Comparative Production, Analysis and Shelf life Studies of Fermented African Oil Bean Seed

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Abstract: Bacillus species were isolated from Ugba produced from naturally fermented African oil bean seed. Proximate composition of the traditional Ugba and *Ugba* produced using controlled fermentation was carried out at different fermentation time (0hr, 24hr, 48hr, 72hr) and the results showed that the moisture content ranges from 53.66%±0.00% and 58.50±0.10%, the ash content ranges from 0.56%±0.04 to 2.73%±0.01, the protein content ranges from 13.56%±0.04 to 15.30%±0.01, the fat content ranges from 3.51%±0.01 to 4.15%±0.05b, the crude fibre ranges from 12.70%±0.01 to 14.36%±0.20 and the carbohydrate content ranges from 4.96%±0.04 to 13.93±0.01. Proximate analysis assay of the products showed that there was significant difference between the pure cultured Ugba and traditional Ugba at different fermentation time (0 hr, 24 hr, 48 hr, 72 hr).Microbial analysis was carried out on the unpreserved '*Ugba*' for 4weeks. Using standard microbiological procedures, the following *Bacillus* species were isolated; *Bacillus subtilis* and *Bacillus megaterium.* However, fermentation period was reduced from 72 hr to 48 hr using the two isolates as mixed culture for the fermentation process. Shelf-life studies of the samples using 10% brine solution showed that the preserved Ugbaand the unpreserved Ugba were significantly different in terms of their keeping quality (6 weeks and 5 days respectively). The unpreserved sample lost its colour, taste, texture and aroma after 5 days while the preserved sample maintained its sensory attributes even after six weeks of storage under room temperature.

Keywords: Fermented African oil bean seed, *Bacillus subtilis*, *Bacillus megaterium,* proximate composition.

INTRODUCTION

The African oil bean, popularly called *Ugba* in Nigeria is a tropical tree in the family *Leguminosae (Mimosoideae).* It is native to tropical Africa, although a representative of the genus exists in tropical South and Central America.

The oil bean seeds contain 4-17% carbohydrate, 44-47% oil which has been found to be rich in oleic acid (Nwokedi, 1975;Odoemelam, 2005) and linoleic acid (Onwuliri *et al*., 2004).Onwuliri *et al*.(2004)reported that the saturated fattyacid, lignoceric acid, occurred in high amounts constituting about 10% of the total fatty acid concentration. Kar and Okechukwu,(1978) reported oil content as low as 38%. Studies also revealed that the oil contains about 75% saturated fatty acids and 25% unsaturated fatty acid.

Studies on the fermenting bacteria of *Ugba* has been documented (Obeta, 1983;Odunfa and Oyeyiola, 1985; Ejiofor *et al*., 1987;Ogueke and Aririatu, 2004). The main fermenting microorganisms have been identified to be proteolytic *Bacillus* sp.notably, *B. licheniformis*, *B. megaterium*, *B. macerans*, *B. circulans* and *B. subtilis*the most predominant(Obeta, 1983).Other fermenting bacteria includes, coagulase negative *Staphylococcus* sp., *Micococcus*sp. *Leuconostoc mesenteroides*; *Lactobacillus plantarum*, *Streptococcus lactis*, *Proteus* sp., *Enterobacter* sp. and *E. coli*. Some workers isolated the yeasts *Candida tropicalis* and *Geotrichum candidum* during fermentation (Ejiofor *et al*., 1987).

The long fermentation time during the processing of *Ugba* for consumption have posed a challenge to the producers. The procedure is tedious and the yield is always small. In addition, *Ugba* fermentation has been left to chance thereby encouraging the growth and proliferation of undesirable organisms. The traditional methods of *Ugba* production had been discussed (Obeta, 1983; Odunfa and Oyeyiola, 1985;Njoku and Okemadu, 1989)). The poor keeping quality of this product is another major problem

encountered by the local processors. The product has a shelf-life of about three (3) days, which implies that within three days of production, the processor has to dispose off his product (Ogbulie *et al*.1998).

MATERIALS AND METHODS

Collection of Samples

The African oil bean seeds were bought from Afor Enyiogu market in Aboh-Mbaise LGA in Imo state, Nigeria. They were visually inspected and defective seeds were discarded. All the chemicals and reagents used in this study were of analytical grade.

Ugba Preparation

Ugba was prepared according to the method of Njoku and Okemadu (1989). The seeds were boiled for 6 hrs and the hard coats dehulled. The cotyledons were cooled, washed to remove dirt and impurities, and then sliced into 4-5x0.1 -0.2 cm.

The cotyledons were washed repeatedly to eliminate the bitter taste and most of the anti- nutrients, then boiled again for another 2 h, cooled and soaked in water for about 12 h. Cotyledons were washed and allowed to drain for 1 h in a basket lined with banana leaves (*Musa sapientum* Linn) and wrapped in portions of about 50 g of slices using another leaf *Mallotus oppositifolius* and incubated for 72 hrs at room temperature 35° C± 2° C.

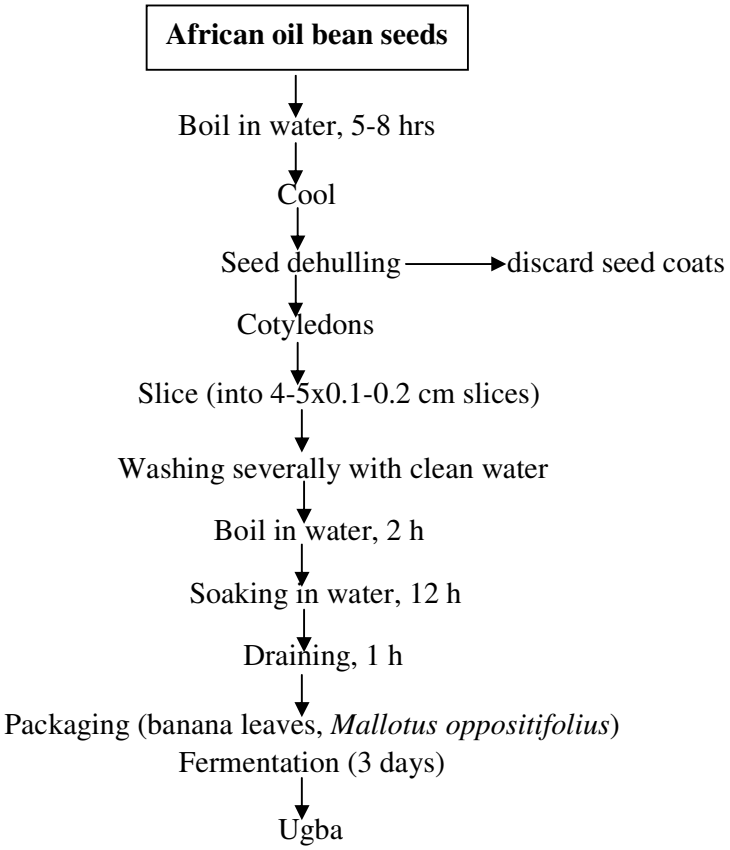

Fig. 1: Flow diagram for the production of Ugba (Njoku and Okemadu (1989)

Isolation of Pure Cultures

Fermenting samples were taken aseptically (using sterile forceps) from the fermenting beans after 72hrs. Ten grams of the sample was transferred into 90mL of sterile 0.1% peptone water as diluent. Subsequent decimal dilutions (10^{-1} 10^{-2},10^{-3}, 10^{-4} ,10^{-5} and 10^{-6}) were made from this diluent. Aliquot portion(0.1ml) from the 10^{-6}dilution was inoculated into pre-sterilized and surface dried nutrient agar medium. Inocula were spread evenly to ensure uniform and countable colonies, Plates were incubated at ambient temperature for 48hours for heterotrophic bacteria (Cheesbrough,2000; Beishir,1987). Colony counts obtained on the media were expressed as colony forming units per gram(CFU/g).

Characterization and Identification of Microbial Isolates

Representative colonies of the isolates were streaked on nutrient agar medium and incubated at ambient temperature for 48hours. Pure cultures resulting from the isolation were subculture and preserved on nutrient agar slants at 4^{o}C. Microbial isolates were characterized based on cultural (colonial), microscopic and biochemical methods with reference to standard manuals(Cheesbrough,2000, Beishir, 1987). The identities of the isolates were cross matched with reference to standard manuals for the identification of bacteria(Buchanan and Gibbon,2000).

Preparation of Pure Culture Isolates as Starter Cultures

Pure cultures of *Bacillus subtilis* and *Bacillus megaterium* isolated from the naturally fermented '*Ugba*' and identified by standard microbiological procedure were inoculated independently into 20 mL sterile 0.1% peptone water and incubated for 24 hrs. After incubation, the cultures were centrifuged and the culture pellets washed repeatedly with sterile saline and distilled water and an aliquot spread on freshly prepared medium to ascertain for viability, purity and biomass of the cells.

Pure Culture Fermentation

Each separate flask was aseptically inoculated with 1 mL suspension of *B. subtilis* and*B. megaterium* using a sterile micropipette and mixed thoroughly with the *Ugba*. Fermentation was allowed to proceed for 72 hr at room temperature with occasional agitation of the flask. To check for purity of the process during the period of fermentation, 1 g of the fermenting slices were removed at 24-hr interval and at the end of fermentation and agitated in 9 mL 0.1 % sterile peptone water. Smear of the sample was streaked on nutrient agar plate for isolation and characterization.

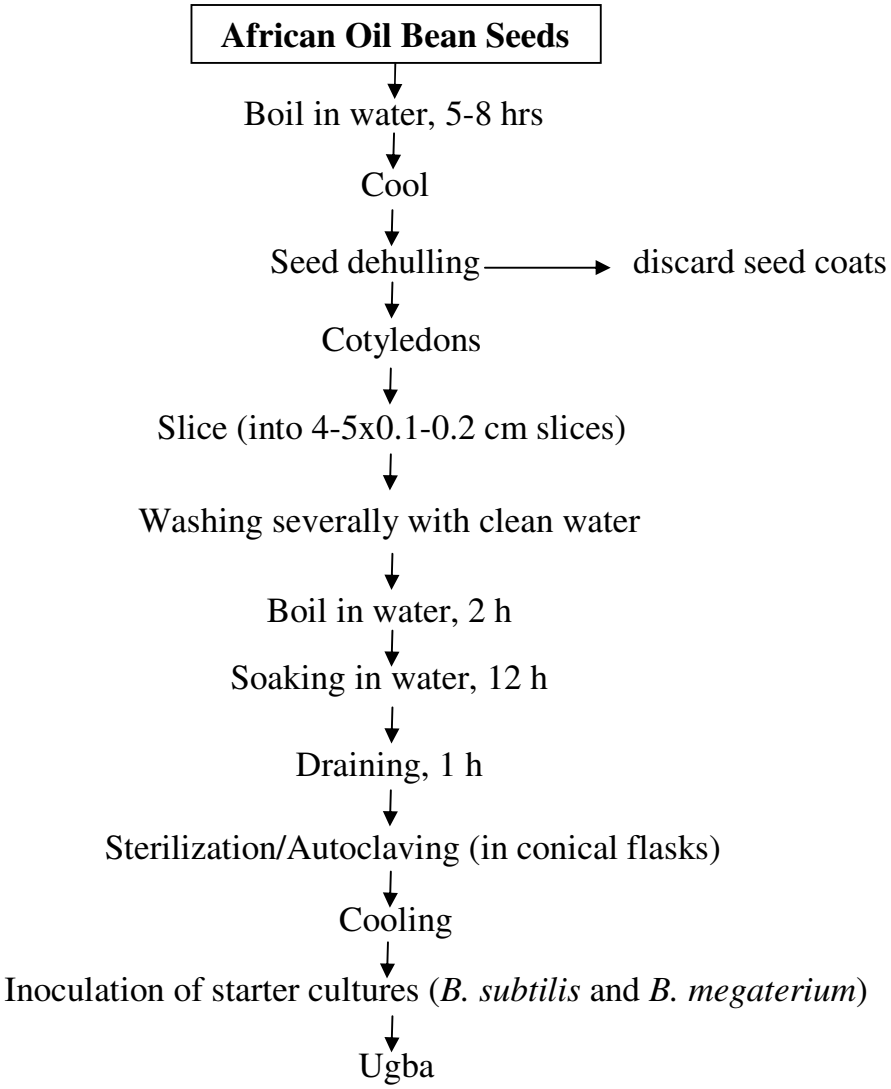

Fig 2: Flow diagram for the production of Ugba from pure cultures (Njoku and Okemadu 1989)

PROXIMATE ANALYSIS OF UGBA

The proximate analysis of the naturally fermented '*Ugba*' and '*Ugba*' prepared from pure cultures was carried out using the standard method of the Association of Official Analytical Chemists (AOAC, 2000).

Ash Content

Two grams (2 g) of the '*Ugba*' was weighed into a crucible of known weight and the crucible placed in a muffle furnace and incinerated at a temperature of 550 °C for 3 hours until the sample turned white and free from carbon. The ash was withdrawn from the furnace and cooled in a desiccator to room temperature and weighed immediately. The mass of the residual incinerate was calculated as % ash content as follows:

% Ash content = mass of ash/mass of original sample x 100

Moisture Content Determination

The moisture content was determined by weighing out 5 g of the '*Ugba*' into a dry petri dish of a known mass, placed in the oven at a temperature of 105 °C for 3 hours. The dried samples withdrawn from the oven and placed in a desiccator to cool and reweighed using the analytical balance. The process was repeated until a constant mass was obtained. The difference in mass as percentage (%) moisture was calculated thus;

% Moisture =(M2-M3)/(M2-M1) x 100

Where; M1= Mass of dish

M2= mass of dish + sample before drying

M3= mass of dish + sample after drying.

Where;

W1= weight of sample before incineration

W2= weight of sample after incineration

W3= weight of original sample

Crude Protein Analysis

The crude protein was determined by Micro Kjeldahl method of standard procedure (A.O.A.C, 2000). Few boiling regulators (glass bead) was placed in the kjeldahl flask, followed by the addition of 15 g of potassium sulphate and 0.5 g of copper sulphate into the kjeldahl and 2 mg of the sample was weighed out on a grease-proof paper, wrapped and transferred to the kjeldahl flask, followed by the addition of 25 ml concentratedsulphuric acid and mixed by gently swirling. The kjeldahl flask was placed on the heating device of kjeldahl apparatus at an angle of 40° from the vertical in a fume hood and heated gently until foaming has ceased. The content in the kjeldahl flask was digested by boiling vigorously until the solution is clear and a light blue-green colour obtained, cooled to room temperature, diluted with 50 ml of

%Nitrogen= Vs – Vb K N acid X 0.01401 X 100/W

Where; Vs= Volume (ml) of acid required to titrate sample

Vb= volume (ml) of acid required to titrate blank

Crude Fibre Determination

Two grams (2 g) of '*Ugba*' was defatted with petroleum ether and boiled in 200 ml of a solution containing 1.25 g H_2SO_4 per 100 ml solution for 30 minutes. The boiled sample was washed with hot water using a twofold muslin cloth to retain particles. The retained particles wasreturned to the flask and boiled again in 200 ml of 1.25 g of NaOHper 100 ml of solution and washed again with hot water and allowed to dry at 105° C to a constant weight and subsequently placed in a muffle furnace at 550° C for 4 hours and finally cooled in a desiccator and reweighed. The mass of the fiber was determined by difference in given by; % Crude fiber =W1-W2/W3 X 100

deionized water transferred to 100ml volumetric flask and made up to the mark with deionized water.

After the digestion, 20 ml of 2% boric acid solution and 2 drops of methyl red indicator was added into the receiving flask under the condenser of the distillation apparatus so that the outlet of the adopter of the delivering tube extended below the surface of the boric acid solution. 10 ml of the digest was transferred into the distillation flask followed by the addition of 35 ml of 40% NaOH and the flask attached immediately to the splash head of the distillation apparatus. The mixture was distilled until 30 ml of the distillate was collected and the conical flask lowered before the distillate collected was terminated. The distillate collected was titrated against 0.1N HCL and the titre value recorded. The blank experiment was set up with all the materials in the procedure above except the sample and the titre value also recorded. The protein content was calculated using the formula below;

N acid = Normality of acid

W= weight of samples in grams

% Crude protein = N X Conversion factor

Where N= Nitrogen

100% Nitrogen in protein = conversion factor that is; 100/16 = 6.25

Crude lipid determination

A soxhlet extraction unit with reflux condenser and a small round bottom flask (250 ml) was used. The flask was weighed after washing and drying and half filled with petroleum ether (boiling pt. 40 – 60 °C) and then fixed back into the unit. Two grams (2 g) of the '*Ugba*' was wrapped with a Whitman filter paper and gradually lowered into the sample holder in the reflux flask. The sample was slowly heated with a heating mantle for 5 hours. During this period, the petroleum ether boiled, %Lipid content = mass of lipid/ mass of sample x 100

Carbohydrate Content Determination

The carbohydrate was calculated as weight difference between 100 and summation of other proximate parameter;

Where M = moisture

P = protein

Microbial Analysis of Ugba

Bacteria isolates from naturally fermented Ugba were characterized and identified with reference to standard manuals (Beishir, 1987;Cheesbrough, 2000). Colony counts obtained on the media were expressed as colony forming units per gram (Cfu/g) to obtain total population (Harrigan and McCance, 1987; Buchanan and Gibbon, 2000).

Sensory Evaluation

Sensory evaluation of the '*Ugba*' samples were determined using a ten-man taste panel comprising elderly women of Mbokecommunity in Ihiagwa, Owerri-West LGA, Imo state, Nigeria.The rating test methods were used and scoring were done using a 5point Hedonic scale. Each panelist was provided with 2g of the fermented *Ugba* to freely evaluate based on taste, aroma, colour, texture, sliminess and overall acceptability. Scoring was done as,5- very good; 4- good; 3- fair;2- poor and 1-very poor/ unacceptable. The score for the samples was analyzed statistically using the analysis of variance – ANOVA tool.

evaporated and covered the sample in the reflux flask and fluxed back to the boiling flask carrying oil. The sample was removed from the sample holder and the apparatus reconnected. Refluxed petroleum ether was recovered by evaporating and the flask containing the lipids was dried in the oven at a temperature of 60 °C for 30 minutes to remove residual solvent, cooled in a desiccator and reweighed. By differences, the amount of oil extracted was determined by difference in weight and thus expressed as percentage as shown below;

Nitrogen Free Extract (NFE (percentage carbohydrate = 100 – (M + P + F + A + F2)

F = fat

F2 = Crude fiber

Shelf-Life Determination

Naturally fermented '*Ugba*' and *Ugba* produced using controlled fermentation were aseptically kept for 4 weeks on the laboratory shelf. Microbiological and organoleptic properties were determined weekly starting from day zero. Microbiological analysis was carried out by transferring ten grams of the Ugba sample into 90ml of the diluents and serially diluted until 10^{-6}dilution was obtained. Aliquot portion (0.1ml) o this dilution was inoculated into the pre-sterilized and surface dried nutrient agar medium. This was incubated at ambient temperature for 48hours. Only plates showing between 30 and 300 colonies were counted. Colony counts obtained on the media were expressed as colony forming units per gram(CFU/g) to obtain total population. The organoleptic properties were determined through sensory evaluation of the Ugba samples using a ten-man taste panelist consisting of elderly women of Mboke village community in Ihiagwa, Owerri-West Imo State, who are very familiar with Ugba. Each panelist was either regular or

occasionally a consumer of Ugba and was not allergic to it. The rating test method was used and scoring was done using a 5-point Hedonic scale. Each panelist was provided with 2g of the test samples and were asked to freely evaluate the coded samples for taste, aroma, colour, texture, sliminess and overall acceptability, comment and score the samples using the scale as follows; 5-very good,4-good, 3-fair, 2-poor,1- very poor/unacceptable. The score or the samples was analyzed statistically using the method of analysis of variance- ANOVA.

Shelf-Life Extension

The shelf-life assessment of the natural and stimulated *Ugba* was preserved in different concentrations of 10% sterile sodium chloride solution. This did not only extend the shelf- life of the Ugba but also preserved its sensory attributes. This was carried out by weighing 20g of the different Ugba samples into plastic containers that are air tight containing 10% sodium chloride solution. The set up was kept on the shelf at ambient temperature for 6 weeks.

Statistical Analysis

The data collected were subjected to analysis of variance (ANOVA). The mean separation values were determined using a Fischer LSD test. Significant difference was defined as $p< 0.05$.

RESULTS/DISCUSSION

The percentage moisture contents of the traditional Ugba and '*Ugba*' produced usingcontrolled fermentation increased as the fermentation time increased. The moisture content of the traditional Ugba and '*Ugba*' produced by controlled fermentation was 53.66% ±0.00% and 57.24±0.05% at 48 hrs.Respectively. It increased progressively to 54.08±0.02% and 58.50±0.10% on the third day (72 hrs).These values were slightly higher compared to the value reported by Pierson *et al*.(1986) which was 46.80%. However, Balogun, (2013), who worked on African oil bean seed which was fermented for 72hrs (3 days) reported a higher moisture content of 95.00%. However, Okechukwu (2012) reported a moisture value of 51.88% for a sample which was fermented for 5 days. High moisture contents of the samples as the fermentation time increased would affect the keeping quality of the final product (Njoku and Okemadu, 1989), The unfermented sample had the least value percentage moisture content of 52.33 ± 0.7%. The moisture content of the samples significantly ($p<0.05$) differed from each other. This is in agreement with results reported by Nwachukwu, et al.(2018).

The ash content of the samples ranged from 0.562±0.04 to 8.22±0.00 and is significantly [$P<0.05$] different. Samples UFO and TU72 had the highest and least ash contents respectively. It can be observed that as the fermentation of the Traditional '*Ugba*' and stimulated '*Ugba*' progresses, the ash contents decreased. The decrease in ash content was significantly [$P<0.05$] high. This indicates that the mineral contents of the samples will be reduced as the fermentation progresses (Okorie and Olasupo, 2014).

The crude fiber contents of the samples decreased during fermentation of the samples. The unfermented '*Ugba*' had the highest percentage crude fiber content of 15.50±0.10% and significantly ($P<0.05$) different from others. The percentage ash content of the samples PU48 (0.80±0.00%) and TU72(0.56 ±0.04%) were significantly ($P<0.05$) the same. Sample PU72 had the least percentage ash content of 0.73±0.03%.

The crude protein of the traditional Ugba and Ugba produced using controlled fermentation increased as fermentation progresses after 48 hours. The crude protein of the stimulated '*Ugba*' fermented for 48hours had the highest percentage protein content of 15.30±0.01%. The percentage protein content of the samples is significantly ($P<0.05$) different except for samples PU24 and TU48 with percentages of 14.47±0.03% and 14.53±0.02% respectively.

Food fortification with '*Ugba*' sample PU48 would go a long way in eradicating protein deficiency and malnutrition (Enujiugha and Akambi, 2008).

Crude fat of samples ranged from 3.51±0.01% to 4.15±0.05%with samples PU72 and UF0 having the least and the highest percentage crude fat content respectively. The crude fat content of the samples was significantly ($p>0.05$) differentexcept for UF0 which was significantly ($p<0.05$) same with sample TU24.

The carbohydrate contents of the '*Ugba*' sample were significantly ($p>0.05$) different from each other. This is in agreement with the values reported by Nwachukwu*,et al*.(2018). The percentage carbohydrate content of the samples did not follow a particular trend as fermentation progresses. The percentage carbohydrate content of the sample TU72 (13.93±0.01%) and TU24 (4.96±0.04%) had the highest and the least values respectively.

The '*Ugba*' samples were evaluated for consumer's acceptance and market value as shown in Table 2. Taste of the '*Ugba*' samples ranged from 1.00±0.00 to 4.67±0.58. At the fourth week the taste of the stimulated '*Ugba*' drastically dropped. The variations in the taste could be due to development of flavor compounds.

The aroma of traditional '*Ugba*' ranged from 2.00±1.53 to 3.67±1.53 and that of stimulated '*Ugba*' 2.67±1.53 to 3.67±1.53. The stimulated '*Ugba*' has an aroma values that were high at the first and second week and decreased at the third and fourth week This was evaluated using a ten-man taste panelist. The rating test method was used and scoring was done using a 5-pointHedonic scale as follows; 5-very good,4-good, 3-fair, 2-poor, 1- very poor/unacceptable. The mean scores of the aroma of the traditional and pure culture fermented Ugba were analyzed statistically using the method of analysis of variance-ANOVA.

The colour and the texture value of the traditional '*Ugba*' and '*Ugba*' produced using controlled fermentation decreased from the third week. This couldbe as a result of the degradation of bio chemical compounds (Oguntoyinbo, 2007). The colour of the pure cultured *Ugba* and traditional *Ugba*was highly acceptable at the first week. The colour value of the stimulated was rated very high on the second week (4.67±0.58) while that of the traditional '*Ugba*' was very low (2.33±1.16). The texture value of the traditional '*Ugba*' was very high (5.00±0.00) on the first week while that of the stimulated '*Ugba*' was high (3.67±1.15) on the same week. In the first week the score for the slimness of the stimulated '*Ugba*' was higher (4.00±0.00) than that of the traditional '*Ugba*' on the same week (1.67±0.57).

The slimness of the traditional '*Ugba*' was very high on the second week (3.00±1.00) than the stimulated *Ugba*(1.00±0.00)and significantly ($p>0.05$) different. Ogueke and Aririatu (2004), reported that sensory evaluation studies showed that the scores for colour, odour, texture/sliminess and taste at 0hr-72hr were significantly different from the scores of the same parameters at 96hr-120hr.

Table 3 shows the total colony counts of bacteria isolated from the *Ugba* samples on nutrient agar during the shelf-life study. The microbial load in week one was higher in the naturally fermented *Ugba* (2.69×10^7) than the stimulated *Ugba* (1.89×10^8). This trend continued in week two to week four as shown in Table 3. However, Ogueke, *et al* reported that bacterial counts increased from 5.0×10^3 at 0hr to 4.7×10^9 at 120hr.

The characteristics of the microbial isolates is shown in Table 4. Both indigenous and contaminating bacteria were isolated from the naturally fermented *Ugba*. *Lactobacillus* and *Bacillus* species are traditional bacteria involved in the fermentation of the *Ugba.* *Pseudomonas* and *Staphylococcus* species are proteolytic bacteria present as contaminants.

*Serratia*is a soil borne bacteria and probably carried over from harvesting of the *Ugba*seed.After six weeks of preserving *Ugba*sample in 10% brine solution, it was observed thatthere was an increase in salinity in the taste of the traditional Ugba and '*Ugba*' produced using controlled fermentation probably due to the process of reverse osmosis which eventually equalized both the '*Ugba*' and the brine solution to the same salinity. The brine decreased slightly and the '*Ugba*' increased in salinity. The aroma of the samples remained the same. The texture of the '*Ugba*' sample was still hard and there was zero sliminess due to the presences of salt which removes water from the *Ugba*. There was a slight change in colour from light brown to dark brown. This is in agreement with what was reported by Ogueke and Aririatu (2004).

Proximate Composition of the *Ugba* Samples at Different Fermentation Time

Table1: Mean Proximate Composition of the *Ugba* Samples produced from African oil bean seed.

Samples	%Moisture Content	%Ash Content	%Crude Fiber	%Crude Protein	%Crude Fat	%CHO
UF (0hr)	52.33±0.70g	8.22±0.00a	15.50±0.10	13.13±0.02	4.15±0.05a	6.70±0.00f
TU (24hr)	53.66±0.00f	2.27±0.03c	14.36±0.20c	13.56±0.04e	4.10±0.02ab	4.96±0.04g
PU (24hr)	55.87±0.03c	2.73±0.01c	14.36±0.20e	14.47±0.03c	4.15±0.05b	8.08±0.02e
TU (48hr)	55.08±0.02d	0.68±0.02f	13.57±0.03d	14.53±0.02c	3.94±0.01c	12.73±0.07b
PU (48hr)	57.24±0.05b	0.80±0.00d	13.15±0.03e	15.30±0.01a	3.70±0.00e	10.23±0.02c
TU (72hr)	54.08±0.02e	0.56±0.04g	13.10±0.02e	14.00±0.00d	3.80±0.01d	13.93±0.01a
PU (72hr)	58.50±0.10a	0.73±0.03e	12.70±0.01f	14.87±0.07b	3.51±0.01f	8.85±0.00
LSD	0.54	0.02	0.06	0.11	0.07	0.50

Mean ± standard deviation of sensory evaluation score.

UF(0hr) = Unfermented '*Ugba*' at 0 hr. of fermentation; TU(24hr) =Traditionally produced '*Ugba*' at 24 hr. of fermentation; PU(24hr.) = Pure cultured '*Ugba*' at 24 hr. of fermentation; TU(48hr) =Traditionally produced '*Ugba*' at 48 hr. of fermentation; PU(48hr) = Pure cultured '*Ugba*' at 48 hr. of fermentation; TU(72hr) =Traditionally produced '*Ugba*' at 72 hr. of fermentation; PU(72hr) = Pure cultured '*Ugba*' at 72 hr. of fermentation.

Proximate composition of *Ugba* sample at different fermentation time is shown in Table 1. Means with the same subscripts within a column are not significantly different (P<0.05).

Table2: Mean Sensory Evaluation for shelf life determination of the *Ugba*Samples produced From African oil bean seed.

Sample	Taste	Aroma	Colour	Texture	Sliminess
TU1	3.00±1.00b	3.33±0.58ab	4.33±0.58a	5.00±0.00a	1.67±0.57c
PU1	3.00±1.73b	3.67±1.53ab	4.33±0.58a	3.67±1.15b	4.00±0.00a
TU2	3.00±0.00b	3.33±1.52ab	2.33±1.16b	1.67±0.58cd	3.33±1.15a
PU2	4.67±0.58a	4.67±0.58a	4.67±0.58a	3.00±1.00bc	3.00±1.00ab
TU3	4.67±0.58a	3.67±1.53ab	4.33±0.58a	3.00±0.00bc	2.00±1.00bc
PU3	3.00±0.00b	2.33±1.53b	2.67±0.58b	1.33±0.58d	1.00±0.00c
TU4	3.00±0.00b	2.00±1.53ab	2.67±0.58b	2.00±0.10cd	3.00±1.00ab
PU4	1.00±0.00c	2.67±1.53ab	1.00±0.00c	1.00±0.00d	1.00±0.00
LSD	1.09	2.10	1.21	1.13	0.92

Mean ± standard deviation of sensory evaluation score.

Means with the same subscripts within a column are not significantly different (P<0.05)

TU1= Traditional '*Ugba*' on the first week of fermentation; PU1= Pure cultured '*Ugba*' on the first week of fermentation; TU2= Traditional '*Ugba*' on the second week of fermentation; PU2= Pure cultured '*Ugba*' on the second week of fermentation; TU3= Traditional '*Ugba*' on the third week of fermentation; PU3= Pure cultured '*Ugba*' on the third week of fermentation; TU4= Traditional '*Ugba*' on the fourth week of fermentation; PU4= Pure cultured '*Ugba*' on the fourth week of fermentation.

Table 3. Total counts (Cfu/g) of Bacteria isolated from natural (traditional) and pure culture fermented Ugba samples on Nutrient Agar.

Sample Code	Total Count
TU1	1.89×10^8
PU1	2.11×10^7
TU^1	2.01×10^8
PU^1	2.69x107
TU2	4.9×10^8
PU2	1.69×10^9
TU3	9.6×10^{10}
PU3	2.51×10^{11}
TU4	1.68×10^8
PU4	1.3×10^8

Where TU = Traditional fermented ugba, PU = pure culture fermented ugba, 1 = 1st week, 2 = 2nd week, 3 = 3rd week, 4 = 4th week

Table 4: Biochemical and sugar Fermentation of Bacterial isolates From Natural Fermented Ugba

CAT	OXI	COA	IN	MR	VP	Cit	URS	NO_3	GLU	SUC	LAC	MAL	XY	Probable identity of isolates
+	-	-	-	-	+	+	-	+	+	+	+	+	+	*Serratia* sp
+	+	-	-	+	-	+	-	-	+	+	-	-	+	*Pseudomonas aeruginosa*
+	-	-	-	-	+	-	+	+	+	+	+	+	-	*Staphylococcus aureus*
+	-	-	-	-	+	+	-	+	+	+	-	-	+	*Bacillus megaterium*
-	-	-	-	+	-	+	-	-	+	+	+	-	-	*Enterococcus faecalis*
+	-	-	-	-	+	+	-	+	+	-	-	+	-	*Bacillus subtilis*
-	-	-	-	+	-	+	-	-	+	+	-	-	+	*Lactobacillus* sp

CAT, catalase; OXI, oxidase; COA, coagulase; IN, indole; MR, methyl red; VP, Vogesproskaeur, CIT, citrate, URS, urease production; No_3; nitrate reduction; GLU, glucose; SUC, sucrose; LAC, lactose; MAL, maltose; XY, xylose.

CONCLUSION

This study has shown that *Bacillus* species are the major fermenting organisms for the production of '*Ugba*' and could therefore be used as starter cultures for a controlled fermentation of the product. This study also showed that fermentation does not only remove the anti- nutrients in Ugba but also add value to it by increasing its nutritional composition since the proximate composition of the product increased as the time of fermentation increased. However, this increased its moisture content thereby making it vulnerable to spoilage. Preservation of the product by the use of brine solution significantly improved the keeping quality of the '*Ugba*' samples. The application of this finding will mitigate the problem associated with poor keeping quality of the product being reported by the local producers

REFERENCES

Achinewhu, S.C., (1982). Composition and food potential of African oil bean (*Pentaclethra macrophylla*) and velvet bean (*Mucunauriens*). *Journal of Food Science.,***47**: 1736-1737.

Adam, M.R. and Mass M.O., (1999). Food Microbiology. 2nd Edn., Royal Society of

Chemistry, Cambridge, ISBN: 978-0-85404-284-5, pp: 390.

Balogun, B.I. (2013). Evaluation of the nutritional potentials of fermented oil bean seed (*Pentaclethra macrophylla* Benth). *Production,Agriculture and Technology Journal.***9**(2).73-87.

Beishir,I.(1987). Microbiology in Practice. A Self –Instruction Laboratory Course. Fourth Edition. Harper and Row Publishers, New York, pp 96-111, 120-130, 238-272.

British Pharmacopoeia Commission, (1993).Biological assay of antibiotics. *Br. Pharmacopoeia*, **2**: A165-A169.

Buchanan, R.E and Gibbon, N.E (2000). Bergeys Manual of Determinative Bacteriology, Williams and Wilkens, Baltimore, USA. Pp 10-50.

Ejiofor, M.A.N., Oti, E. and Okafor J.C., (1987). Studies on the fermentation of seeds of the African oi lbean tree (*Pentaclethra macrophylla*). *International. Tree Crops Journal*., **4**: 135-144.

Fogarty, W.M. and Griffin, P.J., (1973). Production and purification of metalloproteases of *Bacillus polymyxa*. *Applied Microbiology*., 26: 191-195.

Kar, A. and Okechukwu, A.D., (1978). Chemical investigations of the edible seeds of *Pentaclethra macrophylla* Benth. *Plant Foods Human. Nutrition*, **28**: 29-36.

Mbadiwe, E.I., (1978). Nutritional evaluation of seeds of *Pentaclethra macrophylla*. *Plant Foods Human. Nutrition.,***28**: 261-269.

Mbata, T. and Orji, M.U., (2008). Process optimization in the production and preservation of Ugba, a Nigerian fermented food. *International. Journal of Microbiology*., **4**: 2-6.

Njoku, H.O. and Okemadu, C.P. (1989). Biochemical changes during the natural fermentation of the African oil bean (*Pentaclethramacrophylla*) for the production of Ugba. *Journal of Science. Food and Agriculture*., **49**: 457-465.

Nwachukwu, C.D, Enyoh, E.C, Enyoh, C.E and Amobi, C.E.(2018). Effect of fermentation time on the proximate and mineral composition of fermented African oil bean seed 'Ugba'.*Sustainable food production,***2.** 13-20.

Nwokedi, C.I.C., (1975). *Pentaclethra macrophylla Benth* a potential oil seed. Tech. Report No. 14. Nigerian stored Product Research Institute.

Obeta, J.A.N., (1983). A note on the microorganisms associated with fermentation of seeds of the African oil bean tree (*Pentaclethramacrophylla*). *Journal of Applied Bacteriology*., **54**: 433-433.

Odoemelam, S.A., (2005). Proximate composition and selected physicochemical properties of the seeds of African oil bean (*Pentaclethramarcrophylla*). *Pakistan. Journal of. Nutrition*., **4**: 382-383.

Odoemelam, S.A., (2005). Proximate composition and selected physicochemical properties of the seeds of African oil bean (*Pentaclethramarcrophylla*). *Pakistan. Journal of. Nutrition*., **4**: 382-383.

Odunfa, S.A. and Oyeyiola, G.F., (1985). Microbiological study of the fermentation of ugba, a Nigerian indigenous fermented food flavour. *Journal of Plant Foods*, **6**: 155-163.

Odunfa, S.A., (1986). Other Legume-Based Fermented Foods. In: Legume-Based Fermented Foods, Reddy, N.R., M.D. Pierson and D.K. Salunkhe (Eds.). CRC Press, Florida, pp: 219-225.

Ogueke, C.C. and Aririatu, L.E., (2004). Microbial and organoleptic changes associated with ugba stored at ambient temperature. *Nigerian. Food Journal.*, **22**: 133-140.

Okechukwu, R. I. (2012) Changes in nutrient composition of African oil bean meal 'Ugba'*(Pentaclethra macrophylla* Benth) subjected to solid state natural fermentation, *Journal of Applied Bioscience. 5.* 3591-3595.

Onwuliri, V.A., Attah, I. and Nwankwo, J.O., (2004). Anti-nutritional factors, essential and non-essential fatty acids composition of Ugba (*Pentaclethramacrophylla*) seeds at different stages of processing and fermentation. *Journal of Biological Science.*, **4**: 671-675.

Pierson, M.D; Reddy, N.R and Odunfa, S.A. (1986) .Other legume based fermented foods, CRC. Press, New York. Pp. 219-222.