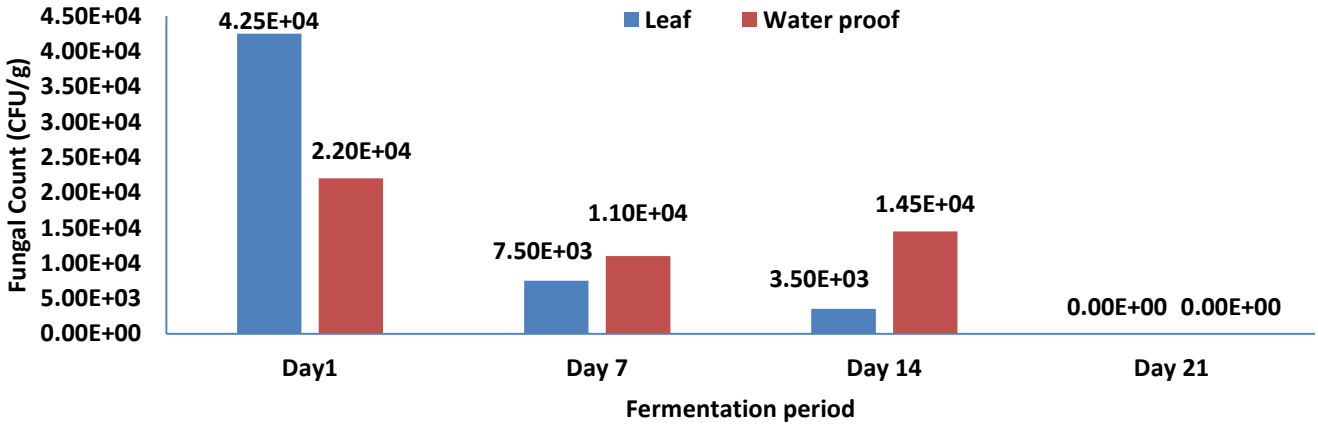

Fig. 3: Change in fungal count During Fermentation of African Oil Bean Seeds

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Discussion

There was a considerable high moisture, crude fibre, and crude protein contents in the fermented Ugba while Ash, Fat and Carbohydrate contents in both samples were low owing to high increase of storage period. The higher the extended period of fermentation, the higher the moisture, crude fibre and crude protein contents increased while a decrease in Ash, Fat and carbohydrate contents were detected.

The moisture content values in the present study were slightly higher than the values (49.32±0.08 to 51.32±0.06 %) reported by Nwokedi. (2018) after 72 hours of fermentation. However, the moisture contents were lower than the 95.0% reported by Balogun (2013). Okechukwu (2012) reported a moisture value of 51.88 % for a sample which was fermented for five days. The crude fat values in the leaves and waterproof across the days were not significantly ($P>0.05$) different but were lower than the range of 17.80±0.08 % to 19.36±0.12 % reported by Obeta (2008) for a 72 hours fermentation.

Thus, this could imply that longer fermentation as observed in the present study might have caused the loss in the crude fat. Enujiugha and Akanbi, (2005) reported high crude fat (53.98 %) after fermentation of the oil bean seed for 72 hours while Okechukwu (2012) reported a value of 37.36% after a 5days fermentation. The protein, ash and carbohydrates contents in the present study varied across the study period but were lower than the values reported by previous studies (Odoemelam, 2005; Okechukwu, 2012). The crude fibres in the present study were higher than the values (2.69±0.09% to 3.22±0.06%) reported by Obeta (2008).

The increase in microbial load during the fermentation could be associated with the presence of fermentable substances that aided the growth of the microorganisms. More so, the presence of microorganisms on the leaves or waterproof used for storage could have increased the microbial load. This increase in microbial load could be associated with presence of indigenous microorganisms in leaf which enhance the quicker spoilage rate of the food.

The high bacterial load in the leaves compared to those in the waterproof could be due to the synthetic nature of the waterproof which unlike the leaves might not be very sufficient for the growth of microorganisms. This is in agreement with Akindahunsi, (2004) who had similar observations. The decrease in the coliform bacterial load and fungal load could be attributed to the depletion of nutrients as a result of the fermentation. In a previous study, it was reported that the late stage of fermentation could result in different changes including nutritional properties and flavours of the food (Okorie and Olasupo, 2014).

The microorganisms isolated from the fermented African oil bean seeds in this present study are well documented. Previous study has reported that these organisms are associated with the fermentation of the African oil bean seeds. In a previous study, Anukam and Reid (2009) reported that *Penicillium* and yeasts amongst other fungal isolates to be associated with the fermentation of African traditional foods. More so, Kabou *et al.*, (2007) reported that during the fermentation of Ugba, *B. subtilis* and *B. licheniformis* were responsible for the production of the typical aroma and flavour.

More so, Molds, Enterobacteriaceae, aerobic mesophilic bacteria, yeasts, gram-negative bacteria rods, lactic acid bacteria, *Bacillus* spp., and yeast have been reported in the fermentation of oil bean seeds. The present study indicated that a number of bacterial sp were involved in the fermentation of the African oil bean seeds to obtain Ugba. The most predominant microbial species in the fermentation of oil bean seeds include *Bacillus* sp. especially *Bacillus cereus* which was predominant from the first to the last day of the fermentation.

In conclusion, this study revealed that the nutritional quality of the oil bean seed improved with longer fermentation days. Also, the microbial counts increased in the first weeks but declined towards the last days of fermentation. The presences of the bacterial and fungal isolates are not of public health implication especially since these organisms were responsible for the fermentation of the African oil bean.

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