Effect of Processing Methods on the Nutritive Value of Ugba (*Pentaclethra Macrophylla* Benth)

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Abstract— *"Ugba", an indigenous Nigerian fermented food condiment is rich in protein, dietary fibre and minerals. Traditional processing method reduces the level of nutrients and minerals in the processed Ugba after long boiling. This study was therefore undertaken to determine the effects of processing methods on nutritional composition of Ugba. The control sample (Cl) was compared with the samples processed with Potash (Ps), Unripe Paw Paw (Pw1), Dry ashed Plantain peels (Pp2), Dry ashed Palm waste (Ag1) and Oil palm waste Og1. The isolation of microorganisms and determination of viable counts were achieved by culture methods and two of the isolated organisms were identified using Molecular studies. Mineral composition of all the samples was determined using the Atomic Absorption Spectrophotometric method. Proximate analyses of raw and processed samples were also evaluated following the official methods of Association of Official Analytical Chemists. The shelf life study of Ugba, was also done using different preservation treatments which include Sun drying, Oven drying, Refrigeration and Freezing. Organoleptic evaluation test was carried out for the processed samples using a 9-point Hedonic scale analysis on a 27- member panel. The data was statistically analysed using one way analysis of variance (ANOVA). The microorganisms isolated from Ugba sample were noted as to ascertain the organisms involved in fermentation. They include Bacillus subtilis, Bacillus licheniformis, Micrococcus varians, Enterobacter asburiae and Escherichia coli. The result of the total viable counts in (cfu/ml) were* 2.79×10^9, 2.60×10^9, 2.68×10^9, 2.61×10^9*l,* 2.5×10^9*l and* 1.97×10^9 *for samples Cl, Ps, Pw1, Pp2, Ag1 and Og1 respectively after 72 hours fermentation. The result of the Proximate analysis showed an increase in protein, moisture and ash content and a decrease in carbohydrate, crude fat and crude fibre for the samples at 72 hours of fermentation. Mineral composition result showed that Sample Ag1 had the highest amount of calcium (33.422 mg/100g) and zinc (2.44 mg/100g) while sample Ps had a little trace of lead (0.003 mg/100g) at 72 hours fermentation. An increase in pH and temperature during fermentation were recorded for all the samples. At the end of the preservation period, the oven dried Ugba was able to retain some amount of microorganisms that can be used as starter culture and was the best preservation method recorded. The result from sensory assessment showed that the sample processed with Ash Ngu (Ag1) was most preferred followed by sample Ps in terms of overall acceptability. The results of this study showed that sample Ag1 was most preferred by the panellist and also had the highest amount of protein and calcium which is highly desired to supplement the nutritional requirement of the populace.*

Keywords— Processing Method, Nutritive Value, Ugba.

I. INTRODUCTION

"Ugba" an importance dense fermented product of African Oil bean seed constitute one of the commonest fermented leguminous foods eaten in the south eastern Nigeria. Ugba has assumed an essential delicacy among Nigerians. This has earned it the popular name "African salad*"*. It can be garnished with pieces of cooked stock fish (Ugba and Okpoloko). The normal fermentation of the seed which currently is still carried out at house-hold level makes the product nutritive, appetizing and safe (Enujiugha *et al.,* 2002).

The oil content in the seed comprises oleic acid and linoleic acid together with crude protein made up of the 20 important amino acids. The increased concentration of essential amino acids makes the seed a possible source of protein and condiment (Ach inewhu, 2002).

The processing of Ugba comprises sorting of the seeds, washing, boiling, dehulling, slicing and reboiling for removal of the unpleasant taste. The processed Ugba are covered in leaves and left to ferment for 3days (Njoku and Okemadu, 2009). Ugba processing similar to other native fermented foodstuffs conventionally depend on natural fermentation commenced by normal microorganisms that are inherent on raw materials, processing utensils, hands of producers and from the local atmosphere as indigenous starters (Jespersen *et al.*, 2004).

Several studies have been done on Ugba which comprise nutrients and other biochemical changes in Ugba associated with microorganisms during fermentation (Kolawole and Okonkwo, 2005, Njoku and Okemadu, 2009). The chief fermenting microbe has been known to be *Bacillus sp*, others include *Lactobacillus plantarum, Staphylococcus* sp., *Leuconostoc mesenteroides, Micrococcus sp., Streptococcus lactis, Enterobacter sp Proteus sp.* and *Escherichia coli*. Some researchers have also isolated the yeast *Candida tropicalis* and *Geotrichum candidum* during fermentation (Ejiofor *et al.,* 2007).

African oil bean seed have been found to contain several nutrients and minerals including calcium, magnesium potassium, phosphorous, zinc, sodium, manganese, iron, and copper which are reduced considerably with long and unrestrained processing methods. *Ugba* fermentation, like most fermented foods, is produced and marketed on a small scale, most especially by rural dwellers that are unable to apply improved methods. There is the need to immediately find ways to improve this problem. The raw unfermented seeds of *Pentaclethra macropyhlla* are uneatable and its regular processing technique is burdensome and decreases the mineral and nutritional value of the seed. Applying diverse processing procedures will decrease the fermentation period and make the process less tiresome while attaining an improved quality product. *Ugba* can serve as a replacement for meat for the poor and can decrease protein-calorie malnutrition and important fatty acid insufficiencies (Oguntoyinbo *et al.,* 2010).

II. MATERIALS AND METHODS

2.1 Sample collection and processing

Samples of African oil bean seeds were obtained from Eke Nibo Market in Anambra State, Nigeria and identified in the Department of Botany in Nnamdi Azikiwe University (NAU), Awka. The Ugba was produced in the Department of Applied Microbiology and Brewing, NAU laboratory using the traditional method. The seeds were divided into 6 portions – one portion was processed using the traditional processing method according to Njoku and Okemmadu (2009) while portion 2-6 were processed with some modification which involve addition of Potash (Ps), Unripe Paw Paw (Pw1), Dry ashed Plaintain peels (Pp2), Dry ashed Palm waste (Ag1) and Oil palm waste Og1 to the traditional processing method. The first portion was first boiled for 6hrs, shredded and re- boiled for 3hrs. It was washed, drained and steeped in cold water for 10hrs after which the de-bittered cooked shreds were wrapped and allowed to ferment for 72hrs. For the portion 2-6, it followed the same methods except for the processing duration that were 2 hrs, 45mins and 5hrs respectively.

2.2 Isolation of Microorganisms.

The unfermented sample (0hr) and those fermented (24hrs, 48hrs and 72hrs) were grounded in a sterile porcelain mortar. Six fold serial dilutions were used for the isolation of microorganisms using 1gram samples from each day of the fermentation. . Nutrient agar was used in plating via pour plate method. Plates were incubated at 37^{o}C for 24hrs. Representative colonies were differentiated on the basis of morphology and colour and then sub-cultured to obtain pure cultures by repeated streaking. Microorganisms were isolated at zero hour and subsequently after every 24 hours. Colonies were counted from the different mixed culture plates and representative colonies were sub-cultured.

2.3 Characterization of Bacterial Isolates

This was done by carrying out colonial morphology (which includes shape of colony, elevation of colony, edge of colony and pigmentation). The purified cultures were identified using biochemical method according to Bergey's manual of Determinative Bacteriology (Buschanan and Gibbons, 2010). The biochemical tests carried out include Gram staining, Sugar fermentation tests, Catalase test, Coagulase, Indole, Citrate, Oxidase, Methyl red, Voges proskauer and Motility.

2.4 Determination of pH and Temperature of Unfermented and Fermented African oil bean samples.

The pH of the samples was determined using a digital pH meter (Jenway, model 3510). It was repeated respectively for 24hrs, 48hrs and 72hrs. The temperature of the sample was determined by inserting a sterile thermometer (wiped with alcohol) into each of the sample on each day of fermentation. The mercury-in-glass thermometer was used. This process was repeated for 24hrs, 48hrs and 72hrs.

2.5 Proximate analysis

The six samples were analysed for moisture, crude protein, crude lipid, crude fibre and ash using standard methods of Association of official analytical chemists (AOAC, 2005). The carbohydrate content was obtained by difference. 5 grams each of the labelled samples were used for each determination. The moisture content of the samples was determined by air oven method at 110^0C. The crude protein was determined by micro-kjeldahl method.

2.6 Mineral composition

Potassium and sodium were determined by digesting the ash of the samples with perchloric acid and nitric acid and then taking the readings on Jenway digital flame photometer/spectronic. Calcium and zinc were determined spectrophotometrically.

2.7 Preservation of *Ugba*

The preservation methods used were freezing, refrigeration, oven drying and sun drying.

2.8 Organoleptic analysis of *Ugba* samples.

The quality parameters of *Ugba* evaluated were colour, aroma/flavour, taste, texture and overall acceptability using a 9- point hedonic scale.

2.9 Statistical Analysis

The data obtained from the organoleptic analysis were subjected to statistical analysis using the one way ANOVA. P-values < 0.05 were considered statistically significant while P-values > 0.05 indicates that there is no significant difference between the *Ugba* samples (American Society of Brewing Chemists, 1987).

III. RESULTS

The varieties of microorganisms present during the fermentation were responsible for the uncontrolled fermentation of Ugba to give its characteristic Ugba smell and colour change. Five organisms isolated from the samples were *Bacillus subtilis, Bacillus licheniformis, Micrococcus varians, Enterobacter asburiae* and *Escherichia coli.* However *Bacillus subtilis and Bacillus licheniformis* were able to survive the fermentation conditions and were recovered at the end of fermentation.

Table 1a gives the results of the total viable counts (cfu/ml) of the six samples Cl, Ps, Pw1, Pp2, Ag1, and Og1 during the 72 hours fermentation period.

At the end of the 72 hours fermentation period, the total viable counts (TVC) were 2.79 x 10^9 (cfu/g), 2.60 x 10^9 (cfu/g), 2.68 x 10^9 (cfu/g), 2.61 x 10^9 (cfu/g), 2.5 x 10^9 (cfu/g) and 1.97 x 10^9 (cfu/g) for samples Cl, Ps, Pw1, Pp2, Ag1 and Og1 respectively. There was gradual increase in the total viable counts from the initial to the end of the fermentation period for all the samples.

There was a significant increase ($p > 0.05$) in TVC as the fermentation period increased in all the six samples. At 0 hour fermentation period, Sample Cl had the highest total viable count with 1.73×10^9 (cfu/g) and Pp2 had the least with 1.23×10^9 (cfu/g). At 72 hours of fermentation, Sample Ps still maintained the highest value of 2.79×10^9 (cfu/g) while sample Og1 had the least value of 1.97×10^9 (cfu/g).

TABLE 1A
CHANGES IN TOTAL VIABLE COUNT (TVC) OF *UGBA* DURING FERMENTATION

	(cfu/g)			
Samples	**0hr**	**24hrs**	**48hrs**	**72hrs**
Cl	1.73×10^9	2.2×10^9	2.41×10^9	2.79×10^9
Ps	1.25×10^9	1.68×10^9	2.05×10^9	2.6×10^9
Pw1	1.0×10^9	1.47×10^9	2.28×10^9	2.68×10^9
Pp2	1.23×109	1.30×109	2.11×109	2.61×109
Ag1	1.2×10^9	1.39×10^9	2.0×10^9	2.5×10^9
Og1	1.11×10^9	1.62×10^9	1.32×10^9	1.97×10^9

There was a significant increase in pH of the Ugba samples as the period of fermentation increased (Fig 2a).The range of pH for the naturally fermented Ugba is between 6.4 and 8.1. As fermentation progressed, there was a rise in temperature from 29°C – 33°C in naturally fermented samples.

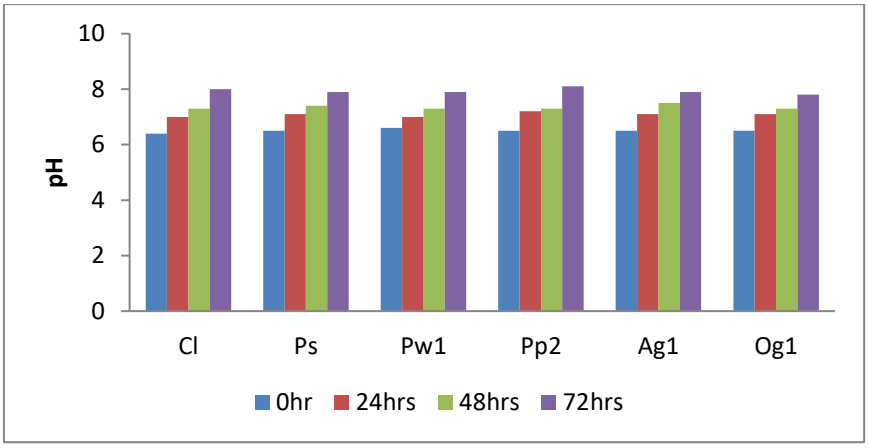

FIGURE 2 a: Changes in pH during the natural fermentation of Ugba.

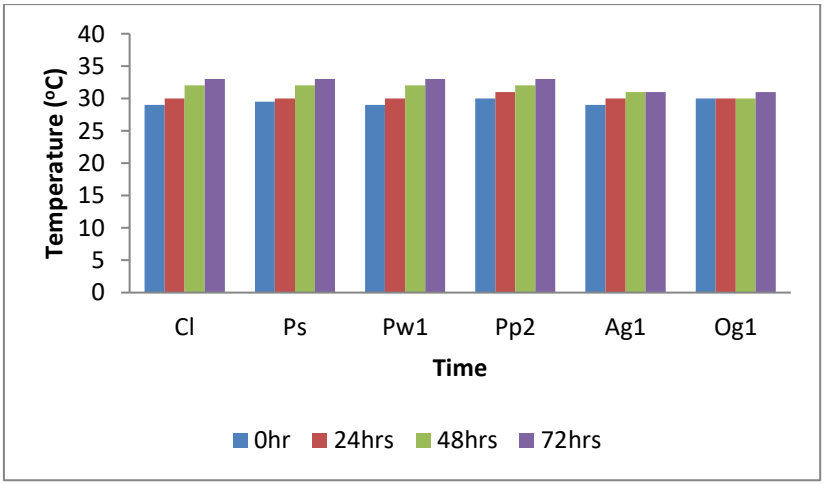

FIGURE 2b: Temperature changes during the natural fermentation of Ugba sample

3.1 Proximate Analysis

The results of proximate composition of the raw and processed Ugba are shown in Table 3a and 3b respectively. All the processed samples of Ugba were high in protein, ash and moisture content, moderate in crude fibre but low in carbohydrate and crude fat. Sample Ag1 had highest value of crude protein (24.15%) ash (3.9%) and crude fat (28.30%) while Og1 had the highest value of crude fibre (3.46%) and carbohydrate (11.62), as well as Pp2 with the highest value of moisture at 42.64% with no significant difference in all the nutrient values ($p>0.05$). Cooking and dehulling led to increase in the protein, ash and moisture content of the samples but with significant reduction in their carbohydrate, crude fat and crude fibre content. Processing of the African oil bean seed using the traditional method (that involves long cooking period) to Ugba led to significant decrease in proximate composition of the sample. However, processing reduced the carbohydrate and crude fat of the products compared with the raw and processed samples.

TABLE 3A
PROXIMATE ANALYSIS OF RAW AFRICAN OIL BEAN SEED.

Component	Composition(%)
Moisture	25.32
Ash	2.4
Crude protein	22.32
Crude fibre	2.13
Crude fat	33.98
Carbohydrate	13.85

From the result of the proximate composition of Ugba processed using different cooking condition, the sample cooked with Ag1 had the highest protein content, ash and crude fat.

TABLE 3B
PROXIMATE ANALYSIS OF UGBA PROCESSED USING DIFFERENT COOKING CONDITION.

Component	Percentage (%)					
	Cl	Ps	Pw1	Pp2	Ag1	Og1
Moisture	40.70	30.78	31.72	29.14	29.64	31.46
Ash	3.25	3.20	2.65	2.66	3.91	2.76
Crude protein	23.45	19.6	18.20	16.8	24.15	22.4
Crude fibre	1.22	3.10	2.61	3.16	3.15	3.46
Crude fat	20.31	27.62	27.29	25.92	28.30	28.22
Carbohydrate	11.07	9.70	11.53	8.82	5.43	11.62

3.2 Mineral Composition

The mineral composition of the raw and processed Ugba are shown in Table 4a, 4b and 4c The raw samples were very high in calcium and sodium but low in zinc and no trace of lead. Processing the seed to Ugba using the traditional method lead to significant decrease in potassium and calcium content of sample Cl, with significant increase in sodium and zinc content. Addition of potash and Ash ngu significantly increased the mineral content of Ugba (sample Ps and Ag1) compared with the control sample (sample Cl).

TABLE 4A
MINERAL COMPOSITION OF RAW AFRICAN OIL BEAN SEED.

Component	Composition(%)
Zinc	0.978
Calcium	27.3
Lead	0
Sodium	8.49
Potassium	1.27

TABLE 4B
MINERAL COMPOSITION OF PROCESSED UGBA SLICES BEFORE FERMENTATION (0 HR).

(Samples µg/ml)	Ag1	Pw1	Pp2	Og1	Ps	Cl
Zinc	0.421	0.436	0.622	0.395	3.304	0.395
Calcium	30.868	30.724	28.802	28.892	21.117	9.538
Lead	-	-	-	-	0.001	-
Sodium	11.349	12.705	8.753	8.782	4.209	9.10
Potassium	2.832	1.867	1.945	2.673	3.276	1.758

TABLE 4C
MINERAL COMPOSITION OF UGBA SLICES AFTER 72 HOURS OF FERMENTATION

(Samples µg/ml)	Ag1	Pw1	Pp2	Og1	Ps	Cl
Zinc	2.44	1.76	0.98	1.82	1.32	1.82
Calcium	33.422	31.11	11.77	10.43	21.37	10.43
Lead	-	-	-	-	0.003	-
Sodium	7.100	17.101	9.100	9.28	17.20	7.024
Potassium	2.832	1.921	2.23	4.42	5.628	1.103

3.3 Preservation of Ugba

Table 5a shows the result of the pH changes observed in Ugba preserved for 24 weeks using different preservation treatments. The pH increased significantly (6.53 – 7.46) as the storage period increased for all the treatments (Table 5a). There was a reduction in microbial count ($3.7 \times 10^6 – 1.7 \times 10^6$cfu/g) of all the samples after 24 weeks storage period. The highest microbial count after 24 weeks was with the refrigerated Ugba which had 2.6×10^6cfu/g while the least of 1.7×10^6cfu/g was observed with oven dried Ugba (Table 5b).

TABLE 5A
CHANGES IN PH OF UGBA DURING STORAGE PERIOD

Preservation period	(weeks)					
Sun drying	4	8	12	16	20	24
Oven drying	7.01	7.02	7.06	7.10	7.13	7.19
Refrigeration	7.05	-	-	-	-	-
Freezing	7.03	7.18	7.28	-	-	-

TABLE 5B
MICROBIAL COUNT (Cfu/ml) OF UGBA DURING STORAGE PERIOD

Preservation period	(weeks)					
Sun drying	3.6	3.0	2.6	2.4	2.3	2.0
Oven drying	3.7	3.2	2.5	2.4	2.1	1.9
Refrigeration	3.7	-	-	-	-	-
Freezing	3.7	3.4	3.3	-	-	-

3.4 Organoleptic Evaluation

Table 6 shows the results of organoleptic evaluation of the Ugba samples. The data were analysed using one way analysis of variance. Duncan partitioning of the mean values of likes and dislikes shows that there was a significant ($P \leq 0.05$) difference in the level of likeness of Ag1, Ps and Cl as against Og1 and Pp2. The percentage level of acceptance was highest for Ag1 at 59% and lowest for Pp2 at 22%. The mean value of the samples Cl, Ps, Pw1, Pp2, Ag1 and Og1 in terms of colour, taste, texture and taste showed that sample Ag1 was 'liked very much' and highly preferred by the panellists.

TABLE 6
ORGANOLEPTIC ANALYSIS ON PROCESSED UGBA SAMPLES

(Samples µg/ml)	Ag1	Ps	Pp2	Og1	Pw1	Cl	Total
Observation N	27	27	27	27	27	27	162
Sum Σx_i	203	192	133	166	180	186	1060
Mean x	7.5185	7.1111	4.9259	6.1481	6.6667	6.8889	6.5432

IV. DISCUSSION

Studying the time spent in cooking the *Ugba*, the samples cooked with Ps, Pw1, Pp2, Ag1, and Og1 had the shortest time as against the sample Cl that took long cooking hours. Sample Ag1 -+had the best colour and taste after the period of fermentation making it the highest acceptable *Ugba* with improved taste and aroma considering the cooking time.

Since the bean seeds were boiled for hours before fermentation, the microorganisms involved in the fermentation could not have originated from the beans. The bacteria involved in the fermentation probably were introduced through air, water, utensils and leaves used in wrapping or by handling during the preparatory stage.

Since protein hydrolysis is the major biochemical change in *Ugba* fermentation (Oyeyiola, 2001), it can be assumed that *Bacillus* spare the main fermenting organisms. They were found to persist until the end of the fermentation (Obeta, 2003) and their number increased throughout the period of fermentation.

Escherichia coli and *Enterobacter asburiae* were also found to be present only in 1 sample (Cl) at the beginning of fermentation but disappeared after 24 hours of fermentation. *E. coli* though fermentative and found in the air and soil has been isolated from some fermentation (Ogunshe *et al*., 2007). The rise in pH which occurred during fermentation could be attributed to the increased production of ammonia during the fermentation due to protein hydrolysis and deaminase activity as was reported for some other fermenting protein foods such as Natto, Koji, Iru, Okpehe, Kawal and Soumbala (Ouoba *et al*., 2007). The increase in pH would encourage the growth of *Bacillus* sp which have been found to grow well at pH 7.8 to 8.0 (Odunfa and Oyeyiola, 2005). The rise in temperature indicated that *Ugba* fermentation is exothermic. The initial increase in temperature has been attributed to the intense metabolic activities of the microorganisms (period of maximal metabolic activity) and represent the most active and important period of the fermentation. From the proximate analysis result obtained, it has been shown that crude protein content of African oil bean seed in the cooked and fermented form have enough nutrients to satisfy protein requirement of population in the developing countries that rely much on starchy staples. The highest content of protein (24.15) was recorded for sample Ag1 and the lowest (16.8) in sample Pp2.The increase in crude protein is in agreement with the work of Campbell-Platt (2000) where the crude protein of dawadawa increased. They attributed this to the organism *Bacillus subtilis* and *Bacillus licheniformis* associated with the fermentation. The progressive decrease in crude fat during the fermentation for all the samples is desirable because fat was broken down into simpler substances which will enhance the digestibility of the product in human

body. The decrease in fat has been reported by Odunfa (2005) to be desirable, since high amounts of fatty acids in foods can cause rancidity thereby making the food taste sour.

The ash content observed is an indication that *Ugba* samples are rich in minerals. The adjuncted samples had higher ash content with sample Ag1 (3.91) and Pw1 the lowest (2.65) which is an indication that this improved *Ugba* are highly rich in minerals.

For the preservation of *Ugba*, the pH increased slightly during the storage period although the pH of the treated samples was lower than fresh *Ugba*. The lower pH obtained was due to the different preservation treatments carried out on the samples. Evaporation of ammonia during drying results in decrease in pH (Parkouda *et al*., 2008). The lowest microbial count obtained for the oven dried was because drying helps to dehydrate both the food and microorganisms. It also helped to concentrate the soluble ingredients in *Ugba*, and these high concentrates prevented the growth of microorganism.

V. CONCLUSION

In a bid to reduce the time spent in Ugba production, the sample processed with Ag1 was found to be the best and had the best taste, flavor, aroma, texture and nutritional composition. The microorganisms responsible for Ugba fermentation were bacteria: *Bacillus subtilis, Bacillus licheniformis, and Micrococcus varians.* Sensory evaluation results showed that the Ugba sample produced by cooking with Ag1 was generally liked by panelists in all parameters tested. This implied that the sample was "overall best". A total of 5 minerals were found in Ugba sample; Sodium, Potassium and Calcium were abundant in the "overall best" sample with no trace of Lead. While the sample Ps (Ugba cooked with potash) which however was moderately liked by panelists because of its characteristic Ugba aroma had traces of lead in its mineral analysis.

It was therefore concluded that since long boiling cause loss of some essential nutrients, wastes time, energy and resources, the introduction of Ag1 to African oil bean seed production improves its flavor, taste and aroma and fermentation brings about the best acceptable Ugba in terms of nutritional composition.

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