Effect of Preparation Method on the Proximate composition and Microbial Quality of Processed/cooked Fermented African Oil Bean Seeds (*Ugba*)

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Abstract: This study was carried out to determine the effect of different cooking methods on the proximate analysis and total microbial content ofUgba, using standard microbiological, proximate and phytochemical methods. Microbiological analysis revealed a progressive increase in the total heterotropic bacteria count and coliform count, from zero hour to the 72 hr for treated samples A,B,C,D. Staphylococcal count revealed almost a similar trend, except that no organism was isolated in treated sample B from zero to 24 hours. Proximate analysis revealed that the moisture content, ash content, crude protein content and crude fat content increased as the fermentation progressed. While the crude fiber and carbohydrate decreased as the fermentation progresses. Sensory evaluation revealed a change in the colour, taste, aroma and texture of the respective samples as the fermentation progresses. However, sample B was the most preferred, followed by sample A in terms of overall acceptability. The result of this study shows that sample A and B were most preferred, and also contains high amount of protein which is highly desired to supplement the nutritional requirement of the populace.

Keywords: Coliform count, Ugba, Microbial load, proximate composition.

INTRODUCTION

Ugba" is the Igbo name for sliced fermented African oil bean seed *(Pentaclethra macrophylla).* The African oil bean seed is called several names in Nigeria, such as "*Apara*" by the Yoruba, "*Ugba*" or" *Ukpaka*" by the Igbo's (Enujiugha and Akanbi, 2005). It is consumed voraciously in the Eastern states of Nigeria as a local delicacy popularly known as "African salad". It is prepared with oil, pepper, fish and salt and sometimes eaten with tapioca, stock fish and garden eggs. It can also be eaten with boiled or roasted yam and cocoyam.

The seeds are fermented under alkaline conditions (Olasupo *et al*., 2016). It is rich in protein and other essential nutrients, serving mainly as a source of protein, with distinctive economic, social and cultural role among the consumers (Ogueke*et al.* 2010).

There is no streamlined method, safety guideline or standards and so production practices and packaging is on individual/family basis. Although fermentation is a means of providing nutritious and palatable food, the safety of fermentation products especially in non-standardized production practices is a major concern that needs adequate research (Olasupo *et al*., 2016). Most studies of African fermented foods have focused on isolation and identification of desirable microorganisms involved in the fermentation process (Gadaga*et al*., 2004; Okorie and Olasupo 2013b). There is limited information on the occurrence and growth of pathogens in African fermented foods. The traditional processing method of fermenting the African oil bean seed into *Ugba* is saddled with problems of product safety and quality inconsistency. The growth and occurrence of organisms of public health importance in *Ugba* is of great concern. Natural fermentation process used routinely in the fermentation of *Ugba* allows participation of diverse microorganisms which may include contaminants, notably, *Escherichia coli* and *Staphylococcus aureus* (Ogueke *et al*., 2010; Eze *et al.,* 2014; Ejiofor*et al.* 1987; Enujiugha and Akanbi 2008; Nwagu *et al.* 2010; Ogueke and Aririatu 2004; Okorie and Olasupo 2013a). Anyanwu*et al.* (2016) and Ogbulie*et al.*(2014) had independently reported the presence of *E. coli*, *Klebsiella* and *Staphylococcus* species in processed *Ugba*. The presence of these organisms in the product is worrisome and portendsdanger.

The lack of uniformity in texture of fermented *Ugba* following different processing methods has increased the diversity of microbial isolates. Nevertheless, good processing and storage techniques that can impact improvements in nutrient and reduce microbial population is essential to producing quality*Ugba* while at the same time ensuring the elongation of its shelf-life. This study reports on the effects of different processing methods (Oly-Alawuba and Anunukem, 2018) in the microbial population of *Ugba.*

MATERIALS AND METHODS

Source of raw material and processing

The African oil bean seed was purchased from Relief Market, Imo State, Nigeria and processed according to the method described by Njoku and Okemadu (1989). The seeds were boiled for 1h, 3h, 6h, and 8h respectively. The hard coats were peeled off and the cotyledons cooled for 10-25min and sliced into tiny portion before washed with water. The sliced cotyledons were further boiled for 1h, cooled and soaked in distilled water for 10 hours to reduce its bitter taste. Thereafter, the slices were drained in a basket and further wrapped in banana leaves and kept at room temperature for0, 24, 48, and 72 h. The first was referred to as zero (0h) sampleswas collected immediately before the slices were wrapped in blanched banana leaves.

Total Viable Count

Microbiological analysis of the *Ugba* sample was carried out following standard methods. Total viable count (TVC) of bacteria and moulds was determined by method described by Vincent(2004). Ten gramme of the unfermented (0 h) and fermented samples (24 h, 48hrs and 72hrs) were ground in a sterile porcelain mortar and serially diluted and aliquot volume of 1ml was inoculated into different media using spread plate method and incubated at appropriate temperature and time. The colonies formed on each plate were counted and the average counts were recorded as colony forming units per ml (Cfu/mL) of the samples.

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).

Purification of Isolates

Discrete colonies were sub cultured into freshly prepared nutrient agar plate to obtain pure colonies. Pure isolates of resulting growth were transferred into nutrient agar prepared in a slant and stored at 4^{o}C for identification and further test.

Characterization and Identification of Microbial Isolates

The organisms were characterized and identified using standard methods and manuals, Holt *et al*., (1994); Barnett and Pankhurst, (1974).

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.

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 was returned to the flask and boiled again in 200 ml of 1.25 g of NaOH per 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 thefiber was determined by difference in given by;% Crude fiber =W1-W2/W3 X 100
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 concentrated sulphuric 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 flaskwas 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 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;
%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
N acid = Normalty 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, 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;

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

Where M = moisture

P = protein

F = fat

F2 = Crude fiber

Sensory Evaluation

The "Ugba" samples were subjected to sensory analysis by 20 members of regular "Ugba" consumers drawn from the university environment. The parameters evaluated included colour, texture, aroma, taste, overall acceptability. These parameters were evaluated after 72 hours of fermentation using a nine - point Hedonicscale of 1= extremely dislike to 9= extremely like.

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

Table 1 shows the total viable counts (Cfu/g) of the four samples A, B, C and Dafter 72 hours fermentation period. At the end of the 72 hours fermentation period, the total viable counts (TVC) were 3.01 x 10^9(Cfu/g), 3.28x 10^9 (Cfu/g), 3.62 x 10^9(Cfu/g)and 4.28× 10^9Cfu/g for samples A, B, C, and D respectively. There was a gradual increase in the total viable counts from the 0hr to the end of the fermentation period for the samples A, B, C and D. There was a significant increase ($p > 0.05$) in THBC as the fermentation period increased in all the four samples. Sample D had highest THBC with 4.0 x 10^8(Cfu/g) at 0-hour fermentation period, 48hrs and 72 hrs and A had the least at 0 hour for the four samples. At 24 hours of fermentation, Sample B had the highest value of 9.8 x 10^8 (Cfu/g). This is in agreement with the result of the study done by Odunfa and Oyeyiola (1984).

Ugba production is by spontaneous inoculation of microorganisms from water used for soaking and steeping (Njoku *et al.,* 1990), from the air and from the packaging materials (Mbajunwa*et al.,* 1998). It is evident that microorganisms isolated from the fermented African oil bean seed were introduced from water, air, and utensils during processing. There was an increase in the number of microorganism during fermentation which may have resulted in the change in colour and texture of the final product. Microbial population increased with increase in time, indicating microbial proliferation as a function of the spontaneous activities of microorganism resulting in short shelf –life due to the increase in moisture content and other proximate composition parameters (Mbata and Orji, 2008).

Colour changes are attributed to the proteolytic activities of microorganisms in *Ugba*. Fermented *Ugba* become soft with time due to the presence of certain bacteria species. The continuous activities of these contaminants were also responsible for the spoilage of *Ugba*.

The microorganisms isolated from the fermented African oil bean seed during the fermentation period (0hr to 72hr) using standard microbiological procedures are as follows *Bacillus cereus, B. subtilis, Streptococcus* sp*, Staphylococcus aureus, Enterobacter* sp*, Klebsiella* sp*, Serratia* sp*, and Citrobacter* sp. These isolated bacterial species is consistent with previous studies (Mba and Orji 2008; Kabuo *et al.*, 2013; Eze *et al.*, 2014). Fungal isolates were also isolated from the sample using standard microbiological procedures. This is in conformity with the work of Eze*et al*. (2014) on the fermentation of the same African Oil Bean Seeds. The fungi isolated from this study *Aspergillus* and *Penicillium* have been known to produce mycotoxin, which exposes consumers to food intoxication (Azubuine, 2006). The local production process of *Ugba* renders the product vulnerable to contamination by pathogenic microorganisms, both bacteria and fungi. The packaging system used locally such as banana leaves (*Musa sapietum* Linn) could be other possible sources of these extraneous organisms into the product. They could have also been introduced through the air, water and utensils used for processing, or the handler. African Oil Bean Seeds have been known to contain proteins, fats and carbohydrates, therefore the microorganisms responsible for the fermentation of African Oil Bean Seeds must be capable of utilizing these food constituents for energy and carbon source. *Bacillus* sp implicated in the fermentation of African Oil Bean Seeds are capable of breaking down these nutrient constituents (Forgarty and Griffin, 1974). *Bacillus* sp is a notable producer of enzymes responsible for the breakdown of proteins, starch and fats into their simple forms. One of the major biochemical changes in African Oil Bean Seeds is the hydrolysis of protein (Chelule *et al.*, 2010) in which *Bacillus* sp produces proteases, an enzyme responsible for the breakdown of proteins into amino acids and short peptide chains. These metabolic activities could be attributed to the high microbial population of *Bacillus* sp., in the fermentation of African Oil Bean Seeds. The co- existence of *Staphylococcus sp*\and *Bacillus* s*p* in the fermenting sample during the first 24 hours was typical of the microflora of fermenting bean Seeds. *Staphylococcus* sp have been associated with fermenting foods of plant origin, especially vegetable proteins (Jideani and Okereke, 2010). According to Isu and Njoku(1997) and Mbajunwa *et al*. (1998), *Bacillus* spare the main starter of the fermentation in African Oil Bean Seeds. *Bacillus* sp*.,* survived throughout the fermentation period, this could be attributed to the ability of *Bacillus* sp*.*, to inhibit the growth of other microbes in the competitive fermenting environment (Oguoke and Aririatu, 2004). This is possible by the release of antibiotics by *Bacillus* sp to inhibit other microbes that may be harmful to the end product. Some of the bacterial and fungal isolates found in the fermenting samples could have been introduced by chance via handling, air, water, utensils and leaves for wrapping (market sample). Microbes such as *Staphylococcus* sp could have been from the skin of handlers during the preparation of the fermented Oil Bean Seeds (Adam and Moss, 1999). The results of proximate composition of the processed *Ugba* are shown in shown in Table 6 and 7 respectively. All the processed samples of *Ugba* were high in protein, ash, crude fat and moisture content, moderate in crude fibre but low in carbohydrate. Sample C had highest value of crude protein (15.92 %), while sample B had the highest value of crude fibre (3.06%) and carbohydrate (13.22%).

Sample C had the highest value of crude fat (18.24%) and moisture content(50.73%) as well as sample D with the highest value of ash at (2.06%). Cooking and dehulling led to increase in the protein, carbohydrate, crude fat, crude fibre content and moisture content of the samples but with significant reduction in their ash content. Processing of the African oil bean seed using the traditional method (that involves long cooking period) to *Ugba* resulted to significant decrease in proximate composition of the sample. From the result of the proximate composition of *Ugba* processed using different cooking condition, the sample C had the highest protein, fat and moisture contents.

Table 1: Total Heterotrophic Bacteria Count ofUgba samples.

Sample	0(hour)	24(hours)	48(hours)	72(hours)
A	1.00×10^8	8.00×10^8	1.98×10^9	3.01×10^9
B	2.00×10^8	9.80×10^8	2.16×10^9	3.28×10^9
C	1.60×10^8	1.24×10^9	2.50×10^9	3.62×10^9
D	4.00×10^8	1.50×10^9	3.00×10^9	4.28×10^9

Sample A= Ugba boiled for 1hour
B=Ugba boiled for 3hours
C=Ugba boiled for 6hours, D= Ugba boiled for 8hours

Table2: Fungi Count of samples of fermented Ugba.

Sample	0(hour)	24(hours)	48(hours)	72(hours)
A	-	-	-	3.00×10^7
B	-	-	-	9.00×10^7
C	-	-	1.00×10^7	9.00×10^7
D	-	-	3.00×10^7	4.00×10^8

Table 3: Coliform Count of samples of fermented Ugba

Sample	0(hour)	24(hours)	48(hours)	72(hours)
A	5.00×10^7	4.70×10^8	6.00×10^8	9.50×10^8
B	1.00×10^8	4.90×10^8	6.90×10^8	1.10×10^9
C	1.30×10^8	5.30×10^8	7.60×10^8	1.08×10^9
D	2.20×10^8	5.50×10^8	7.30×10^8	1.28×10^9

Table 4: Staphylococcalcount of samples of fermented Ugba.

Sample	0(hour)	24(hours)	48(hours)	72(hours)
A	1.00×10^7	5.00×10^7	3.20×10^8	4.10×10^8
B	-	-	3.90×10^8	6.20×10^8
C	1.00×10^7	-	3.90×10^8	7.00×10^8
D	1.00×10^7	2.50×10^8	5.00×10^8	1.00×10^9

Table 5: Proximate Composition of the *Ugba* sample

DAY	SAMPLE	%Moisture content	%Ash	%Crude protein	%Crude fat	%Crude fibre	%CHO
1	A	49.69	1.86	13.92	16.95	3.16	14.12
	B	49.48	1.93	13.08	17.80	3.20	14.51
	C	50.51	1.88	14.50	18.11	3.14	11.86
	D	48.31	1.97	14.38	17.80	3.05	14.49
2	A	49.72	1.91	14.88	17.74	3.09	12.66
	B	49.52	1.95	13.90	17.95	3.10	13.58
	C	50.63	1.96	14.68	18.20	3.02	11.51
	D	49.05	1.99	14.50	17.86	2.94	13.66
3	A	49.80	1.97	16.89	17.85	2.95	10.54
	B	49.56	1.94	14.76	18.30	3.00	12.44
	C	50.80	1.89	17.00	18.25	2.96	9.10
	D	50.11	2.05	15.10	17.89	2.85	12.00
4	A	49.99	2.06	17.50	17.99	2.86	9.60
	B	49.60	2.16	14.85	18.50	2.96	12.35
	C	51.00	2.19	17.50	18.40	2.91	9.70
	D	50.36	2.22	16.41	18.09	2.80	10.12

Key; Day 1 represents zero hour, Day 2 represents 24 hours, Day 3 represents 48 hours, Day 4 represents 72 hours.

Table 6:Mean Proximate composition of the *Ugba* sample

Samples	% Moisture content	% Crude protein	% Crude fat	% Crude fibre	% Crude Ash	% CHO
A	49.80	15.79	17.63	3.02	1.95	11.80
B	49.54	14.14	18.13	3.06	1.99	13.22
C	50.73	15.92	18.24	3.00	1.98	10.54
D	49.45	15.09	17.91	2.91	2.06	12.56

Table 7: Sensory evaluation for the processed ugba

Sample	Texture	Colour	Aroma
A(0- 24hours)	Hard	Creamy brown	Mild ammonia gas
A(48hours)	Hard	Slight dark brown	strong ammonia gas
A(72hours)	Soft	Dark brown	Stronger ammonia gas
B(0-24hours)	Hard	Creamy brown	Mild ammonia gas
B(48hours)	Hard	Slight Dark brown	Strong ammonia gas
B(72hours)	Soft	Dark brown	Stronger ammonia gas
C(0-24 hours)	Soft	Brown	Mild ammonia gas
C(48 hours)	Soft	Brown	Strong ammonia gas
C(72 hours)	Very soft	Brown	Stronger ammonia gas
D(0-24 hours)	Soft	Brown	Mild ammonia gas
D(48 hours)	Very soft	Darker brown	Strong ammonia gas
D(72 hours)	Very soft	Darker brown	Stronger ammonia gas

Key: Sample A= Ugba boiled for 1hour
B= Ugba boiled for 3hours
C= Ugba boiled for 6 hours
D= Ugba boiled for 8 hours.

CONCLUSION

Different preparation methods (such as boiling time) of "*Ugba*" increased the protein contents of the samples. Studies have shown that several microbes are involved in spontaneous and non-spontaneous fermentation of variety of food substrates to bring about the digestibility, nutrient availability, flavour enhancement and improvement on the overall quality of fermented food. The nutritional quality of the slices increased as shown in protein, moisture content, ash and crude fat contents as fermentation progressed. The total viable count revealed that the fermented products can still be consumed after 72 hours fermentation time but cannot be kept for longer time because of the uncontrollable increase in microbial activities which will cause the product to deteriorate. For the sensory evaluation, sample B was most preferred in colour, aroma,while in terms of texture, taste and overall acceptability samples B and A were most preferred by panelists.

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