Bioscience and Bioengineering
Vol. 4, No. 2, 2018, pp. 41-51
http://www.aiscience.org/journal/bio
ISSN: 2381-7690 (Print); ISSN: 2381-7704 (Online)

Effects of Thermal Processing and Fermentation on African Oilbean (Pentaclethra Macrophylla) Seeds During Ugba Production

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Abstract

Boiled slices of African oil bean (Pentaclethra macrophylla) seeds were subjected to a 72-hour traditional fermentation to produce ugba for the evaluation of the effect of boiling and fermentation on the nutritional status of the seeds. Bacterial isolates recovered from the fermenting substrate include Bacillus, Streptococcus, Salmonella, Escherichia coli, Pseudomonas, Staphylococcus, Klebsiella, Micrococcus, Lactobacillus and Proteus species while Saccharomyces cerevisiae, Penicillium, Aspergillus, Fusarium and Rhizopus spp were the fungi isolated. Bacillus and Lactobacillus spp were the predominant bacterial starter cultures because they persisted from the beginning till the end of the fermentation. Saccharomyces cerevisiae was the predominant fungus. Fermentation and boiling improved the nutritional values of the protein, fat, fibre, moisture and ash contents of the ugba samples after 72 hours offermentation with an increase in pH value towards alkalinity. However, both processes decreased the carbohydrate content of the ugba. Sensory evaluation showed that the laboratory-produced ugba sample compared with the local market sourced ugba in sensory properties. At the end of the fermentation, food borne spoilers and pathogens that were part of the contaminants of the fermenting samples were eliminated by the microbial metabolites thus making ugba safe for human consumption. The ugba can also stay for some days without spoilage in the absence of refrigeration.

Keywords

Boiling, Fermentation, Nutritional Status, Oil Beanseed, Ugba

Received: September 10, 2018 / Accepted: September 27, 2018 / Published online: October 25, 2018

1. Introduction

Ugba is the Igbo name for sliced, fermented African oilbean 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 [1]. It is consumed mostly in the eastern states of Nigeria as a local delicacy popularly known as "African salad" prepared with oil, pepper, fish and salt. It could also be prepared with tapioca, stockfish and garden eggs. It can be eaten with boiled or roasted yam andcocoyam [2, 3]. Ugba is a traditional food generally prepared in homes as a small family business. Its method of preparation varies from one place to another resulting in a non-uniform product [4]. Ugba is produced traditionally by boiling the seeds overnight for easy removal of the seed coat [5]. The cotyledons are sliced and cooked until they are soft with reduced bitter taste. The sliced ugba is washed about five times or more and fermented for three days by spontaneous fermentation [5].

Ugba is a rich source of protein, vitamins and minerals, with a high demand for local and export consumption [6].

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Previous research showed that fermentation gives a better nutritional product than the raw seed [7, 8]. [11] reported that fermentation of African oil bean seeds to produce ugba softens the cotyledon, improves its digestibility and nutrients availability. [10 and 11] observed that the source of microorganisms in fermenting seeds come from handling processing, utensils used in processing and the leaves used in packaging. The result is uncontrolled fermentation. This is due to the activities of microorganisms involved in the fermenting process that remains viable till the product is consumed or continues fermentation till the product becomes unacceptable. The deterioration or spoilage of the product is evidenced by organoleptic changes in colour, texture, appearance, odour and taste [12]. Cooked oil bean seed is not edible, hence the need for fermentation. The consumption of the Ugba could pose as a means of addressing the prevailing protein energy malnutrition (PEM) in developing countries [13]. This work was aimed at determining the microbiological and nutritional changes associated with the thermal processing and fermentation of African oilbean (Pentaclethra macrophylla) seeds for ugba production.

2. Materials and Methods

2.1. Sample Collection

A total of 25 African oil bean seed samples weighing 201g used for this study were purchased from a local market in Aba, Abia state Nigeria and transported in an air-tight polythene bag to the National Root Crops Research Institute, (NRCRI) Umudike, Umuahia, for analyses. Already prepared ugba was also purchased from the local market for sensory comparison with the laboratory prepared ugba sample.

2.2. Laboratory Preparation of Ugba

The traditional technique used by the local producers from Umuahia and Aba was followed in the preparation of the ugba samples. The oil bean seeds were cleaned and boiled in an autoclave at a temperature of 121°C and a pressure of 15 psi for 2 hours to soften the hard brown testa (shell). The shells were removed and the cotyledons were cooled and washed, drained and rewashed with cold water several times. The washed cotyledons were cut into long thin slices with a sharp knife into 4-5 x 0.1-0.2 cm slices. The slices were boiled for another 30 minutes, cooled and washed and later soaked in water for 24 hours. They were washed again to remove the bitter taste and then allowed to drain in a basket for 1 hour. The slices were wrapped in seven small packets with banana leaves and lightly tied. These small packets were placed in a basket to ferment at room temperature for 3 days to yield Ugba. These steps are represented in Figure 1 Samples were aseptically taken from these small packets of ugba for the various analyses.

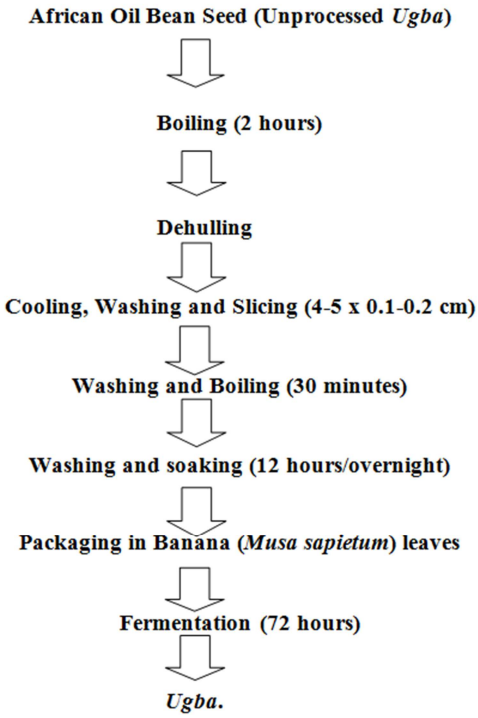

Figure 1. Flow chart for fermentation of oilbean seed for ugba production.

2.3. Samples Inoculation and Incubation

2.3.1. Serial Dilution

One gram of each of the sample was thoroughly mashed with sterile laboratory pestle and mortar and serially diluted and 0.1 ml aliquots of suitable dilution was inoculated aseptically by spread plate methods on Nutrient, MacConkey, De Man Rogosa sharpe and Sabourand Dextrose agar plates previously prepared for the isolation of bacteria and fungi [14]. The bacterial media was fortified with fluconazole. The bacterial plates were incubated at 37°C for 24-48 days while the fungal plates were incubated at 22°C for 5 days. The total viable counts were determined according to [15].

2.3.2. Bacterial Identification

The isolates were sub-cultured and identified based on their morphological and biochemical characteristics as well as Gram staining and sugar fermentation tests [16]. The pure cultures were transferred into MacCartney Bottles and stored at 4°C in refrigerator [17, 18].

2.3.3. Fungal Identification

A drop of methanol was placed on a clean slide and a portion of fungi growth was cut and was tested in the methanol. A drop of lactophenol cotton blue was added, a cover slip was then placed on it gently to which it was observed under microscope with ×40 objectives. The picture seen was compared with an identification chart [17].

2.4. Antimicrobial Susceptibility Test of Bacterial Isolates

This was carried out using Kirby-bauer disk diffusion method. Each of the bacterial isolates was aseptically streaked evenly using sterile swab stick on separate Mueller Hinton agar plates previously prepared and antibiotics disks selected based on the Gram's reaction of the isolate were placed on the plate using sterile forceps. The plates were incubated at 37°C for 24 hours and the cleared areas were measured and recorded in millimetre as resistant, intermediate or sensitive.

2.5. Proximate Analyses of Boiled and Fermented Oil Bean Seeds

The analyses of the sample for fat, crude protein, crude fiber, Ash and moisture contents were carried out and the temperature, pH and titratable acidity were determined according to [19].

2.5.1. Moisture Content Determination

Flat silica Petri dish was washed and dried for about 1 hour in the oven to cool and weighed again after which 2.0 g of the sample was weighed and added into the Petri-dish. The sample was transferred into the oven for drying at 105°C for 3 hours. The sample was then cooled in the desiccator and the weight of the sample plus the dish was taken. The percentage moisture loss was calculated.

Calculation:

$$\% \text{ Moisture} = \frac{W2-W3}{W2-W1} \times 100$$

Where:

W_1 = Initial weight of the empty crucible

W_2 = weight of crucible + sample before drying

W_3 = final weight of crucible + sample after drying

Total solid =100 - % moisture.

2.5.2 Crude Fiber Content Determination

The crude fiber content in the sample was determined according to [19]. 2.0g of mashed sample was placed in a 600 ml beaker and 200 ml of 1.25% H_2SO_4 solution was added into the beaker. The sample was heated for 30 minutes on a heating mantle. The sample was cooled before filtering. The residue was placed on a weighed crucible and allowed to dry in an oven at 150ºC until a constant weight was obtained. The dried sample was burnt to ashes in a muffle furnace, then cooled in a desiccator and re-weighed.

% Crude fiber = weight loss on ashing/Weight of original sample x100

$$\frac{\text{Weight loss on ashing}}{\text{Weight of original sample}} \text{ x } 100$$

2.5.3. Ash Content Determination

Ash Content Determination

A clean silica dish was weighed to a constant weight. 2.0 g of each sample was measured into the dish. The sample was ignited using a heating mantle in the fume cupboard until charred and no more smoke given off (pre-ashing). Using a pair of tong, the sample was transferred into a muffle furnace at a temperature of 550°C until fully ashed. The percentage ashed was calculated as follows:

Calculation:

$$\% \text{ Ash} == \frac{\text{Weight of ash}}{\text{Weight of original sample}} \times 100$$

$$= \frac{W3-W1}{W2-W1} \times 100$$

Where:

W_1 = weight of empty dish

W_2 = weight of dish + sample before drying

W_3 = weight of dish + ash

2.5.4. Fat Content Determination

The method of solvent extraction in a Soxhlet reflux apparatus was used. 250ml boiling flasks were thoroughly washed and dried in an oven. They were transferred into the desiccators to cool and each was weighed accordingly after which the Soxhlet reflux apparatus was set up. 5.0 g of the sample was accurately weighed and put into a labeled thimble with a cotton wool. 200 ml of petroleum ether was filled in the boiling flasks and heated using a heating mantle set at 100°C. The Soxhlet apparatus was allowed to reflux for about 6 hours. The thimble was carefully removed and the petroleum ether was recollected using a rotary evaporator and drained into a bottle for re-use. The flask was removed and dried at 105°C for 1 hour in an oven. The flask was then transferred from the oven into desiccators using a pair of tong and allowed to cool. After cooling, the flask containing the oil was then weighed.

Calculation:

$$\% \text{ Crude Fat Content} = \frac{W2-W1}{W3} \times 100$$

Where:

W_1 = weight of the empty extraction flask

W_2 = weight of the flask and the oil extracted

W_3 = weight of the sample.

2.5.5. Carbohydrate Determination

The Nitrogen free extract method was used. The carbohydrate was calculated as weight by different between 100 and the summation of other proximate components as nitrogen free extract (NFE).

Calculation:

$$\% \text{ N F E} = 100 - \% (a + b + c + d + e)$$

Where:

a = protein content

b = fat content

c = fibre content

d = ash content

e = moisture content.

2.5.6. Protein Content Determination

The protein content was determined by Kjeldahl method according to [19]. 0.2g of the sample was weighed into a filter paper and transferred into a neat dried Kjeldahl flask. Exactly 25ml of Conc. Sulphuric acid was added to the flask and 2 tablets of selenium catalyst. The flask was heated gently in a fume cupboard using a heating mantle in an inclined position and allowed to digest (digest is complete when the liquid is clear and free from black or brown colour). The flask was allowed to cool and was diluted with 200ml of distilled water. A distillation apparatus consisting of 500ml flask with stopper carrying a dropping funnel and a splash head adaptor and a vertical condenser in which a straight delivery tube is attached was used. Approximately 50ml of boric acid solution was measured into 500ml Erlenmeyer titration flask and a few drops of screened methyl red indicator were added and the Erlenmeyer flask placed on the receiving end of the delivery tube dipping just below the level of the boric level. Some anti-bumping agent granules and 75ml of NaOH solution were added to the distillation flask. Exactly 50ml of distilled water was added and was gently shaken to ensure mixing of contents. The flask was connected to distillation bulb and boiled vigorously until about 100ml of the distillate was obtained. The distillate was titrated with 0.1M Hcl till first trace of pink colour.

Calculation:

$$\% \text{ N} = \frac{\text{Tv} \times 1.4 \times 0.1}{\text{W}}$$

Where:

W = weight of sample in grams

Tv = Titre value

% crude protein = N X conversion factor

2.5.7. Determination of Total Titratable Acidity

The titratable acidity was determined by the alkaline titrimetric method expressed in percent (%). The acid produced was determined by the titration of 10ml of fermenting ugba sample dissolved in deionized water with 0.1N NaOH using phenolphthalein as an indicator 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.

The acidity was calculated using the formula below

$$\% \text{ TTA} = = \frac{100}{\text{W}} \times \text{titre} \times \text{N}$$

Where: N = normality of titrant, W = weight of sample used

2.5.8. Determination of pH

One gram of ugba sample was ground to a paste and added to 9ml of deionized water contained in test tubes. The electrode of Pye, Unicam pH meter, model 291 was dipped into the solution and the reading was taken.

2.5.9. Temperature Determination

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

2.5.10. 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, appearance, taste and overall acceptability. These parameters were evaluated after 72 hours of fermentation and compared with the local market ugba.

2.6. Statistical Analysis

Mean occurrences were used to determine the microbial load of the various samples. One way ANOVA was used to investigate the significant difference in the nutritional contents of the sample. Each test was conducted at 95% confidence interval, $P \leq 0.05$. The data were analysed using the program IBM SPSS Version 22.

3. Results

Table 1 shows the cultural and biochemical attributes of the bacterial isolates from ugba. They are Bacillus spp, Proteus mirabilis, Stapholococcus. aureus, Micrococcus spp, Streptococcus spp, Escherichia coli, Pseudomonas aeruginosa, Salmonella spp, Lactobacillus spp and Klebsiella spp.

Table 1. Colonial morphology and biochemical identification of bacterial isolates from ugba production.

Colony Morphology	Microscopy	Gram Reactions	Catalase	Coagulase	Voges proskauer	Cittate Utilization	Oxidase	Urease
Opaque, flat, irregular pinhead,	Long/short rods	+	+	-	+	+	+	-
Large, Circular, Smooth, mucoid	Rods	-	+	-	-	+	-	+
Opaque, golden yellow, spherical	Irregular cocci in clusters	+	+	+	+	+	-	+
Pigmented in shades of yellows	Cocci in Pairs/tetrads	+	-	-	ND	+	-	+
Creamy gold, Smooth, Spherical	Short cocci in clusters/chains	+	-	-	+	-	-	-
Pink, Irregular, Smooth, Entire	Rods	-	+	-	-	-	-	-
Irregular, Bluish green, mucoid	Slender short rods	-	-	-	+	-	+	+
Colourless, with black centres	Rods	-	+	ND	-	-	-	-
Raised, Convex, smooth, opague	Long slender rods	+	-	-	-	-	-	-
Pink, circulsr, translucent, entire	Single rods	-	+	+	+	+	-	+

Colony Morphology	Microscopy	Indole	Motility	Methyl Red	Fructose	Glucose	Galactose	Lactose
Opaque, flat, irregular pinhead,	Long/short rods	-	+	-	A	AG	A	A
Large, Circular, Smooth, mucoid	Rods	-	+	+	A	-	-	A
Opaque, golden yellow, spherical	Irregular cocci in clusters	-	-	+	A	AG	A	A
Pigmented in shades of yellows	Cocci in Pairs/tetrads	-	-	ND	A	AG	-	-
Creamy gold, Smooth, Spherical	Short cocci in clusters/chains	ND	-	ND	-	+	-	+
Pink, Irregular, Smooth, Entire	Rods	+	+	+	A	AG	A	A
Irregular, Bluish green, mucoid	Slender short rods	-	+	-	-	A	-	-
Colourless, with black centres	Rods	-	+	+	A	A	A	-
Raised, Convex, smooth, opague	Long slender rods	ND	+	ND	A	A	A	A
Pink, circulsr, translucent, entire	Single rods	-	-	-	ND	AG	A	A

Colony Morphology	Microscopy	Mannose	Xylose	Mannitol	Sucrose	Maltose	Glycerol	Probable Organisms
Opaque, flat, irregular pinhead,	Long/short rods	A	A	A	A	A	A	Bacillus spp
Large, Circular, Smooth, mucoid	Rods	-	A	-	A	A	A	Proteus mirabilis
Opaque, golden yellow, spherical	Irregular cocci in clusters	A	A	A	A	A	-	Staphylococcus. aureus
Pigmented in shades of yellows	Cocci in Pairs/tetrads	ND	A	A	A	-	AG	Micrococcus spp
Creamy gold, Smooth, Spherical	Short cocci in clusters/chains	ND	-	-	+	-	+	Streptococcus spp
Pink, Irregular, Smooth, Entire	Rods	-	A	A	A	A	ND	Escherichia coli
Irregular, Bluish green, mucoid	Slender short rods	-	A	A	-	A	ND	Pseudomonas aeruginosa
Colourless, with black centres	Rods	+	-	+	-	+	-	Salmonella spp
Raised, Convex, smooth, opague	Long slender rods	+	A	A	A	A	-	Lactobacillus spp
Pink, circulsr, translucent, entire	Single rods	A	A	A	A	A	-	Klebsiella spp

ND= Not Done, A=Acid, AG=Acid and Gas, + = Positive, - = negative.

Table 2 shows the microscopy and cultural morphology of the fungal isolates. The isolates are Aspergillus spp, Penicillium spp, Fusarium spp, Yeast cells and Rhizpous spp.

Table 2. Microscopy and colonial morphology of fungal isolates from ugba production.

S/no	Colony characteristics	Microscopic appearance	Probable organism
1.	Black in colour, large mass ofcolony.	Conidiospores aseptate with hyphae.	Aspergillus spp
2.	Velvety, fuzzy, green, dark in colour	Green spores with whitish hyphae.	Penicillium spp
3.	Brown-greenish to white-greenish with dark zonation	Long and Branched Monophialides	Fusarium spp
4.	Moist, small shiny whitish colony.	Ova, spherical shape.	Yeast cells.
5.	Rapid growth, white to pale	Sparsely septate broad hyphae	Rhizpous spp

Table 3 shows the mean counts of microbial isolates (cfu/ml) from ugba production. All the bacterial isolates on TAPC and MRS increased significantly from the beginning to the end of the 72 hours fermentation while bacterial isolates on SSA, MacConkey agar and TFC decreased significantly as the fermentation progressed.

Table 3. Mean counts of microbial isolates (cfu/ml) from ugba production.

Fermentation Time (h)	TAPC	TCC	SSC	TFC	TLABC
0	2.1×10^6	4.9×10^5	3.1×10^5	6.1×10^6	2.6×10^6
12	2.5×10^6	4.1×10^4	2.9×10^4	5.9×10^5	2.8×10^6
24	3.3×10^6	3.4×10^4	2.3×10^4	5.2×10^5	3.1×10^6
36	3.9×10^7	3.0×10^3	2.0×10^3	4.9×10^4	3.9×10^7

Fermentation Time (h)	TAPC	TCC	SSC	TFC	TLABC
48	4.7×10^7	2.1×10^3	1.7×10^3	4.3×10^4	4.4×10^7
60	5.4×10^8	1.7×10^3	1.5×10^3	3.8×10^3	5.3×10^8
72	8.9×10^8	1.1×10^3	1.0×10^3	3.0×10^3	7.5×10^8

TAPC-Total aerobic plate counts. TCC-Total coliform counts. TSSC-Total Salmonella-Shigella counts. TFC-Total Fungal Count. TLABC-Total Lactic Acid Bacterial Count.

Table 4 shows the bacterial succession during ugba production. Among all the isolates, only Bacillus and Lactobacillus spp stayed till the end of the fermentation.

Table 4. Bacterial succession during ugba production.

		Period of Fermentation (h)						
S/N	Organisms	0	12	24	36	48	60	72
1.	Staphylococcus aureus	+	+	+	+	+	-	-
2.	Micrococcus spp.	+	+	+	+	+	-	-
3.	Bacillus spp.	+	+	+	+	+	+	+
4.	E. coli	+	+	+	+	-	-	-
5.	Lactobacillus spp	+	+	+	+	+	+	+
6.	Klebsiella spp.	+	+	+	-	-	-	-
7.	Streptococcus spp.	+	+	+	+	+	-	-
8.	Pseudomonas spp.	+	+	+	-	-	-	-
9.	Proteus spp.	+	+	+	+	+	+	-
10.	Salmonella spp	+	+	+	+	-	-	-

+ Present. – Absent

Table 5 shows the fungal succession during ugba production fermentation. Only Saccharomyces cerevisiae persisted till the end of the fermentation.

Table 5. Fungal succession during ugba production.

		Period of Fermentation (h)						
S/NO	Organism	0	12	24	36	48	60	72
1.	Saccharomyces cerevisiae	+	+	+	+	+	+	+
2.	Fusarium spp	+	+	+	+	+	+	-
3.	Aspergillus spp.	+	+	+	+	+	+	-
4.	Rhizopus spp.	+	+	+	+	+	-	-
5.	Penicillium spp.	-	+	+	+	+	+	-

+ = Present, - = absent

Table 6 shows the percentage occurrence of the bacterial and fungal isolates. Bacillus spp had the highest occurrence (18.3%) while Salmonella and Klebsiella spp occurred least (3.5%) respectively.

Table 6. Percentage occurrences of bacterial isolates during ugba production.

Isolates	Number	%
Staphylococcus aureus.	15	13.0
Micrococcus spp.	13	11.3
Bacillus spp.	21	18.3
E. coli	5	4.3
Lactobacillus spp	19	16.5
Klebsiella spp.	4	3.5
Streptococcus spp.	12	10.4
Pseudomonas spp.	5	4.3
Proteus spp.	18	15.7
Salmonella spp	4	3.5
TOTAL	115	100

Table 7 shows the percentage occurrence of fungal isolates during ugba production. Saccharomyces cerevisiae has the highest occurrence (26.5%) while Rhizopus spp was the least (13.3%)

Table 7. Percentage occurrence of fungal isolates during ugba production.

Isolates	Number	%
Saccharomyces cerevisiae	22	26.5
Fusarium spp.	17	20.5
Aspergillus spp.	15	18.1
Rhizopus spp.	11	13.3
Penicillium spp.	18	21.7
TOTAL	83	100

Table 8 shows the antibiotics susceptibility pattern of the bacterial isolates. The highest sensitivity was from Cotrimoxazole, Tetracycline and Chloramphenicol (21 mm) respectively

Table 8. Antibiotic susceptibility of the bacterial isolates from ugba production.

	Cotrimoxazole (10ug)	Cloxacillin (20ug)	Erythromycin (30ug)	Augmentin (30ug)
Escherichia coli	17.00	11.00	12.00	17.00
Staphylococcus aureus	13.00	10.00	15.00	18.00
Klebsiella spp	21.00	10.00	10.00	15.00
Pseudomonas aeruginosa	3.00	5.00	17.00	20.00
Streptococcus spp	15.00	9.00	18.00	8.00
Bacillus spp	10.00	13.00	7.00	12.00
Lactobacillus spp	9.00	8.00	5.00	9.00
Proteus spp	5.00	10.00	15.00	12.00
Micrcoccus spp	11.00	11.00	4.00	10.00
Salmonella spp	8.00	3.00	15.00	14.00

Table 8. Continued.

	Gentamicin (10ug)	Stretomycin (30ug)	Tetracycline (30ug)	Chloramphenicol (30ug)
Escherichia coli	20.00	10.00	2.00	17.00
Staphylococcus aureus	18.00	6.00	13.00	15.00
Klebsiella spp	15.00	8.00	3.00	20.00
Pseudomonas aeruginosa	18.00	12.00	21.00	13.00
Streptococcus spp	14.00	4.00	18.00	18.00
Bacillus spp	18.00	5.00	20.00	21.00
Lactobacillus spp	15.00	3.00	4.00	4.00
Proteus spp	15.00	15.00	5.00	17.00
Micrcoccus spp	12.00	10.00	9.00	19.00
Salmonella spp	16.00	15.00	2.00	18.00

Table 9 shows the temperature, TTA and pH values of the laboratory prepared ugba. The highest temperature (32.3±0.2 ^{o}C) was recorded at 36th hour while the highest pH (8.1±0.2) and the lowest TTA (0.52±0.1) were recorded on the 72nd hour.

Table 9. Temperature, TTA and pH values of the fermented ugba.

	Time (h)
Parameters	0 12 24 36 48 60 72
Temperature	27.6±0.2 28.8±0.1 30.6±0.3 32.3±0.2 32.1±0.1 31.1±0.2 30.2±0.2
TTA	0.52±0.1 0.61±0.1 0.77±0.2 0.85±0.1 0.96±0.1 1.14±0.2 1.22±0.1
pH	5.7±0.1 6.2±0.1 6.7±0.2 7.1±0.3 7.4±0.1 7.7±0.1 8.1±0.2

Table 10 shows the proximate composition of the raw, boiled and fermented ugba. The fermented ugba has the highest moisture, crude protein, crude fibre, ash and fat content than others while the raw oilbean seed had the highest carbohydrate content.

Table 10. Proximate composition of ugba samples.

Samples	Moisture	Crude protein	Crude fibre	Ash	Carbohydrate	Fats
Raw oil bean seed	34.33±0.1	24.32±0.17	4.36±0.1	2.1 ± 0.1	3.19 ± 0.1	18.72±0.4
Boiled oil bean seed	34.97±0.4	26.15±0.13	4.58±0.1	2.4 ± 0.2	2.11 ±0.1	23.11±0.1
Fermented ugba	35.44±0.2	29.68±0.9	4.86±0.3	2.8 ± 0.1	1.59 ±0.2	25.23±0.2

Mean±SD :Values with same superscript along the columes are not significantly different, $P \leq 0.05$

Table 11 shows the sensory evaluation of the laboratory prepared ugba and ugba bought from the local market. The two types of ugba have the same colour, appearance, slimness, aroma, texture, taste and overall acceptability.

Table 11. Sensory evaluation of the laboratory fermented ugba and ugba bought from the local market.

Samples	Colour	Appearance	Slimness	Aroma	Texture	Taste	Over Acceptability
Laboratory Ugba	Brownish	Normal	Slightly slim	Normal	Soft	Normal	Accepted
Market Ugba	Brownish	Normal	Slightly slim	Normal	Soft	Normal	Accepted

4. Discussion

The present research aimed at determining the microbiological and nutritional changes associated with the thermal processing and fermentation of African oilbean (Pentaclethra macrophylla benth) seeds for ugba production. The oil bean seeds were fermented spontaneously for 72 hours and became edible as characterized by its appearance and aroma when the substrate became soft darkened and had a characteristic strong aroma resembling the smell of ammonia. The bacterial isolates (Bacillus species, Staphylococcus aureus, Streptococcus spp, Escherichia coli, Proteus spp, Lactobacillus spp, Pseudomonas aeruginosa, Salmonella spp, Klebsiella spp and Micrococcus spp) and the fungal flora (Aspergillus spp, Rhizopus spp, Fusarium spp, and Penicillium spp) recovered from the fermentation are similar to those reported by [20].

Since the oil bean seeds were boiled before fermentation, the fermenting microbes must have come from the handling (during the slicing of the boiled seeds), the knife, the well water used in soaking the sliced oil bean seeds, the containers, the air and the banana leaves used in wrapping the sliced oil bean seeds. This finding is in agreement with the findings [10, 11]. The traditional fermentation process that employs chance inoculation of starter culture renders the substrate vulnerable to contamination by pathogenic and spoilage microorganisms. However, these mixed starter cultures contribute individually towards a better fermentation, flavour and aroma formation that are characteristic of well fermented ugba.

The major constituents of the ugba seeds are proteins, fats and carbohydrates, thus, microorganisms responsible for ugba fermentation must be capable of utilizing these food sources. Most of the microorganisms isolated here possess such abilities [21, 22].

Lactobacillus spp and Bacillus spp persisted to the end of the fermentation. This means that these bacteria tolerated the various metabolites produced by the mixed starter cultures throught out the fermentation time. This finding is in agreement with the work of [10]. These two bacteria possessed the enzymes proteases that hydrolyzed the complex proteins found in the oil bean seeds. This is in agreement with the findings of [23 and 24]. Bacillus spp has been identified as the main microorganisms responsible for the fermentation of African oil bean seeds. In this work, the microbial and oganoleptic changes associated with the thermal processing and fermentation of African oilbean seeds for ugba production shows that Bacillus, Lactobacillus and Proteus spp are proteolytic in nature and so worked in hydrolyzing the oil bean seeds [25] and therefore are responsible for the observed increase in free amino acids (FAA) that were always recorded during production of the ugba. Bacillus species is the major fermenting organism in ugba processing, and has been responsible for the obtained texture, aroma and palatability of the fermented ugba [26].

Increase in pH towards alkalinity has been attributed to the abundant production of ammonia during the fermentation due to protein hydrolysis and deaminase activity. This is in agreement with the findings of [11, 4, 27]. Since protein hydrolysis is a major biochemical change in ugba fermentation, this could be associated with Bacillus species which are the major fermenting organism as observed in this work.

The total aerobic plate counts were low during the first 12-24 hours, but this increased tremendously towards the later part of the fermentation period. Generally, the counts of food borne pathogens recovered from the fermenting oil bean seeds increased gradually up to the 36^{th} hours of fermentation, but decreases rapidly as fermentation progresses to the end point. This is in agreement with the findings of [10]. This could be due to the production of antimicrobials such as bacitracin by Bacillus spp, bacteriocins, hydrogen peroxides, CO_2, diacethyl by Lactobacillus spp which inhibited and eliminated these bacteria from the fermenting sample as reported also by [27].

The co-dominance of Staphylococcus species and Bacillus species in the fermenting sample up to the 48^{th} hour of fermentation was because S. aureus has been associated with fermenting foods of plant origin, especially vegetable proteins [28]. S. aureus exists as opportunistic-natural flora of the human skin and nasal cavity, hence is easily disseminated through handling. Its presence in food poses a high risk of gastroenteritis due to its production of enterotoxin, hence its absence in the fermented ugba sample is a sign of food safety. Most of the isolated organisms in this study are not majorly true fermenters of ugba, but could be involved in the fermentation process as contaminants.

Members of the Enterobacteriaceae: E. coli, Klebsiella spp., and P. aeruginosa and Salmonella species were encountered in significantly low amounts especially at the early stages of production and gradually started reducing after 36-48 hours fermentation in accordance with the reports of [29, 30]. These microbial genera did not survive till the end of the fermentation, presumably because of the modified environment such as increase in pH towards alkalinity, activities metabolites such as bacteriocin, CO_2, alcohols (produced S. cerevisiae) might have eliminated these pathogens towards the end of the fermentation. This proves that fermentation eliminates pathogens from ugba thus guaranteeing it as a safe food for human consumption.

The fungi isolated from this study reduced in number with increase in fermentation time. A. flavus is known to produce mycotoxin with obvious health hazards when consumed with food. Fermentation was able to destroy this fungal and deactivate any mycotoxin it possibly produced in the fermenting medium thus presenting ugba as a safe food for human consumption. The elimination of Fusarium, Rhizopus and Penicillium spp (potential food spoilers) from the end product ensures that ugba can last for some days without spoiling especially in the local homes without refrigeration as storage means.

In percentage occurrences of all bacterial isolates, Bacillus spp dominated followed by Lactobacillus and Proteus spp. S. cerevisiae dominated other fungal isolates in occurrence.

The bacterial isolates showed most sensitivity to Chloramphenicol, Augmentin, Gentamycin and least to Tetracycline. This indicates that bacterial infections arising from post-fermentation contamination of ugba could be treated adequately using Chloramphenicol, Augmentin and Gentamycin as the first drugs of choice.

The increase in pH values of the sample has been attributed to the abundant production of ammonia and other products of protein decomposition during the fermentation due to protein hydrolysis by deaminase activity [27]. This increase in pH would also encourage the growth of Bacillus spp., which has been found to grow well at pH 7.0 to 8.0, since protein hydrolysis is a major biochemical change in Ugba fermentation.

The temperature of the ugba fermentation increased from 27.6 to 32.3°C within the first 24-36 h of fermentation and

later dropped to 30.2°C at the end of fermentation. This is in agreement with the findings of [25, 4]. Thus ugba fermentation is exothermic reaction. This initial increase in temperature has been attributed to the intense metabolic activities of the microorganisms (period of maximum microbial activity) and represents the most active and important period of the fermentation. This is because enzyme studies have revealed that the α-amylase, proteolytic and lipolytic enzyme attain their maximum activity levels within 24-36 h of fermentation.

The moisture content of the sample was highest among the three and this could lead to short shelf-life of the ugba products because increase in moisture content could lead to deterioration of the product upon storage. The increase in moisture throughout the fermentation period was also observed by [11, 27]. Increase in moisture content of the boiled oil bean seed shows a significant effect of thermal processing which caused swelling and softening of the seed due to thermal hydrolysis of the seeds. The further increase in moisture content of ugba was due to the hydrolytic proteolysis by the aerobic respiration of the microorganisms during which water is formed by terminal reduction of oxygen. The moisture content of boiled and sliced ugba recorded here was higher than that reported by [31] but lower than the moisture content of same sliced oil bean seeds reported by [32].

There was a slight increase in ash content of ugba sample at the end of fermentation (72 hours).

The increase in ash content of the fermented products in this study could be attributed to increased metabolic activities of the fermenting microorganism leading to the hydrolysis of some anti-nutritional factors that locked up some minerals in the raw seeds [33, 34]. Similar result was observed by [6] in fermented African oil bean seeds but values lower than these were recorded by [35] in fermented seeds of Jatropha curcas. This implied that boiling and fermentation of African oil bean seeds increase the mineral content.

The fat content of the boiled and fermented products increased progressively. Boiling and fermentation greatly hydrolyze some higher molecular fats in the substrates leading to increase in edible fats in ugba. The fat content of boiled and fermented African oil bean seed in this study compared favourably with fat content of fermented Jatropha curcas [35]. This result implies that ugba is a good source of fatty acids and glycerol oil and can be grouped under oil rich plant foods. It could also be a source of edible oil for domestic and industrial uses.

In this study the fibre contents of the African oil bean seed slices during fermentation slightly increased. The values obtained here were comparable to the value for ugba and fermented Bambara nut reported by [31, 36] respectively. Fibre has a role in providing roughage and aiding digestion. Reports have shown that fibre consumption may reduce occurrence of diseases like diabetes, coronary heart disease, colon cancer and various digestive disorders [37]. Consumption of fibre also softens stool in the body [38]. Hence, ugba could prove helpful in disease prevention.

Crude protein content of ugba sample increased significantly possibly due to the hydrolysis of complex inedible proteins found in oil bean seeds. Thermal hydrolysis increased the protein content and fermentation increased it further by the production of free amino acids by the proteolytic microbes. The protein value must have been increased further by the addition of single cell proteins from the microbes that fermented the sample. Similar result was reported by [5]. However, higher crude protein values were reported by [39], in fermented African locust bean. The results obtained in this study were similar to crude protein content of fermented melon seeds [39] and ugba [31] respectively. Thus, ugba could be used as a source of protein in diet or protein supplement in developing countries where their staple foods are starchy root tubers and cereals and where conventional sources of protein like egg, meat, fish and milk are relatively scarce and costly to procur. Mashed ugba could be used too as weaning food supplements when mixed with ogi (pap) in these areas too due to its good protein content.

The carbohydrate content of boiled oil bean seeds decreased and further dropped with fermentation. This observation was probably due to the hydrolytic effect of microbial amylases converting carbohydrate into sugars which they used for their various metabolic activities [40]. The value recorded here for ugba was lower compared to the value for ugba and fermented African locust bean as reported by [31] and [39] respectively. However, carbohydrate contents of the unfermented oil bean seed in this study compared favourably with the carbohydrate content of oil bean seed flour [32].

Sensory evaluation of the ugba sample showed significant similarities in term of colour, appearance, slimness, aroma, texture, taste and overall acceptability with the market samples. This comparisons show that the microbial isolates from the laboratory prepared ugba sample were similar to the isolates from the market ugba.

5. Conclusion

The fermentation of African oil bean Pentaclethra macrophylla seeds resulted in higher nutrients availability. Boiling led to loss in some of the nutrients found in the oil bean seed. Bacillus spp was the dominant fermenting starter culture followed by Lactobacillus spp. Fermentation

eliminated food borne pathogens and spoilage microbes associated with the spontaneous fermentation of the substrate thereby making ugba a safe food for human consumption. The elimination of food spoilers ensures that ugba will last for some days before it spoils in the absence of refrigeration.

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