Microbiological, Nutritional and Organoleptic Changes Associated with Fermented Oil Bean (*Pentaclethra macrophylla* Benth) Seed (*ugba)* Stored at Ambient Temperature

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Abstract: *Ugba* is the Igbo name of the fermented African Oil bean seeds (*Pentaclethra macrophylla* Benth). It is a traditional food condiment generally produced by natural fermentation and is an important and cheap source of protein for people whose staple foods are deficient in protein. The study was carried out to determine the microbiological, nutritional and organoleptic changes associate with *ugba* when stored at ambient temperature. The oil bean seeds were processed traditionally and subjected to a four day spontaneous fermentation to produce the *ugba*. One gram of the fermenting and fermented samples was serially diluted and 0.1 ml aliquots was plated by spreading on appropriate media for the isolation of bacteria and fungi. The isolates were purified and then characterized. The *ugba* sample was then stored in an air-tight container for 7 days. Each day, the sample was examined for sensory properties and acceptability by a team of panelists. The bacterial isolates recovered during the fermentation were *Staphylococcus aureus*, *Escherichia* coli, *Proteus vulgaris, Pseudomonas aeruginosa, Bacillus* and *Lactobacillus* species while *Aspergillus niger*, *Saccharomyces cerevisiae, Rhizopus* species*, Candida* and *Fusarium* spp were the fungal isolates recovered from the sample. Only *Bacillus* spp and *Lactobacillus* spp were isolated at the end of the fermentation while *Saccharomyces cerevisiae* was the only fungus isolated at the end of the fermentation. The food borne pathogens and spoilage organisms were eliminated from the final product making the *ugba* safe for consumption. The *ugba* was found to be high in protein (27.64 %) and fat (24.93 %) but low in ash, fibre and carbohydrate. Acceptability of *ugba* was best on the 2nd and 3rd days of storage with acceptability scores of 79.22 and 78.9% respectively. Beyond the 4th day of storage, the *ugba* was not found acceptable for consumption. In conclusion, the production of *ugba* is by a mixed microbial flora. However, only health organisms GRAS (Generally Regarded As Safe) (*Bacillus* and *Lactobacillus* species) remained at the end of the fermentation as the contaminating food borne pathogens and spoilage organisms were eliminated from the final product making the *ugba* safe for human consumption. Fermentation also increased the protein and fat contents of the *ugba* thereby making it a good supplement for animal protein especially in rural areas where animal protein is very expensive to provide.

Key words: Fermentation, microbiological, nutritional, oil bean seed, sensory, storage

INTRODUCTION

Ugba is the Igbo name for sliced, fermented African oil bean seed (*Pentaclethra macrophylla* Benth). The African oil bean seed is called several names in Nigeria, such as Apara by the Yoruba, *Ugba* or *Ukpaka* by the Igbos (Enujiugha and Akanbi, 2005). It is consumed mostly in the Eastern States of Nigeria as a local delicacy commonly known as "African salad" prepared with oil, pepper, fish and salt. It could also be prepared with tapioca, stock fish and garden eggs. It can be eaten with boiled or roasted yam or cocoyam (Okafor *et al.,* 1991; Mbajunwa *et al.,* 1998). Fermentation of African oil bean seed involves the traditional technique of natural fermentation with microorganisms of substrate and environmental compositions. The aim of fermentation is to extend shelf life, inhibit pathogenic and spoilage microorganisms, impacts desirable sensory qualities, with enhanced nutritional values and aided digestibility. The ability to preserve food is directly related to the level of technological development.

The fermentation of oil bean seed for *ugba* production is by mixed microbial fermentation that occurs spontaneously through protein breakdown by several microorganisms which include *Lactobacillus* species, *Micrococcus* species, *Staphylococcus* species, *Leuconostoc mesenteroides, Proteus* species and *Escherichia coli* (Mbata and Orji, 2008). Past studies on the microbiology of fermentation of African oil bean seeds have identified *Bacillus* species as the major microorganism responsible for the fermentation. It was noted by Ogueke and Ariratu (2004) that *Bacillus* and *Proteus* species are proteolytic. So they dominate the fermentation process and thus are responsible for the observed increase in free amino acids (FAA) always recorded during the production of *ugba.* The major problem with *ugba* is the restricted availability due to its short shelf life.

During the fermentation process, *Bacillus subtilis* plays a role in modifying the substrate biochemically, nutritionally and organoleptically (Isu and Njoku, 1997). Although the predominant species responsible for *ugba* fermentation is *Bacillus subtilis,* other species including *B. pumilus, B. megaterium, B. lichenformis* have also been recovered from the product (Diawara *et al.,* 1998). Microorganisms involved in the production of *ugba* are introduced through the air, water, utensil, the handler or the plant leaves. So, no starter culture is used.

Different groups of bacteria belonging to the genera: *Bacillus, Micrococcus, Leuconostoc, Staphylococcus* and family members of Enterobacteriaceae have been reported by several authors as contributing to the individual fermentations (Enujiugha and Akanbi, 2008); (Obeta and Ugwuanyi, 1996). The unfermented seeds are known to harbor varieties of microbial species like *Aspergillus*, *Staphylococcus*, *Micrococcus*, *Penicillium*, and *Bacillus*. Although, it is believed that only bacteria species are involved in the fermentation of the seeds (Obeta, 1983). Other species disappear in the course of fermentation (Olasupo *et al.,* 2016).

An important value in the consumption of *ugba* is in addressing protein energy malnutrition (PEM) challenges and the case of its adoption by local producers. *Ugba* is currently gaining large acceptance and is consumed all round Nigeria as well as West African sub region (Anyanwu *et al.,* 2015). It has been recommended by previous researchers to be a good source of low cost palatable protein and has a great potential to serve a general condiment for the food like Okra soup. The aim of the present study was to determine the microbiological, nutritional and organoleptic changes associated with *ugba* stored at ambient temperature.

MATERIALS AND METHODS

Sample collection

Fifty (50) oil bean seed samples were purchased from Orie-Ugba market in Umuahia City and Ndoruu market Umudike, Abia State and transported in an air tight polythene bag to CES Laboratory, Ahiaeke for analyses.

Laboratory preparation and fermentation of oil bean seeds

The traditional technique used by local producers from Umuahia was followed for the preparation of the *Ugba* (Anyawu *et al.,* 2015). The seeds were sorted, cleaned and boiled in a pot for 2hours to soften the hard brown testa (shell). The shells were removed and the cotyledons were cooled, washed, drained and rewashed with tap water several times. The washed cotyledons were then cut into long thin slices with a sharp knife into 4-5 x 0.1-0.2 cm slices. The slices were boiled for another 30minutes, cooled, washed and then soaked in water for 24 hours. They were washed again to remove the bitter taste and then allowed to drain in a basket for 1 hour. The slices were wrapped in four small packets with banana leaves and lightly tied. These small packets were placed in an aluminum pot to ferment spontaneously at room temperature for 4 days leading to the production of *ugba*. Samples were aseptically taken from these

small packets of *ugba* at regular intervals for microbiological, proximate and organoleptic analyses.

Microbial Analysis of *Ugba* Sample

Ten-fold serial dilution of samples were done. Spread plate culturing techniques (Enujiugha and Badejo, 2002) was used to isolate and enumerate microorganisms in the sample. One gram of each processed and fermented *ugba* samples were aseptically taken, blended and homogenized in 9 ml of distilled water (10^{-1} dilution). Then serial dilutions of homogenates were made to 7 test tube and 0.1 ml aliquot was taken from 10^{-5} dilution and spread aseptically using the spread plate technique in triplicates on sterile Nutrient agar plates for total heterotrophic bacterial count and on MacConkey and Mannitol Salt agar media for determination of total coliforms and staphylococci counts. Samples were taken every 24 hrs every for determination of microbial succession during the fermentation time. The sample was also inoculated on Sabouraud Dextrose Agar (SDA) by spread plate method for fungal isolation. The media for bacteria isolation were incubated at 35^{o}C for 48 hrs while the SDA plates were incubated at 22^{o}C for 5 days. Inoculated media showing 30-300 colonies were counted using the digital illuminated Gallenkamp colony counter. Colony counts were expressed as colony forming units per gram of the sample. All counts were done in triplicate and average values were reported. The bacterial isolates were sub-cultured on appropriated media and incubated for 24 hrs at 35^{o}C to get pure cultures which were later identified using cultural, morphological, biochemical characterization using the schemes of Enujiugha and Badejo (2002).

Storage and Organoleptic Studies of *Ugba*

The *ugba* sample was stored for 7 days in an air tight sterile 500 ml glass beakers after fermentation. From there, samples were aseptically collected daily for both microbiological, nutritional and organoleptic studies.

Fungal Identification

A drop of methanol was placed on a clean slide and a portion of fungi growth was cut and was tested in the menthol. A drop of Lactophenol cotton blue was added, a cover slip was then placed on it gently to which it was observed under the microscope with x40 objective. The picture seen was compared with an identification chart (Cheesebrough, 2016).

Proximate Analysis of *Ugba*

The analysis of the sample for fat, crude protein, crude fiber, ash and moisture contents were determined using standard methods as described by Onwuka (2018); Halold *et al.*, 1991).

Moisture content determination

This was done using the gravimetric method. A measured weight of the sample (5g) was dispensed into a previously weighed moisture can. The sample in the can was dried in the oven at 105^{o}C for four (4) hours and cooled in a desiccator and weighed. It was returned to the oven for further drying during which it cooled and weighed at hourly interval until no further reduction in its weight (constant weight) was attained.

The formula below was used to calculate the moisture content.

Calculation:

$$\% \text{ moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where:

W_1= initial weight if the empty crucible

W_2= weight of crucible + sample before drying

W_3= final weight of crucible + sample after drying

Total solid = 100 - % moisture

Crude fibre content determination

Crude fibre was determined as the quantity of residue left after the removal of acid and alkali labile materials, fats and ash. The Weenade gravimetric method (Onwuka, 2018) was employed. A measured weight, 5g, of the sample was boiled in 150ml of 1.25% H_2SO_4 solution under reflux for 30minutes.

It was washed repeatedly with hot distilled water using a two-fold muslin cloth to retain the particles. Carefully, the washed sample was transferred quantitatively back into the flask and boiled for another 30minutes in 1.25% NaOH solution. Thereafter, it was washed again thoroughly in hot distilled water and flushed with normal hexam transferred with a crucible to remove any traces of fat , and dried in the oven for 30minutes 100^0C, cooled in a dessicator and weighed. The crude fibre contained in the sample was burnt out in the furnace (as in Ash determination) leaving the ash in the crucible which was cooled and weighed.

The formula below was used to calculate the crude fibre content.

$$\% \text{ crude fibre} = \frac{W_2 - W_3}{W} X \frac{100}{}$$

Where:
W = Weight of sample analysed
W_2 = Weight of sample and crucible after boiling, washing and drying
W_3 = Weight of crucible and sample after burning washed dried sample in furnace

Protein content determination

The protein content was determined by Kjeldahl method in which the nitrogen content is determined and multiplied with the factor 6.25 to obtain the protein content (Onwuka, 2018; Halold *et al*., 1991). One gram of the sample was weighed into a filter paper and transferred into a neat dried Kjeldahl flask. Exacted 10ml of Concentrated sulphuric acid was added to the flask in the presence of a pinch of selenium catalyst. The flask was heated gently in a fume cupboard using a heating mantle in an inclined position and allowed to digest (digest is complete when the liquid is clear and free from black or brown color). The flask was allowed to cool and was diluted with 100ml of distilled water. A distillation apparatus consisting of 500ml flask with stopper carrying a dropping funnel and a splash head adaptor and a vertical condenser in which a straight delivery tube is attached was used. Approximately 50ml of boric acid solution was measured into 500ml Erlenmeyer titration flask and a few drops of screened methyl red indicator were added and the Erlenmeyer flask placed on the receiving end of the delivery tube dipping just below the level of the boric level. Some anti-bumping agent granules and 75ml of NaOH solution were added to the distillation flask. Fifty ml of distilled water was added and was gently shaken to ensure mixing of contents. The flask was connected to distillation bulb and boiled vigorously until about 100ml of the distillate was obtained. The distillate was titrated with 0.1ml HCI till first trace of pink color

$$\% \text{ Protein} = \% N_2 \times 6.25$$

$$\% N_2 = \frac{100}{W} \times \frac{14 \times 0.02 \times (B-T) \times Vf}{1000 \quad Va}$$

Where W= weight of sample in grams
B = Titre value of reagent blank
T = Titre value of sample
Vf = Total value of digest
Va = volume of digest distilled

Fat content determination

This was determined using the continuous solvent extraction gravimetric method (Kurt *et al.,* 1996). Accordingly, a measured weight of the sample (5g) was wrapped tightly in a previously weighed 15cm porous paper (Whatman No 1 paper). The wrapped sample was put in a porous thimble and placed in a Soxhlet reflux flask. The reflux flask was mounted on to an oil extraction flask containing about 200ml of n-hexane.

The upper end of the reflux flask was coupled to a condenser. The solvent in the flask was heated to vaporize and condense into the reflux flask enveloping the wrapped sample (extracting the oil in it). When the reflux flask is filled up, the oil extracted siphons into the extraction flask and the cycle continues. The processes of vaporization, condensation, extraction and reflux was allowed to go on repeatedly up to fourteen (14) refluxes. The extracted wrapped samples were with the aid of a long pair of forceps and dried while the solvent was measured.

The fat content was calculated as the percentage weight of the oil extracted from the sample using the formula below

Calculation:

$$\% \text{ crude fat content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where:

W_1 = Weight of the empty filter paper

W_2 = Weight of the paper and sample before extraction

W_3 = Weight of the paper and sample after fat extraction and drying.

Ash content determination

Ash content was determined by the ash gravimetric method (Onwuka 2018, Nelson, 2003). In this regard, a measured weight of the sample 5g, was weighed into a previously weighed crucible and burnt to ashes in a muffle furnace at 500-550^{0}C for about 2hours (until a grey ash material was seen). The crucible and its content was cooled in a dessiccator and weighed.

By difference in weight, the weight of the Ash was determined and expressed as a percentage weight of the sample using the formula below.

$$\% \text{ Ash} = \frac{W_2 - W_1}{W} X\ 100$$

Where:

W = weight of sample analyzed

W_1 = weight of empty crucible

W_2 = weight of crucible and ash

Carbohydrate content determination

The nitrogen free extract method was used. Carbohydrate was calculated as weight difference between 100 and the summation of other proximate components as nitrogen extract (NFE) (Bo Milber, 2003)

Calculation: % N F E =100- % (a + b + c +d +e)

Where:

A= Protein

B= Fat content

C= Ash content

D= Fibre content

E= Moisture content.

Sensory Evaluation

The stored *ugba* sample was subjected to sensory evaluation analysis by 10 members of regular *ugba* consumers drawn from the university environment. The parameters evaluated included taste, appearance, aroma, texture and general acceptability. These parameters were evaluated after during the seven days the *ugba* was stored.

RESULTS

The morphological and biochemical identification (Table 1) of the bacterial isolates from the sample yielded the following organisms: *Bacillus* spp, *Lactobacillus* spp, *Staphylococcus aureus, Escherichia coli, Proteus vulgaris* and *Pseudomonas* spp.

The morphological characteristics of the fungal isolates from the sample is presented in Table 2. The isolates are *Aspergillus*

niger, *Candida* spp., *Fusarium* spp., *Rhizopus* spp and *Saccharomyces cerevisiae*.

The bacterial succession from the oil bean seed fermentation is shown in Table 3. Six bacteria isolates were recorded on the first day of fermentation and these are *Bacillus* spp, *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus* and *Lactobacillus* species. At the end of the fermentation, only *Lactobacillus* and Bacillus spp were isolated from the *ugba* sample.

The fungal succession during oil bean seed fermentation is presented in Table 4. Five isolates namely: *Rhizopus* spp, *Aspergillus niger*, *Penicillium* spp, *Fusarium* spp and *Saccharomyces cerevisiae* were recovered from the sample at the beginning of the fermentation. But at the end of the fermentation, only *Saccharomyces cerevisiae* was isolated from the sample.

In Table 5 is shown the proximate compositions of the *ugba* sample during fermentation. Moisture content was in the range of 31.99 - 38.01 % from Day 1 to Day 4 while protein was in the range of 22.17 to 27.64 %. For the same number of days, fat content was in the range of 22.58 to 24.93 %; fibre was in the range of 2.66 to 3.94 %; ash content was in the range of 1.60 to 2.08 while carbohydrate was in the range of 5.3 to 17.24 %.

Tables 6.1-6.5 show the results for the sensory evaluation of the *ugba* sample during the period of storage.

Table 7 has the mean scores of the sensory evaluation of the *ugba* sample based on the organoleptic properties. The *ugba* was most acceptable to the panelists at the 2nd and 3rd days of storage with mean acceptability scores of 7.13 and 7.10 respectively on a 9-Point hedonic scale. This gave acceptability scores of 79.22% and 78.9% respectively. The least acceptable *ugba* were the samples on the 1st and 4th days of storage with mean scores of 5.57(61.9%) and 5.30(58.9%) respectively. There was no significant difference between the values of the two *ugba* samples. From Day 5 to 7 of storage, the *ugba* was rejected by all the panelists and hence scored zero in all the parameters evaluated.

Table 1: Morphological and biochemical identification of bacterial isolates from *Ugba* production

Colony Morphology	Microscopy	Gram reaction	Catalase	Coagulase	Indole	Hydrogen sulphide	Oxidase	Citrate Utilization	Motility	Gas	Glucose	Lactose	Galactose	Mannitol	Sucrose	Fructose	Probable isolate
Dull green,spore forming colonies on blood agar	Long rods	+	+	N/A	-	+	N/A	+	-	-	AG	A	A	AG	A	A	*Bacillus* spp
Colourless, swarmy, flat and smooth colonies on blood characteristic pungent smell	Rod shaped	-	-	N/A	-	N/A	-	+	+	+	-	A	-	-	A	A	*Proteus vulgaris*
Golden yellow, round colonies on blood and mannitol agar	Irregular cocci in clusters	+	+	+	-	N/A	N/A	N/A	-	-	AG	A	A	AG	A	A	*Staphylococcus aureus*
Non-lactose fermenting, flat colonies with characteristic rusty metallic smell on MacConkey agar	Long, Slender rod	-	+	N/A	-	N/A	+	+	+	+	A	-	-	A	-	-	*Pseudomonas aeruginosa*
Raised, convex, opaque, long slander and smooth colonies	Long slender Rod shapes	+	-	-	N/A	+	-	-	+	-	AG	A	A	AG	A	A	*Lactobacillus* spp
Pink, irregular, smooth and entire colonies	Rod shaped	-	+	-	+	-	-	-	+	+	G	+	A	+	+	A	*Escherichia coli*

KEY:
+ = Positive
- = Negative
N/A = Not applicable
A = Acid

Table 2: Morphological identification of the fungal isolates from the *Ugba* sample

Macroscopic characteristics	Microscopic appearance	Possible Isolate
Black in colour, large mass of colony	Conidiospores aseptate with hyphae	*Aspergillus niger*
Velvety, fuzzy, green, dark in colour	Green spores with whitish hyphae	*Penicillium* sp
Brown-greenish to white-greenish with dark zonation	long and branched monophialides	*Fusarium* sp
Rapid growth, white to pale	Sparsely septate broad hyphae	*Rhizopus* sp
Moist, small shiny whitish colony	Oval, spherical shape	*Saccharomyces cerevisiae*

Table 3: Bacterial succession during *ugba* production

Days	***Lactobacillus* spp**	***E. coli***	***Proteus vulgaris***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Bacillus* spp**
Day 1	+	+	+	+	+	+
Day 2	+	+	+	+	+	+
Day 3	+	-	-	-	+	+
Day 4	+	-	-	-	-	+

KEY: + = Present;
- = Absent

Table 4: Fungal succession during *ugba* production

Days	***Saccharomyces cerevisiae***	***Rhizopus* spp**	***Aspergillus niger***	***Penicillium* spp**	***Fusarium* spp**
Day 1	+	-	+	-	+
Day 2	+	-	+	+	+
Day 3	+	+	+	+	-
Day 4	+	-	-	-	-

KEY: + = Present;
- = Absent

Table 5: Proximate composition of oil bean seed during fermentation

DAYS	**Moisture content**	**Protein content**	**Fat content**	**Fibre content**	**Ash content**	**Carbohydrate content**
1	31.99^{d} ±0.50	22.17^{d}±0.10	22.58^{c}±0.54	3.94^{d}±0.09	2.08^{c}±012	17.24^{d}±1.33
2	34.51^{c}±0.14	26.20^{c}±0.10	23.54^{b}±0.52	3.53^{c}±0.09	1.95^{b}±0.07	11.91^{c}±0.90
3	36.67^{b}±0.41	26.84^{b}±0.01	24.53^{a}±0.22	2.99^{b}±0.08	1.62^{a}±0.04	7.35^{b}±0.77
4	38.01^{a}±0.83	27.64^{a}±0.01	24.93^{a}±0.05	2.66^{a}±0.11	1.60^{a}±0.24	5.35^{a}±0.85

Values show means of triplicate analysis ± standard deviation. Figures with different superscripts in the columns are significantly different P (≤0.05)

Table 6.1: Sensory evaluation of the *ugba* sample (Day 1)

Panelists	Taste	Appearance	Aroma	Texture	General Acceptability
1	2	9	5	6	6
2	4	9	8	6	**7**
3	5	9	9	9	8
4	4	8	9	9	8
5	5	9	7	8	**7**
6	3	9	2	3	4
7	2	8	3	5	4
8	3	7	4	5	5
9	1	8	4	4	4
10	2	7	3	5	4
TOTAL	**27**	**83**	**54**	**60**	**57**
MEAN	**2.7**	**8.3**	**5.4**	**6.0**	**5.7**

Scale of Acceptability: 0 – Rejected. 1- 4 Poor acceptability. 5 - Average acceptability. 6 - 9 High acceptability

Table 6.2: Sensory evaluation of *ugba* sample (Day 2)

Panelists	Taste	Appearance	Aroma	Texture	General Acceptability
1	6	8	9	7	8
2	5	8	8	7	7
3	7	8	9	6	8
4	6	7	7	7	7
5	7	8	8	7	7
6	7	7	9	6	7
7	6	7	9	7	7
8	5	6	9	7	6
9	5	7	8	7	7
10	8	8	7	8	8
TOTAL	**62**	**74**	**83**	**69**	**72**
MEAN	**6.2**	**7.4**	**8.3**	**6.9**	**7.2**

Scale of Acceptability: 0 – Rejected. 1- 4 Poor acceptability. 5 - Average acceptability. 6 - 9 High acceptability

Table 6.3: Sensory evaluation of *ugba* sample (Day 3)

Panelists	Taste	Appearance	Aroma	Texture	General Acceptability
1	5	7	7	6	6
2	5	7	8	5	6
3	6	8	7	6	7
4	7	7	8	6	7
5	6	7	8	5	6
6	6	7	7	6	7
7	5	8	7	6	7
8	6	8	8	7	7
9	6	7	7	6	7
10	6	7	7	7	7
TOTAL	**58**	**73**	**74**	**60**	**69**
MEAN	**5.8**	**7.3**	**7.4**	**6.0**	**6.9**

Scale of Acceptability: 0 – Rejected. 1- 4 Poor acceptability. 5 - Average acceptability. 6 - 9 High acceptability

Table 6.4: Sensory evaluation of *ugba* sample (Day 4)

Panelists	Taste	Appearance	Aroma	Texture	General Acceptability
1	4	6	3	2	4
2	3	5	2	3	3
3	4	5	3	2	5
4	5	6	3	2	4
5	4	5	3	3	3
6	5	5	4	3	4
7	5	6	3	3	4
8	5	5	2	4	4
9	4	6	3	3	4
10	5	5	3	2	4
TOTAL	**44**	**54**	**29**	**27**	**37**
MEAN	**4.4**	**5.4**	**2.9**	**2.7**	**3.7**

Scale of Acceptability: 0 – Rejected. 1- 4 Poor acceptability. 5 - Average acceptability. 6 - 9 High acceptability

Table 6.5: Sensory evaluation of *ugba* sample (Days 5, 6 and 7)

	Taste			Appearance			Aroma			Texture			General Acceptability		
Panelists	Day 5	Day 6	Day 7	Day 5	Day 6	Day 7	Day 5	Day 6	Day 7	Day 5	Day 6	Day 7	Day 5	Day 6	Day 7
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
MEAN	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Scale of Acceptability: 0 – Rejected. 1- 4 Poor acceptability. 5 - Average acceptability. 6 - 9 High acceptability

Table 7: Mean scores of the sensory evaluation of the *ugba* sample during storage

Days	Taste	Appearance	Aroma	Texture	General Acceptability
1	2.73^{c}±0.25	8.10^{a}±0.53	5.30^{c}±0.26	5.83^{b}±0.76	5.57^{b}±0.11
2	6.23^{a}±0.25	7.13^{b}±0.11	8.20^{a}±0.17	6.80^{a}±0.26	7.13^{a}±0.23
3	5.93^{a}±0.11	6.73^{b}±0.25	7.30^{b}±0.26	6.10^{a}±0.96	7.10^{a}±0.17
4	4.47^{b}±0.50	3.73^{c}±0.25	2.97^{d}±0.50	2.90^{c}±0.17	5.30^{b}±0.26
5	0	0	0	0	0
6	0	0	0	0	0
7	0	0	0	0	0

Values show means of triplicate analysis ± standard deviation. Figures with different superscripts in the column are significantly different P(≤0.05)

DISCUSSION

Six genera of bacteria namely *Bacillus, Staphylococcus, Escherichia, Lactobacillus, Proteus* and *Pseudomonas* and five genera of fungi namely- *Saccharomyces, Rhizopus, Aspergillus, Fusarium* and *Penicillium* were isolated. *Ugba* processing involves a traditional method with mixed bacterial fermentation. Although, the oil bean seeds were boiled before fermentation, the

fermenting microorganisms must have come from the handling during slicing of the boiled seeds, the knife used, the wet water used in soaking the sliced oil bean seeds (Okorie and Olasupo, 2013). Traditional fermentation for production of *ugba* employs chance inoculation of starter culture. This renders the substrate vulnerable to contamination by pathogenic and spoilage microorganisms.

However, these mixed starter cultures contribute individually towards a better fermentation, flavor and aroma formation that are characteristic of well fermented *ugba*. The activities of these micro-organisms enhance the organoleptic properties of the consumed *ugba* delicacy. Fermentation process has a major aim in extending self-life, inhabiting spoilage and pathogenic microorganisms and imparting desirable sensory qualities with improved nutritional value and digestibility. The number and types of microorganisms isolated from this research is in agree with the works of Okorie and Olasupo, (2013); Nwokeleme and Ugwuanyi, (2015).

On bacterial succession during the oil bean seeds during fermentation, the result shows variations in the bacteria flora of the oil bean seeds during fermentation. Six bacteria isolated were recorded on the first day of fermentation. However, only *Lactobacillus* and *Bacillus* species persisted till the end of the fermentation. *Bacillus* species have been identified as the main microorganisms responsible for the fermentation of African oil bean seeds. They are proteolytic and therefore they dominated during the fermentation process. It was reported by Prabir *et al.* (2014) that species of *B. subtilis* group have been considered to be "Generally Regarded As Safe" (GRAS) by the U.S. Food and Drug Administration and their role in the fermentation of locust bean has been thoroughly investigated. *E. coli* and *Staphylococcus aureus* which are food borne pathogens was eliminated during the fermentation process. Bacteria such as *E. coli* and *Staphylococcus aureus* are capable of causing food-borne infections in the absence of major food pathogens such as *Clostridium perfringens, Clostridium botulinum, Salmonella, Shigella* and *Vibro* spp which were not isolated from the sample. Coagulase-positive *S. aureus* is known is of public health concern as it causes food poisoning (Frazer and Westhoff, 2000). *Pseudomonas aeruginosa* and *Proteus vulgaris* were lost after 48 hours of fermentation.

These two organisms which are known to cause food spoilages were also eliminated by microbial metabolites produced in the fermentation medium by Lactic Acid Bacteria LAB generally are known producers of metabolites such as organic acids, bacteriocins, peroxides, diacetyl which possess antimicrobial properties on food borne pathogens and these help to eliminate pathogens and spoilage bacteria during the fermentation (Foegeding *et al.*, 1992). The production of bacteriocins by these lactic acid bacteria is thought to support domination of the producer in the microbial community by competitive exclusion (Ogueke *et al.,* 2010). This means that fermentation makes *ugba* safe for human consumption. This result also shows that only gram positive bacteria were present till the end of the fermentation.

Study on fungal succession during the oil bean seeds fermentation revealed that although *Rhizopus* spp, *Aspergillus niger*, *Penicillium* spp, *Fusarium* spp and *Saccharomyces cerevisiae* were isolated at the beginning of the fermentation, *S. cerevisiae* was the only yeast isolated at the end of the fermentation. This result agrees with the findings of Odunfa and Oyewole (2010). *A. niger* which is a food borne pathogen known for the production of aflatoxins which cause aflatoxicosis was eliminated by the fermentation process. *A. niger* (a food borne pathogen) as well as *Rhizopus* spp, *Penicillium* spp and *Fusarium* spp which cause food spoilages must have been eliminated by the antagonistic effects of the metabolites produced by Lactic acid bacteria (Foegeding *et al*., 1992). This increases the safety of *ugba* for human

consumption as fermentation has been found to confer safety on food products (Achi, 2005).

From the values obtained on the changes in the proximate composition of the oil bean seeds during fermentation, there was a progressive significant increase (P≤0.05) in the moisture, protein (22.17 to 27.64 g/100g) and fat (22.58 to 24.93 g/100g) contents of the *ugba* up to the last day of fermentation. The increase in the protein content could be due to the breakdown of the fermenting bacteria and fungi on the product which thereby added the microbial cellular proteins (single cell proteins) to the protein content of the fermented oil bean seed. This indicates that the *ugba* samples are rich in amino acids which are the building block of protein. This finding agrees with the report of Omafuvbe *et al.* (2004). This is a positive effect of fermentation on the nutritional composition of *ugba* condiment.

There was also a progressive significant decrease (P≤0.05) in the ash (2.08 to 1.60 g/100g); carbohydrate (17.24 to 5.35 g/100g) and fibre (3.94 to 2.66 g/100g) contents of the *ugba* condiment. This finding is in agreement with the results of Omotosho *et al.* (2017). Ash content gives an idea of the mineral composition of the samples and shows that the samples are not rich in minerals. The changes in nutrient composition and in soluble nutrients during the fermentation of *ugba* could have been made possible by the enzymatic activities of the fermenting organisms as they utilize the nutrients for growth and development (Odibo *et al.,* 1990; Enujiugha, 2003).

Looking at the mean scores of sensory evaluation of *ugba* based on the organoleptic properties during the 7-days storage, the *ugba* was most acceptable to the panelists on the 2nd and 3rd days of storage with mean acceptability scores of 7.13 and 7.10 respectively on a 9 point hedonic scale.

CONCLUSION

This work has shown that the fermentation of oil bean seed for the production of *ugba* is by a mixed microbial flora. However, only health (GRAS, Generally Regarded As Safe) organisms (*Bacillus* and *Lactobacillus* species) remained at the end of the fermentation. Fermentation also increased the protein content (from 22.17 to 27.64) of the *ugba* thereby making it a good supplement for animal protein especially in rural areas where animal protein is very expensive to provide. It was also found that the *ugba* was best acceptable on the 2nd and 3rd days of storage.

RECOMMENDATION

Since *ugba* was found free from food borne pathogens, spoilage organisms and was found to be high in protein content, we recommend that more people should appreciate the healthy nature of the food and consume it more.

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