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Microorganisms associated with the fermented and non-fermented African oil bean "ugba" (*Pentaclethra macrophylla* Benth)

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ABSTRACT

An investigation was carried out on the microorganisms associated with fermented and non-fermented African oil bean meal "ugba" (*Pentaclethra macrophylla* Benth). The moulds isolated are *Mucor* sp., *Rhizopus* sp., *Aspergillus nidulans*, *A. fumigatus and Paecilomyces* sp. Some bacterial species were also isolated. They include *Staphylococcus* sp., *Bacillus* and *Pseudomonas* spp. The yeast were *Geotrichum* sp., *Torulopsis* sp., and *Hansenula* sp.. Fermented ones had more microbial load at the range of 4.5×10^3 - 5.0×10^3 cfu/g. pH was acidic in the non-fermented (5.7) and alkaline in the fermented ones (8.2). Proximate analysis and mineral composition was done using standard methods. The results showed that non-fermented oil bean had high percentages in protein, fat, fibre, K, Na and P, while fermented had high percentages in moisture content, K, Na and P. Some of the microorganisms isolated during the study are harmful while some are harmless. The harmless microorganisms could offer an alternative answer in protein production in tomorrow's Nigerian Biotechnological development.

KEYWORDS: fermentation, fungi, bacteria, yeast, oil bean

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INTRODUCTION

The presence of microorganisms in food materials can be indeed an advantage in the processing of food by fermentation [1]. "Ugba" also called "ukpaka" a Nigerian indigenous fermented food, rich in protein is obtained by solid state fermentation of the seeds of African oil bean tree (*Pentaclethra macrophylla*). It is a popular food delicacy in Nigeria especially among the Ibo ethnic group where it serves as snacks, side dish or as a food condiment. It is an essential food item for various traditional ceremonies where it is mixed with slices of boiled stockfish, garnished with vegetable and consumed by all socio-economic class. The method of production varies from one produce to another resulting in a non-uniform product with short shelf life [2].

The oil bean seeds are obtained from the African oil bean tree (*Pentaclethra macrophylla*) a large perennial leguminous plant that grows to a height of 25m. The leaves are small and reddish when young and gradually turn to dark green [3]. As in the fermented action of other foods in West Africa, the fermentation of "ugba" is a traditional family act done at home with rudimentary utensils which may act as source of contaminant [4]. Some bacteria found in fermented foods produce toxins that can cause illness or death in man. This occurs when the fermented food exceed their shelf life or are deteriorated. The moulds are also not left out because they are the principal spoilage agents of

"Ugba" evidenced by the different off-colours observed during the fermentation period [5].

Some of these microorganisms associated with food poisoning include *Clostridium*, *Bacillus*, *Staphylococcus*, *Aspergillus* spp., etc. [1]. Most of these microorganisms enter during the processing as contaminants from the water used in washing the raw material and equipments used in production.

This study was designed to isolate some micro-organisms associated with the fermented and non-fermented oil bean.

MATERIALS AND METHODS

The raw "ugba" were purchased from a market in Owerri. The oil bean was hydrothermally treated. The softened seed coat was manually removed to extract the cotyledons. The cotyledons were sliced into small pieces (4.5cm x 0.5cm), (see Plates 1 and 2).

The slices were boiled for 45 minutes and soaked for 12 hours with water at room temperature to remove the bitter components of oil bean seeds which are water soluble. After the soaking period, it was drained in a cleaned basket and covered with a clean banana leaf (*Musa sapientum linn*) and kept in a warm place. After some time, the slices were then wrapped in the banana leaf and tied with a string into packages. Each package weighed about 30g. The wraps were then packed inside a container and sterilized in the autoclave at 121°C for 15 minutes and then allowed to ferment in a warm place (30°C - 40°C) for two to three days (see Fig. 1).

Plate 1. Picture of fermented sliced African oil bean.

Plate 2. Picture of non-fermented sliced African oil bean.

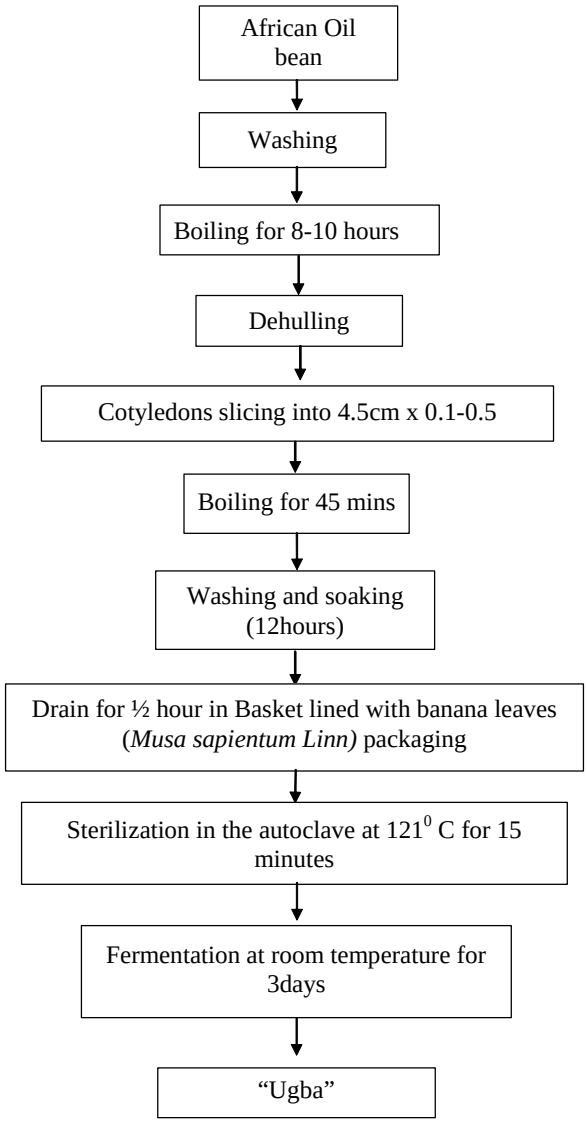

Fig. 1. A block diagram for the laboratory preparation of "Ugba".

Microbial enumeration and isolation

The media of choice were nutrient agar and sabouraud dextrose agar. The media were prepared according to the manufacturer's instruction and allowed to set in petri plates. In the case of the sabouraud dextrose agar, the medium was fortified with chloramphenicol to prevent bacterial growth.

Ten - fold serial dilutions of the samples were performed using 1gm of sample in 9ml 0f 0.85% (w/v) sterile sodium chloride solution, as diluents with vigorous agitation. A 0.1ml aliquot of the appropriate dilution was spread plated in duplicates on surfaces of the appropriate medium [6]. The plates were then incubated between 24 and 96 hours at room temperature. Following incubation, the colonies were randomly selected based on their colonial characteristics and streaked for purity on nutrient agar and sabouraud dextrose agar, respectively.

Characterization and identification of the isolates

Fungal isolates were examined macroscopically and then microscopically using the needle mount technique and identified following the schemes of Alexopoulos and mims [7] and Barnett and hunter [8].

The bacterial isolates were examined for colonial morphology as well as for cell micromorphological and biochemical characteristics according to the methods described by Gerhardt *et al.* [9]. The schemes/ methods of Harrigan and McCance [10] and Cruickshank *et al.* [11], were used for the identification of the bacteria to the generic level.

Proximate analysis of the samples

Chemical characterization of the samples via proximate analysis was carried out to determine the nutritional and mineral composition of the samples determined using the method of AOAC [12].

RESULTS

Table 1 represents the bacterial and fungal loads of the fermented and non-fermented "ugba". The bacteria isolates were *Staphylococcus* sp., *Bacillus* sp. *and Pseudomonas* sp.. The fungal species were *Rhizopus* and *Mucor* spp., *Aspergillus nidulans, A. fumigatus, Paecilomyces* sp., *Geotrichum* sp., *Torulopsis and Hansenula* spp. (see Table 1).

The total aerobic (viable) bacteria count of "ugba" was more in the fermented "ugba" sample with a range of 4.5×10^3 - 5.0×10^3 cfu/g than in the non-fermented "ugba" with 3.5×10^3 - 4.0×10^3 (see Table 2).

A total viable count of fungal isolates was also more in fermented "ugba" with a range of 2.0×10^1 - 2.2×10^1 cfu/gm than that of non-fermented with a range of 1.0×10^1 - 1.5×10^1 cfu/gm (see Table 3).

Table 4 showed the result of the temperature and pH value. It was observed that the temperature varied during the fermentation process with a range of 28°C - 36°C. The pH value of non-fermented oil bean meal was found to be 5.7 while that of fully fermented one was found to be alkaline (8.2). The pH increases with time/ days.

Proximate analysis showed a decrease in the percentage composition of crude protein, fat, fibre etc. contents of the fermented oil bean meal (see Table 5). Also mineral compositions of the samples are shown in Table 6. Non-fermented oil bean had Mg, K, Na and P more than fermented while fermented had high values of Zn, Mn and Fe, more than the non-fermented oil bean. (see Table 6).

Table 1. Occurrence of the isolates in fermented and non-fermented "ugba".

Organisms	Fermented "ugba"	Non-fermented "ugba"
Bacillus sp.	+	+
Staphylococcus sp.	+	-
Pseudomonas sp.	+	-
Rhizopus sp.	+	+
Mucor sp.	+	+
Aspergillus nidulans sp.	+	-
A. fumigatus	+	-
Paecilomyces sp.	+	-
Geotrichum sp.	+	+
Torulopsis sp.	+	+
Hansenula sp.	+	+

Table 2. Total aerobic (viable) bacteria count of "ugba" sample.

Sample	Dilution factor	No. of bacteria isolates per gram sample		Total count per gram sample (cfu/g)		Average count (cfu/g)
A	10^{-3}	40	35	4.0×10^3	3.5×10^3	3.7×10^3
	10^{-4}	20	16	2.0×10^4	1.6×10^4	1.8×10^4
	10^{-5}	10	8	1.0×10^5	0.8×10^5	0.9×10^5
B	10^{-3}	50	45	5.0×10^3	4.5×10^3	4.7×10^3
	10^{-4}	30	20	3.0×10^4	2.0×10^4	2.5×10^4
	10^{-5}	20	15	2.0×10^5	1.5×10^5	1.75×10^5

Sample A: Non-fermented "ugba"
B: Fermented "ugba"

Table 3. Total viable counts of fungal isolates from "ugba" sample.

Sample	Dilution factor	Fungal counts (cfu/g)		Total count of sample (cfu/g)		Average count (cfu/g)
A	10^{-1}	10	15	10×10^1	1.5×10^1	1.25×10^1
	10^{-2}	8	6	0.8×10^2	0.6×10^2	0.7×10^2
B	10^{-1}	20	22	2.0×10^1	2.2×10^1	2.1×10^1
	10^{-2}	12	10	1.2×10^2	1.0×10^2	1.1×10^2

Table 4. Temperature and pH changes during the oil bean meal fermentation.

Sample	Time (H)	pH	Temperature (°C)
Non-fermented "ugba"	0	5.7	36
Fermented "ugba"	12	6.68	28
	24	7.28	31
	36	7.40	31
	48	7.60	28
	60	8.18	32
	72	8.20	33

Table 5. Proximate analysis.

% composition	Fermented "ugba"	Non-fermented "ugba"
% Moisture content	51.88	28.61
% Ash	0.24	0.32
% Fibre	2.80	4.50
% Protein	18.86	28.02
% Fat	20.98	37.16

Table 6. Mineral composition of fermented and non-fermented "ugba".

Mineral composition (mg/100g)	Fermented	Non-fermented
Calcium	0.07	0.10
Magnesium	0.06	0.17
Potassium	20.40	49.60
Phosphorus	30.20	52.30
Sodium	57.80	60.21
Zinc	1.74	11.76
Manganese	1.84	15.70
Iron	9.49	9.67

DISCUSSION AND CONCLUSION

The various microorganisms reported have been observed by some other researchers who worked on related materials [13, 2, 14 and 5]. Some of the microorganisms are harmful while some are harmless. The harmless microorganisms could offer an alternative answer in protein production in the form of single cell protein (Protein from microorganism). Some of the harmful microorganism like *Aspergillus* and *Rhizopus* spp. formed black and blue patches on the "ugba" which may lead to Ugba spoilage. The aspergilla produce aflatoxins which are the most widely studied of all mycotoxins [15]. The various bacteria observed were able to utilize the high nutritional materials in the "ugba" for growth and proliferation.

It should be noted that these "ugba" are not prepared under aseptic conditions, so it could be contaminated by flies, air or even by the hands of the processors, containers and leaves during production [16].

Most of the microbes are thermotolerant that is why they survived both the hot water used in the production to wash the sliced Ugba and autoclave heat.

Fermented "ugba" had the highest microbial load observed in this work. The reason may be that, fermentation results in the production of intermediates that could be utilized by secondary invaders [17]. Thermophilic yeast like *Hansenula* and *Torulopsis* spp. were also isolated and they also aided in fermentation process. The pH value of the unfermented oil bean was found to be acidic (5.7). As the fermentation progressed, the pH becomes alkaline. This tallied with the findings of Njoku *et al.* [14], who reported that the biochemical activities involved in "ugba" fermentation tends to reduce the acidity of fresh unfermented "Ugba" thereby making it alkaline.

Considering protein and fat content of the oil bean seed, the results showed a decrease in both the percentage of crude protein and fat content (see Table 5). The unfermented "ugba" had higher values than the fermented ones. The decrease presumably may be as a result of the fermentative activities of the micro-organisms.

Both the fermented and unfermented "ugba" were relatively rich in K, Na, P, and Fe. It is therefore obvious that the "ugba" is rich in essential minerals and may contribute significantly to the dietary mineral requirement of pregnant/lactating/ mothers and infants.

The public health significance of the isolated organisms cannot be over emphasized. The various stomach complaints received after the consumption of some fermented "Ugba" should be due to the presence of some of the harmful microbes. Following the high percentage of protein, ash, fibre, fat and essential mineral content, its central position as a delicacy in parts of Eastern Nigeria cannot be discouraged. However, care should be taken during the preparation as the sample done in the laboratory has less microbial load when compared with that of traditional methods of preparation. As a result of this, rural education as regards to hygiene and sanitation should be taught to rural women who are the role producers of this food supplement.

Also there is need for a large scale cultivation of "ugba" plant since most of the old trees are producing little or nothing to supplement the high carbohydrate food intake of our people. The harmless micro-organisms could also offer an alternative answer in protein production in tomorrow's Nigerian Biotechnological development.

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