Comparative Studies of Ugba Qualities Using Six(6) Processing Methods

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Abstract: "Ugba an indigenous Nigerian fermented food condiment is rich in substantial amount of 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 (CI) and the modified samples (Ps, Pwl, Pp2, Agl and Ogl) which were processed using 5 different cooking materials (adjuncts) were compared. The microorganisms isolated from Ugba sample were noted as to ascertain the organisms' involved in fermentation. Proximate analysis and mineral composition were carried out on the sample. The result showed an increase in protein, moisture and ash content and a decrease in carbohydrate, crude fat and crude fibre for the samples at 72hoursof fermentation. The sample after 72 hours fermentation contained an appreciable amount of calcium, potassium, sodium and zinc. Sample Agl had the highest amount of calcium (33.422) and zinc (2.44) while sample Ps had a little traces of lead (0.001) at 72 hours fermentation. It was also recorded for the sample, an increase in pH value and temperature. The result of the total viable counts were 2.60 x 10^9cfu/ml, 2.79 x 10^9cfu/ml, 2.68 x 10^9fu/ml, 2.61 x 10^9cfu/ml, 2.5 x 10^9cfu/ml and 1.97 x 10^9cfu/ml for samples CI, Ps, Pwl, Pp2, Agl and Ogl respectively after 72 hours fermentation. No mould growth was found in the unfermented and fermented Ugba slices for all the products. At the end of 72 hours fermentation, sensory evaluation test on a 9-point hedonic scale using a 27- member panel was carried out on the six samples, and the scores statistically analysed using analysis result from sensory assessment showed that the sample processed with Ash Ngu (Agl) was most preferred followed by sample Ps in terms of overall acceptability. At the end of the storage period, the oven dried ugba was able to retain some amount of microorganisms that was used as starter culture. The result of this study shows that sample Agi was most preferred by panellist and also had the highest amount of protein and calcium which is highly desired^o supplement the nutritional requirement of the populace. »

Key words: 'Ugba', fermentation, condiment, traditional processing, nutrition.

Introduction

"Ugba" a value added solid substrate fermented product of African Oil bean seed is one of the common fermented legumes predominately consumed by the Ibos and other smaller ethnic groups of the south eastern Nigeria. Fermented African oil bean seed have become a very important delicacy in the life of Nigerians. Its use as food among the south eastern populace of Nigerian and as a delicacy across the different tribes in Nigeria has increasingly been shown and consequently the ready to eat dish is called "African salad". It is also mixed with slices of boiled stock fish (Ugba and Okpoloko). The natural fermentation of the seed which at present is still done at the house-hold level renders the production nutritious, palatable and non-toxic (Enujiugha *et al.*, 2002).

The oil bean seeds contain carbohydrate, oil; which has been found to be rich in Oleic acid and Linoleic acid. Also they have been found to contain crude protein which contains the 20 essential amino acids. The high content of essential amino acids makes the seed a potential source of protein and flavouring (Achinewhu, 1982).Production of Ugba is still an age old traditional family method in the rural area. The processing involvessorting, washing of the seed followedby boiling, dehulling, shredding and boiling again to remove the bitter taste. Raw processed Ugba are wrapped in leaf and allowed to ferment for 72 hours (Njoku and Okemadu, 1989). Ugba production like many other indigenous fermented foods traditionally relies on spontaneous fermentation initiated by natural microorganisms that are found on raw materials/ingredients, on the processing utensils/equipment, on the hands of producers and from the local atmosphere as natural starters (Jespersen *et al.*, 1994).

Many researches have been carried out on Ugba which include nutrient and other biochemical changes in Ugba associated with microorganisms during fermentation (Kolawole and Okonkwo, 1985 and Njoku and Okemadu, 1989). Only bacteria are involved in the fermentation. The main fermenting microorganisms have been identified to be proteolytic *Bacillus sp*, others are *Staphylococcus* sp., *Micrococcus sp.*, *Leuconostocmesenteroides*, *Lactobacillus plantarum*, *Streptococcus lactis*, *Proteus sp.*, *Enterobacter sp*. And *Escherichia coli*. Some workers isolated the yeast *Candida tropicalis* and *Geotrichumcandidum* during fermentation (Ejiofor *et al.*, 1987).

African oil bean seed contains several nutrient and minerals such as potassium, phosphorous, calcium, magnesium, sodium, manganese, iron, copper and zinc which are significantly reduced by some long and uncontrolled processing techniques. Improving the long

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processing method helps to bring to minimal the nutrient and mineral leached out during processing.

Ugba fermentation, like most fermented foods, is produced and marketed on a small scale. Many people, most especially the rural ones are unable to acquire necessary foods for a healthy life. This is due to low food availability, profound poverty and lack of nutrition education. There is the need to urgently find ways to ameliorate this problem. Among such ways is a deeper research into some unutilized or underutilized crops. The raw unfermented seeds of *Pentaclethramacropyhlla* are inedible and its natural processing method is cumbersome and reduces the mineral and nutritional value of the seed. Using different processing/cooking methods and microorganisms will shorten the production/fermentation time and make the production less tedious while achieving a better quality product. *Ugba*can serve as a substitute for low-income earners and can reduce protein-calorie malnutrition and essential fatty acid deficiencies (Oguntoyinbo *et al.*, 2010).

The study was aimed at comparing the quality of Ugba produced using 6 different processing method.

Materials and Methods

Samples of African oil bean seeds were obtained from Eke Nibo Market in Anambra State and identified in the Department of Botany in Nnamdi Azikiwe University, Awka. *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 (1989) while portion 2-6 were processed with some modification to the traditional processing method. The first portion were first boiled for 5-8hrs, shredded and second boiled for 1-2hrs. It was washed, drained and steeped in cold water for 10hrs after which the de-bittered cooked shred were wrapped and allowed to ferment for 72hrs. For the portion 2-6, it followed the same methods except for the first boiling, second boiling and cold steeping that were 2 hrs, 45mins and 5hrs respectively.

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°C. The crude protein was determined by micro-Kjeldahl method.

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.

Isolation of microorganisms.

The unfermented sample (0hr) and those fermented (24hrs, 48hrs and 72hrs) were grounded in a sterile porcelain mortar. Serial dilution method was used for the isolation of microorganisms from 1gram samples from each day of the fermentation. One gram of sample was added to 9ml of sterile distilled water and shaken to get 10^{-1} dilution. Then 1ml of this dilution was transferred to another 9ml of sterile distilled water, and shaken to obtain 10^{-2} dilution. Another 1ml was taken from 10^{-2}dilution into 9ml sterile water. The process was repeated till 10^{-6} dilution was obtained. Nutrient agar was used in plating via pour plate method. Plates were incubated at 37°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.

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 (Buchanan and Gibbons, 1974). The biochemical tests carried out include Gram staining, Sugar fermentation tests, Catalase test, Coagulase, Indole, Citrate, Oxidase, Methyl red, Voges Proskauer and Motility.

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.

Preservation of Ugba

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

Sensory evaluation of *Ugba* samples.

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

Statistical Analysis

The data obtained from the sensory evaluation were subjected to statistical analysis using the one way anova*http://turner.faculty.swau.edu/mathematics/math241/materials/*. 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).

Results

The results of proximate composition of the raw and processed ugba are shown in Table 1a and 1b 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 1a: Proximate Analysis of Raw African oil bean seed.

Component	Composition(%)
Moisture	25.32
Ash	2.40
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 carbohydrate

Table 1b: Proximate Analysis of Ugba processed using different cooking condition.

Component	Percentage (%)					
Cl Ps	Pw1	Pp2	Ag1	Og1		
Moisture	28.61	36.78	37.72	42.64	35.14	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	20.35	27.62	27.29	25.92	28.30	28.22
Carbohydrate	14.94	9.70	11.53	8.82	5.43	11.62

The mineral composition of the raw and processed Ugba are shown in Table 2a, 2b and 2c 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 sodium and calcium content of sample Cl, with significant increase in potassium 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 2a. Mineral Composition of Raw African Oil Bean Seed.

Components	Composition (µg/ml)
Zinc	0.978
Calcium	27.3
Lead	0
Sodium	8.49
Potassium	1.27

Table 2bMineral 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	1.347
Calcium	30.868	30.724	28.802	9.538	21.117	28.892
Lead	0.00	0.00	0.00	0.00	0.001	0.00
Sodium	9.10	12.705	8.753	8.782	11.349	4.209
Potassium	2.832	1.867	1.945	2.673	3.276	1.758

Table 2c Mineral composition of Ugba slices after 72 hours of fermentation

	(Samples)					
	A.g1	Pw1	Pp2	Og1	Ps	Cl
Zinc	2.44	1.76	0.98	1.82	1.32	0.923
Calcium	33.422	31.11	11.77	10.43	21.37	20.892
Lead	0.00	0.00	0.00	0.00	0.001	0.00
Sodium	9.024	17.101	9.100	9.28	7.100	17.20
Potassium	2.832	1.921	2.23	4.42	5.628	1.103

The varieties of microorganisms present during the fermentation are responsible for the uncontrolled fermentation of Ugba to give its characteristic Ugba smell and colour change. Five organisms were isolated from Ugba samples. They are *Bacillus subtilis, Bacillus licheniformis, Micrococcus varians, Enterobacter asburiae* and *Escherichia coli.* Two organisms each of the five organisms were able to survive the fermentation conditions and were recovered at the end of fermentation. The recovered organisms from the samples were *Bacillus subtilis and Bacillus licheniformis.* No mould (cfu/ml) was observed in these tests for all the samples Cl, Ps, Pw1, Pp2, Ag1 and Og1 for the 72 hours of fermentation.

Table 3a 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 2.60 x 10^9 (cfu/ml), 2.79 x 10^9 (cfu/ml), 2.68 x 10^9 (cfu/ml), 2.61 x 10^9 (cfu/ml), 2.5 x 10^9 (cfu/ml) and 1.97 x 10^9 (cfu/ml) 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 x 10^9

(cfu/ml) and Pp2 had the least with 4.2 x 10^8 (cfu/ml). At 72 hours of fermentation, Sample Ps had the highest value of 2.79 x 10^9 (cfu/ml) while sample Og1 had the least value of 1.97 x 10^9 (cfu/ml).

Table 3a: Changes in total viable count (TVC) of Ugba during fermentation

Samples	0hr	24hrs	48hrs	72hrs
	(cfu/ml)			
Cl	1.73×10^9	2.2×10^9	2.41×10^3	2.60×10^9
Ps	1.25×10^9	1.68×10^9	2.05×10^9	2.79×10^9
Pw1	8.0×10^8	1.47×10^9	2.28×10^9	2.68×10^9
Pp2	4.2×10^8	1.30×10^9	2.11×10^9	2.61×10^9
Ag1	4.7×10^8	1.39×10^9	2.0×10^9	2.5×10^9
Og1	8.4×10^8	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 4a).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

Preservation Of Ugba

Table 4a 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 4a). There was a reduction in microbial count ($3.7 \times 10^6 - 1.7 \times 10^6$cfu/ml) 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/ml while the least of 1.7×10^6cfu/ml was observed with oven dried Ugba (Table 4b).

Table 4a: Changes in pH of Ugba during storage period

Preservation method	Preservation period (weeks) 4	8	12	16	20	24
Sun drying	7.01	7.02	7.06	7.10	7.13	7.19
Oven drying	6.84	7.03	7.13	7.14	7.27	7.33
Refrigeration	7.05	-	-	-	-	
Freezing	7.03	7.18	7.28	-	-	-

Sensory Analysis

Table 6 shows the results of sensory evaluation of the Ugba samples. The data were analysed using one way analysis of variance. 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 panelists.

Table 4.20 ANOVA table

Source	Sum of Squares (SS)	Degree of freedom	Mean of square	F statistic	p-value
Treatment	112.8642	5	22.5728	9.2830	$9.3250\ e^{-08}$
Error	379.3333	156	2.4316		
Total	492.1975	161			

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, 1981), it can be assumed that *Bacillus* spare the main fermenting organisms. They were found to persist until the end of the fermentation (Obeta, 1983) and their number increased throughout the period of fermentation.

Escherichia coli and *Enterobacter asburiae* was 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 abundant 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, 1985). The rise in temperature indicates 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 (1980) 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 (1985) 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 adjunct 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.

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 tasters in all parameters tested. This implied that the sample was "overall best". A total of 5 minerals were analyzed 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 panelist because of its characteristic Ugba aroma had traces of lead in its mineral analysis.

It was therefore concluded that since long boiling causes 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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