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Proximate Composition, Biochemical and Microbiological Changes Associated with Fermenting African Oil Bean (*Pentaclethra macrophylla* Benth) Seeds

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Abstract The proximate composition, biochemical changes and microbiology of fermenting *Pentaclethra macrophylla* (Ugba) seeds were evaluated. Studies were carried out to screen for microorganisms associated with the natural fermentation of the oil bean seeds. Bacterial isolates obtained include species of *Bacillus*, *Streptococcus*, *Salmonella*, *Micrococcus*, *Lactobacillus* and *Proteus*. Fungal isolates include Yeast, species of *Penicillium*, *Aspergillus*, *Fusarium* and *Rhizopus*. Total aerobic counts (TAC) ranged from 1.5 x 10^6 to 2.5 x 10^6 cfu/g, while total coliform counts (TCC) ranged from 1.7 x 10^3 to 7.2 x 10^3 cfu/g. More so, total lactic acid bacterial counts ranged from 2.6 x 10^5 to 4.6 x 10^5 cfu/g. Among the various bacterial isolates obtained from the fermenting Ugba, *Bacillus* and lactic acid bacteria were dominant from the beginning to the end of the fermentation the oil bean seeds. The proximate composition of the fermenting seeds showed the presence of protein, fats, fibre, carbohydrates, and ash. Temperature variations in oil bean seed fermentation showed higher temperatures in the purchased Ugba compared to the laboratory Ugba after 72 hours fermentation. There were significant reduction in pH and titratable acidity as the fermentation time progressed, showing that temperature, pH and titratable acidity of fermenting African oil bean seeds were affected by the metabolic activities of resident microorganisms.

Keywords: *Pentaclethra macrophylla, Ugba, proximate, biochemical, temperature, titratable acidity*

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1. Introduction

Fermentation is the technique of biological conversion of complex substrates into simple compounds by various microorganisms such as bacteria and fungi. [1]. Fermented foods are prepared from plant and animal materials by processes in which microorganisms play important role in modifying the substrate physically, naturally and sensorily. Food fermentation has over the years become a part of the cultural and traditional norm among the indigenous communities in Africa. Different parts of West Africa are renowned with their own favorite food that has evolved over centuries, depending on the customs, tradition and religion [2]. Fermentation, according to [3] is one of the oldest and most important traditional food processing and preservation techniques. Food fermentation involves the use of microorganisms and enzymes for the production of foods with distinct quality attributes that are quite different from the original agricultural raw materials. Fermentation in food processing is the conversion of carbohydrates to alcohol, using yeast and/or bacteria, under anaerobic conditions [4]. Many of the food fermentations are natural and/or controlled fermentation consisting of different species and genera of yeast, fungi and/or bacteria [5,6]. These microorganisms can cause desirable changes in various foods, which distinguish them from the ones, that are responsible for undesirable changes, including bad flavor and spoilage [5].

Traditional food fermentation; a biotechnological process produced by taking advantage of the natural microbiota associated with fresh food materials, is one of the most practical, economical and widely applied empirical methods to preserve and often enhance organoleptic and nutritional quality of fresh foods; and it has been unique to historical countries in different parts of the world. In Africa, we have alcoholic and non-alcoholic beverages that are mainly cereal based [7].

The prevailing population pressure in Nigeria, as in other less-developed countries, has resulted in an increasing demand for wild under-exploited nutritious plant products with aesthetic and organoleptic appeal in the daily diet [8]. The common edible portions of most under-utilized plants are the seeds, which in some cases are cooked or roasted and eaten directly as snack foods e.g. conophor nut and bambara groundnut, while some are cooked and fermented for use as soup and sauce ingredients e.g. African oil bean, locust bean, castor bean and melon. There are various plant seeds that are fermented and used as food in some rural parts of Nigeria, among which are, 'iru' from African locust bean (*Parkia*

biglobosa), 'ogiri' from castor bean (*Ricinus communis*), 'okpei' from mesquite seed (*Prosopis africana*) and 'Ugba' from African oil bean (*Pentaclethra macrophylla*).

African oil bean seed (*Pentaclethra macrophylla*) popularly called *Ugba* in the local parlance belong to the leguminous family *mimosa cease.* It is frequently cultivated in forest areas. At maturity the pods explode and disperse the seeds. The raw seed is a potential source of edible protein, energy and fatty acids [9]. The seed is then allowed to undergo fermentation before consumption. Ugba is an alkaline- fermented food, the traditional production is done with rudimentary equipment. It is used sometimes as part of a main meal or as an additive. However, it plays an important role in the diet of the populations residing in South Eastern Nigeria.

2. Materials and Methods

2.1. Source/Collection of Samples

Ugba samples used for this study were randomly obtained from a single production batch for laboratory analysis and others were obtained from local commercial producers in Umuahia market. The purchased samples were collected in sterile polyethylene bags and were sent immediately to the laboratory. The traditional technique practiced by the local producers from Umuahia was followed in the laboratory preparation of Ugba.

2.2. Laboratory Preparation of Ugba

The traditional method of preparing Ugba was employed in the laboratory to ferment the product. The processing of the large brown glossy seeds of the African oil bean to obtain 'Ugba' involved the following; the oil bean seeds were boiled in an autoclave at a temperature of 121°C and a pressure of 15 pounds per square inch (psi) for 1 h to soften the hard brown testa (shell). The shells were removed and the kernels washed, drained and rewashed with cold water several times. The washed cotyledons were cut into long thin slices. These slices were mixed with salt, wrapped in small packets with leaves and lightly tied. These small packets were placed in a basket to ferment at room temperature for 3 days to yield 'Ugba'.

2.3. Isolation of Microorganisms

Fermented sample (Ugba) was taken aseptically from traditionally fermented oil bean. One gram of the sample was thoroughly mashed with laboratory pestle and mortar and mixed with 9 ml of normal saline water as diluents in a McCartney bottle and the content was thoroughly shaken. Subsequent serial dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}) were made from this solution by adding serially 1 ml of solution from preceding concentration to 9 ml of diluents, using sterile syringe. A loopful (0.1 mL) of various dilutions were transferred separately to agar plates using streaking method in triplicates of Nutrient Agar (for bacteria), de Man Rogosa Sharpe (MRS) agar (for lactic acid bacteria), *Salmonella Shigella* agar (SSA) and Potato Dextrose agar (PDA) with streptomycin for fungi.

Bacteria counts were made on nutrient agar plates incubated at 30°C for 24 hours. The total number of colonies developed were counted and expressed as cfu/g of the original sample. Colonies were differentiated on the basis of morphology and counts of different colonial types. Fungal plates were incubated at 37°C for 3 to 5 days and specific counts of de Man Rogosa Sharpe (MRS) Agar, plates were incubated at 30°C for 3 days.

2.4. Characterization and Identification of Isolates

Colonies obtained after incubation were sub-cultured on Nutrient agar which was incubated for 24 hours at 30°C. The cultural characteristics of isolates on the agar plates were observed. The motility of the isolates was examined using hanging drop technique. Gram staining reactions and cell morphology from heat fixed smears were done. The identification procedures for the microorganisms were carried out using [10] methods. Pure cultures of the different organisms isolated were sub-cultured and preserved on agar slants at refrigeration temperature (4°C).

2.5. Proximate Analysis of the Samples

The parameters determined were crude protein, carbohydrates, fat, moisture content, ash, crude fibre and dry matter. These parameters were determined according to methods of [11] and [12].

2.6. Physicochemical Analysis

There are several physicochemical parameters in the environment which influence the presence, growth, diversities and abundance of microorganisms in Ugba samples. pH and tritrable acidity were determined as described by [13,14].

2.6.1. pH Determination

For the determination of pH of Ugba samples 1 g was ground using an electric blender and thereafter dissolved in 9 ml of deionized water contained in test tubes. This was measured using Pye, Unicam pH meter, model 291 equipped with glass electrode. The pH value displayed by the pH meter before taking the actual reading was noted, the meniscus of the pH meter was then allowed to stabilize. The reading was done immediately the Ugba sample arrived to the laboratory.

2.6.2. Temperature Determination

This was done using calibrated mercury in glass thermometer. A hole was bored through the wrapped samples and the temperature readings were taken at 12 hours intervals.

2.6.3. Measurement of Titratable Acidity (%)

The titratable acidity expressed in percent (%) acid produced was determined by the titration of 10 ml of fermenting Ugba sample dissolved in deionized water with 0.1N NaOH using phenolphthalein as an indicator [15] until the end point (pink colour) is achieved. The percentage total titratable acidity (%TTA) was calculated as:

100/volume of sample x Normality NaOH used (0.0002) x Titre value

3. Results

The fermenting oil bean underwent spontaneous fermentation and became edible Ugba identified by its appearance and aroma. The smell of the fermenting oil bean became prominent after 4 days (48 hours) of fermentation; the substrate became soft darkened and had a characteristic strong aroma resembling the smell of ammonia. Diverse groups of microorganisms were isolated during the course of fermentation, they include: *Bacillus* species, *Staphylococcus* aureus, *Streptococcus* species, *Escherichia coli*, *Proteus* species, *Lactobacillus* species *Pseudomonas aeruginosa*, *Salmonella* species and *Micrococcus* species (Table 1).

Table 1. Microscopic, biochemical and sugar fermentation profile of bacterial isolates

Isolate	Gram Reaction	Biochemical Tests								Sugar Fermentation								Probable organism
		Catalase test	Casein hydrojysis	Starch Hydrolysis	Voge-Prokaeur	Citrate Utilization	Oxidase Test	H_2S Production	Urease Test	Fructose	Galactose	Glucose	Lactose	Maltose	Mannitol	Sucrose	Xylose	
A	+	+	+	+	+	+	+	+	+	A	A	A	A	A	A	A	A	*Bacillus* species
B	-	-	+	+	-	+	-	-	+	A	-	-	A	A	-	A	A	*Proteus* species
C	+	+	+	-	+	+	-	-	-	A	A	AG	A	A	A	A	A	*Staphylococcus aureus*
D	+	-	-	-	+	+		-	+	A	-	A	-	-	A	A	A	*Micrococcus* species
E	+	-		+	+	-	-	-	-	-	-	+	+	-	-	+	-	*Streptococcus* species
F	-	+		-	-	-	-	-	-	A	A	A	A	A	A	A	A	*Escherichia coli*
G	-	-	+	-	+	-	+	+	+	-	-	A	-	A	A	-	A	*Pseudomonas aeruginosa*
H	-	+	+	-	-	-	-	+	+	+	+	AG	-	+	+	-	-	*Salmonella* species
I	+	-	+	+	-	-	-	-	-	A	A	A	A	A	A	A	A	*Lactobacillus* species

+: Positive, -: Negative, A: Acid production, AG: Acid and Gas Production

Table 2 shows the mean of triplicate values of fermenting oil bean seeds for total aerobic count, *Salmonella* and *Shigella* count, fungal count, coliform count and lactic acid bacteria count on MRS agar.

Table 2. Mean counts of microbial isolates

Fermentation Time (Hours)	Colony Forming Units (CFU)				
	TAPC	CC	SSC	FC	LABC
0	$2.5x10^6$	$7.2x10^3$	$2.9x10^3$	$8.1x10^3$	$2.6x10^5$
24	$2.1x10^6$	$6.3x10^3$	$3.2x10^3$	$5.6x10^3$	$3.1x10^5$
48	$2.4x10^6$	$8.3x10^3$	$2.1x10^3$	$6.0x10^3$	$3.8x10^5$
72	$2.0x10^6$	$2.0x10^3$	$1.9x10^3$	$7.5x10^3$	$4.0x10^5$
96	$1.5x10^6$	$1.7x10^3$	$1.6x10^3$	$8.5x10^3$	$4.6x10^5$

TAPC-Total aerobic plate count: CC-Total coliform Count: SSC-Total Fungal Count: TLABC-Total Lactic Acid Bacteria Count

Table 3 and Table 4 shows the percentage occurrence of bacterial and fungal isolates respectively, from fermenting oil bean seeds. Fungal species include: *Aspergillus* species, Yeast, *Penicillium* species, *Rhizopus* species and *Fusarium* spp.

Table 5 shows the proximate composition of fermented oil bean seeds.

Table 3. Percentage occurrences of bacterial isolates from laboratory produced and market Ugba

Isolates	No of Isolates Positive	% Occurrence
Bacillus species	20	17.9
Streptococcus species	14	12.5
Staphylococcus aureus	19	16.9
Escherichia coli	6	5.4
Salmonella species	3	2.7
Micrococcus species	11	9.8
Pseudomonas aeruginosa	5	4.5
Lactobacillus species	18	16.1
Proteus species	16	14.2
Total	112	100

Table 4. Percentage occurrences of fungal isolates from laboratory produced and market Ugba

Isolates	No of Isolates Positive	% Occurrence
Yeast	15	22.7
Penicillium species	18	27.3
Aspergillus species	17	25.8
Fusarium species	6	9.1
Rhizopus species	10	15.2
Total	66	100

Table 5. Proximate Composition of fermenting African Oil bean seeds

Composition	Values (%)
Protein	16.45
Fat	19.72
Fibre	7.34
Ash	6.12
Moisture	34.34
Carbohydrate	15.03
Dry matter	65.66

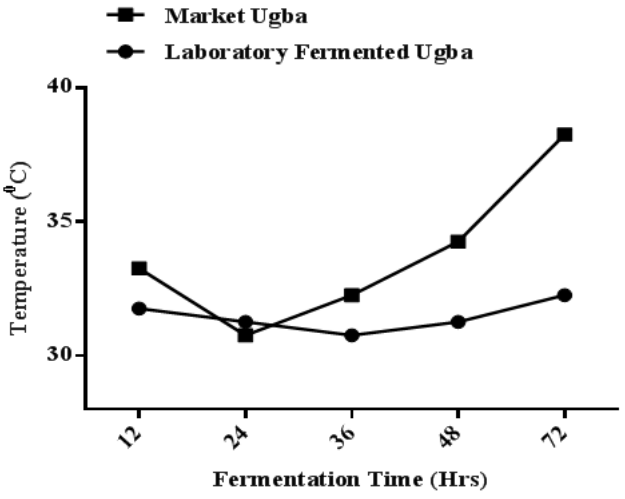

Figure 1. Temperature variations of Ugba bought from the market and Ugba produced in the laboratory

Figure 1 shows the variation in temperature during the course of fermentation of both control and laboratory fermenting oil bean seeds.

Figure 2 show the variations in pH over the fermentation duration of fermenting oil bean seeds bought from the market (control) and the laboratory produced fermenting oil bean seeds.

Figure 3 shows the variations in titratable acidity of fermenting oil bean seeds.

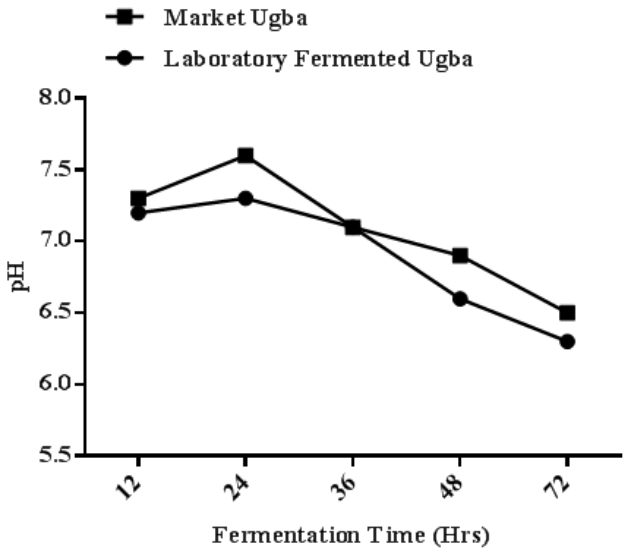

Figure 2. pH variations of Ugba bought from the market and Ugba produced in the laboratory

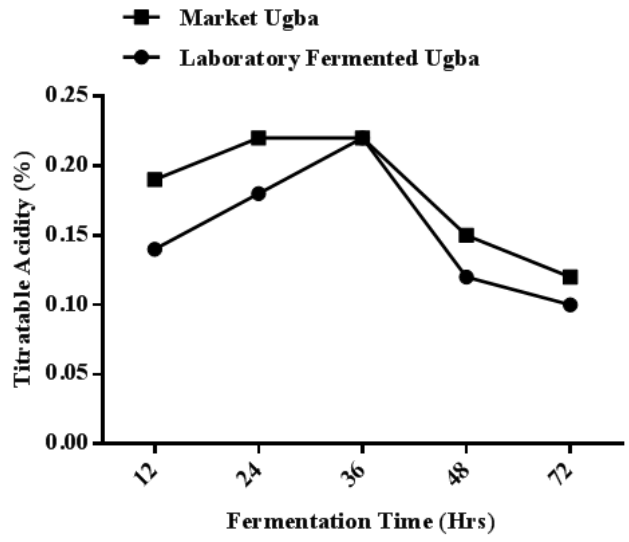

Figure 3. Variations in Titratable acidity of Ugba bought from the market and Ugba produced in the laboratory

4. Discussion

In this work, the proximate composition, biochemical changes and microorganisms associated with Ugba fermentation were evaluated.

Table 1 show the various isolated bacterial species from fermenting ugba. They include; *Bacillus* species, *Streptococcus* species, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* species, *Micrococcus* species, *Pseudomonas aeruginosa*, *Lactobacillus* species and *Proteus* spp. These isolated bacterial species was partly in agreement with observations made by [16].

The number of organisms increased tremendously from the beginning to the end of the fermentation as shown by the microbial counts in Table 2. More so, fungal species (Yeast, *Penicillium* species, *Aspergillus* species, *Fusarium* species and *Rhizopus* species) were also isolated from fermenting Ugba.

Since the major constituents of these Ugba seeds are proteins, fats and carbohydrates, the microorganisms responsible for Ugba fermentation must be capable of utilizing these food sources. Most of the microorganisms isolated from fermenting Ugba are known to posses such abilities [17].

The predominant organism was *Bacillus* species and was present throughout the 72 hours fermentation period. *Escherichia coli* and *Salmonella* species were encountered in significantly low amounts and gradually started reducing after 48 hours fermentation. The number of *Lactobacillus* species was low during the first 48 hours, but generally increased towards the later part of the fermentation period.

Since protein hydrolysis is a major biochemical change in Ugba fermentation [2], it can be assumed that *Bacillus* species are the major fermenting organisms. The co-dominance of *Staphylococcus* species and *Bacillus* species in the fermenting sample during the first 24 hours was typical of the microflora of fermenting bean seeds. *Staphylococcus* species have been associated with fermenting foods of plant origin, especially vegetable proteins [18].

The dominance of *Bacillus* and *Lactobacillus* species towards the end of the fermentation period could be attributed to the production of bacitracin and organic acids and especially bacteriocins [19]. The antibiotic may have inhibited the growth of these bacteria and the disappearance of some bacterial species towards the end of the fermentation; which was in agreement with [20].

These bacterial and fungal species involved in the local fermentation of Ugba, probably were introduced by chance. The air, water, utensils and leaves used in wrapping the oil bean seeds during the preparatory stages could have been the source of inoculation. For example, *Staphylococcus* species are more commonly associated with the skin and hence are easily disseminated through handling [21].

The percentage occurrences of all bacterial and fungal isolates are shown in tables 3 and 4 respectively. *Bacillus*, *Staphylococcus* and *Lactobacillus* species showed high percentage occurrences.

The proximate composition of fermenting oil bean seeds was evaluated as shown in Table 5. Protein, fat, fiber, carbohydrate and ash had 16.45, 19.72, 7.34, 15.03 and 6.12% composition respectively. This result was in agreement with the work of [22].

Figure 1, Figure 2 and Figure 3 shows the biochemical changes associated with the various fermentation stages of 12 hour intervals to produce the fermented product. The biochemical changes of both market and laboratory samples were evaluated and compared. The pH of the market and laboratory ugba during the course of the fermentation was almost the same except for a slight increase in pH between 12 and 24 hours by the market sample. Furthermore, variations in temperatures during the

fermentation periods were also noted. Apart from the later part of the fermentation time (48-72 hours), the temperatures of both sample were fairly the same (Figure 1).

Titratable acidity of the fermenting oil bean seeds was evaluated between fermentation days. A slightly higher percentage titratable acidity was observed in the market sample within the first 24 hours fermentation after which both samples shared close percentage titratable acidity (Figure 3).

5. Conclusion

Present findings indicated that a number of bacterial and fungal species are involved in the fermentation of the African oil bean seeds to obtain Ugba. The most predominant microbial species in the fermenting oil bean seeds include *Bacillus* and *Lactobacillus* species. The predominance of the fermentation stages by these organisms might have contributed to the gradual decline in microbial load towards the end of the fermentation period. This could be further researched to obtain pure starter co-cultures of *Bacillus* and *Lactobacillus* species to be used in African oil bean seed fermentation and also to increase the shelf-life of Ugba.

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