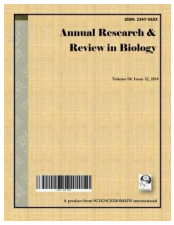

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Microbiological and Nutritional Qualities of Fermented Ugba (*Pentaclethra macrophylla*, Bentham) Sold in Mbaise, Imo State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author OLO designed the study. Author NCJA wrote the protocol and interpreted the data. Author OLO anchored the field study and gathered the initial data. Author NCJA performed preliminary data analysis. Authors CNI and BA managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was carried out to investigate the microbial load and nutritional qualities of fermented Ugba
Study Design: This research study was done using random sampling technique.
Place and Duration of Study: Mbaise Markets, Mbaise, Imo State; Department of Microbiology, Madonna University, Elele Campus, Rivers State, between October 2014 and March 2015.
Materials and Methods: A total of 20 samples of ugba were purchased from different locations in Mbaise markets - Orie-Ikpa, Nkwo-Ogwu, Orie-Amaigwe and Afor-Ajaala. Pour plate technique was used for enumeration while streak plate method was used for isolation. Nutrient agar, MacConkey agar, Eosin methylene blue agar and Sabouraud dextrose agar were used to determine total aerobic plate count, coliform count, *Escherichia coli* and fungal count respectively.
Results: For Total Aerobic Plate Count (TAPC), Afor-Ajaala had the highest count (8.05±0.11 $\log_{10}$ cfu/g) while Orie-Ikpa had the lowest count (7.98±0.14 $\log_{10}$ cfu/g). For Colony Count (CC), Orie-

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Ikpa had the highest count (5.88±0.17 $\log_{10}$ cfu/g) while Orie-Amaigwe had the lowest count (5.75±0.15 $\log_{10}$ cfu/g). For *E. coli* Count (EC) counts, Nkwo-Ogwu market had the highest (4.34±0.40 $\log_{10}$ cfu/g) while Orie-Amaigwe had the least (4.12±0.30 $\log_{10}$ cfu/g). For Fungi Count (FC), Nkwo-Ogwu had the highest count (5.27±0.48 $\log_{10}$ cfu/g) while Orie-Ikpa had the least count (5.03±0.48 $\log_{10}$ cfu/g). Bacteria isolated include *Staphylococcus* sp (80%), *Bacillus* sp (95%), *Lactobacillus* sp (90%), *Klebsiella* sp (35%) and *Escherichia coli* (35%). Fungi isolates were *Aspergillus* spp (45%), *Penicillium* spp (35%) and *Saccharomyces* spp (100%). The proximate analysis (%/100 g) yielded crude protein content (29.3), ash content (8.83), crude fibre (5.75), moisture content (33.7), lipid (18.32), Carbohydrate (3.55) and dry mass (66.23).

Conclusion: This study revealed that consumers of Ugba processed or sold in poorly sanitized condition are exposed to food poisoning and gastroenteritis, hence, the need to control microbial growth on Ugba sold in Imo State. However, Ugba prepared and sold in a hygienic environment serves as an adequate proteinaceous food.

Keywords: Ugba; bacteria; fungi; TAPC; CC; EC and FC.

1. INTRODUCTION

Ugba (or Ukpaka as it is called in Igbo language), popularly called African salad, is a ready-to-eat food, which is produced by the fermentation of African oil bean seeds (*Pentaclethra macrophylla*, Bentham). African oil bean seed is from a woody plant predominant in the rain forest areas of West and Central Africa belonging to the family Leguminosaea, sub-family Mimosoidae [1]. Ugba is a proteinaceous delicacy consumed by millions of people in the South-Eastern zone of Nigeria [2]. The fermented product is rich in fats, protein and carbohydrate [3]. During the fermentation process, *Bacillus subtilis* plays significant roles in modifying the substrate biochemically, nutritionally and organoleptically [4]. Although the predominant species responsible for Ugba fermentation is *Bacillus subtilis*, other species like *B. pumilus, B. megaterium, B. lichenformis* have also been found [5]. Ugba production is locally done through a mixed wild bacteria fermentation of the sliced, boiled and soaked African oil bean seeds. Microbial population of Ugba is introduced through the air, water, utensil, banana leaves or the handler; no starter culture is used for the traditional method. Microorganisms involved are predominantly *Bacillus*, *Micrococcus* and *Lactobacillus*. Other organisms isolated from Ugba include *Pseudomonas*, *Staphylococcus*, *Enterobacter*, *Leuconostoc*, *Corynebacterium* and *Alkaligenes* [4,6,-10]. Ugba is currently gaining wide acceptance and consumed all round Nigeria, as well as West African sub region. It has been recommended to be a good source of low cost palatable protein and has a great potential to serve a general condiment for food like Okra soup.

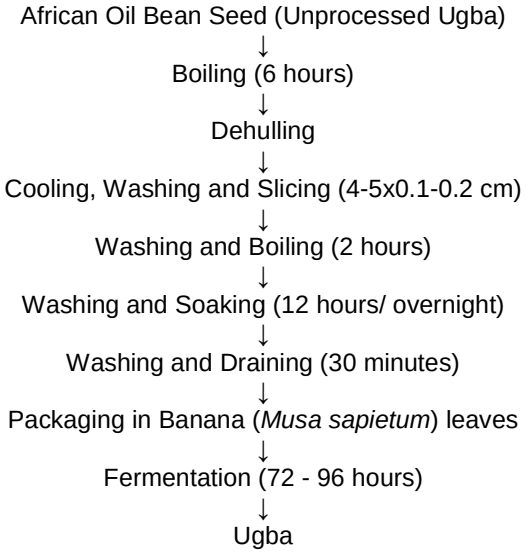

Fig. 1. Processes involved in Ugba production

Previous reports on Ugba have been mainly on the biochemistry and microbiology of fermentation and analysis for safe processing and handling [11-14]. Currently, Ugba is produced on a home industry scale which varies from place to place, resulting in a non-uniform product. The microbial count recorded during the traditional fermentation [4,7] is indicative of poor hygiene and sanitary practices during processing. These are suggestive of the probability of Ugba being a vehicular agent for the dissemination and propagation of some of the implicated pathogens which are known to be responsible for several unreported gastroenteritis and other associated ill health conditions in Nigeria [4].

The problem of occurrence and growth of pathogens in Ugba, like most other fermented food products, cannot be overruled as the general hygienic conditions of the processors, the equipment used, water and other raw materials cannot be said to be free of potential pathogens. The use of Hazard Analysis Critical control program (HACCP) to reduce microbiological hazard associated with food to safe level during production and distribution have been documented [15]. In addition, implementation of HACCP in food processing and industry to detect contamination of product by pathogenic microorganisms has been reported [16]. The relative significance of Ugba as protein supplement, the increasing acceptance and the potential to act as vehicular agent for some food borne pathogens, necessitate the need to apply and implement HACCP.

This study was aimed at investigating the microbial load and nutritional qualities of fermented Ugba sold in Mbaise markets, Imo state.

2. MATERIALS AND METHODS

2.1 Study Area

Mbaise is a region and a people located in Imo State, South-Eastern Nigeria. Set in the heart of Igbo land, it includes several towns and cities. The name "Mbaise" means “Five cities” in Igbo language, and was derived from five cities: Agbaja, Ahiara, Ekwereazu, Ezi na Ihite and Oke Uvuru. The area of Mbaise (encompassing three Local Government Areas) is about 404 km²: Aboh Mbaise (185 km²), Ahiazu Mbaise (111 km²), and Ezinihitte Mbaise (108 km²). The Mbaise Slogan is Seat of Sages. Ugba is a cherished local food of an average Mbaise indigene and is to them, what “Abacha” (Igbo name for Tapioca, an African salad processed from sliced fermented cocoyam tubers) is to an Anambra State indigene.

2.2 Sample Collection and Processing

A total of 20 packaged Ugba samples were obtained from Orie-Ikpa, Nkwo-Ogwu, Orie-Amaigwe and Afor-Ajaala markets in Mbaise, Imo state. 10g each of the samples were marshed with sterile blender into 90 mL of sterile 0.1% peptone water as diluent to get homogenized slurry stock culture. Serial dilution was done for each sample to obtain 6 dilutions (10^{-1}-10^{-6}) by diluting 1 in 9 mls of sterile peptone water, first from stock culture, then from subsequent dilutions.

2.3 Enumeration of Organisms

0.1ml each of the dilution was inoculated using pour plate technique for total aerobic plate count (TAPC) on Nutrient agar (CM003, Oxoid), coliform count (CC) on MacConkey agar (CM0050, Oxoid), *E. coli* count (EC) on Eosin methylene blue agar (CM0069, Oxoid) (at 35-37℃ for 20-24 hours) and fungi count (FC) was done using while 1 mL of the inoculum on Sabouraud dextrose agar (at room temperature for 3-7 days). All culture media used were prepared according to manufacturer’s instruction. Plates showing between 30 and 300 colonies were counted using the digital illuminated colony counter (Gallenkamp). Colony counts were expressed as colony forming units per gram of sample. All counts were done in triplicate and average values were reported.

2.4 Isolation of Bacteria

Isolates were sub-cultured using streak plate technique to obtain pure cultures. Presumptive isolates were identified by observing their morphology on the agar plates. Gram staining and biochemical tests were carried out. Catalase, coagulase, DNase and mannitol fermentation were positive for *Staphylococcus;* Indole, Urease, Voges-Proskauer and Citrate tests were used to identify *Klebsiella* and *E. coli*; *Bacillus* and *Lactobacillus* were identified using catalase, gelatin liquefaction, urease, and starch hydrolysis tests. All isolates were subjected to sugar fermentation tests. Fungi were identified through microscopy using acetone and Lactophenol. The bacterial isolates were confirmed using

Microgen™ identification test kits. The tests were run according to manufacturer's instruction.

2.5 Proximate Analysis of Fermented Ugba Samples

The proximate analysis of the samples was carried out in the Department of Microbiology, Madonna University, with the help of a biochemistry expert.

2.6 Statistical Analysis

Mean occurrences were used to determine the microbial load of various samples. One way ANOVA was used to investigate the significant difference in the microbial load in the different markets sampled. Each test was conducted at 95% confidence interval, $P<0.05$ at the appropriate degrees of freedom (d.f.). A P-value of $P<0.05$ was considered significant. The data were analysed using the programme IBM SPSS Version 22.

3. RESULTS

Table 1 presents the mean count of Ugba in Mbaise markets. TAPC shows that Ugba purchased from Afor-Ajaala market had the highest count (8.0±0.11 $\log_{10}$ cfu/g) but not significantly different ($P>0.05$, $P= 0.20$) from that obtained from Orie-Ikpa market which had the lowest count (7.98±0.14 $\log_{10}$ cfu/g). CC indicates that Ugba purchased from Orie-Ikpa had the highest count (5.88±0.17 $\log_{10}$ cfu/g) while Orie-Amaigwe had the lowest count (5.75±0.15 $\log_{10}$ cfu/g). The difference in counts was not statistically significant ($P>$ 0.05, $P=$ 0.20). EC showed that Nkwo-Ogwu market had the highest mean *E. coli* count (4.34±0.40 $\log_{10}$ cfu/g) while samples from Orie-Amaigwe market had the lowest count (4.12±0.30 $\log_{10}$ cfu/g). FC of Ugba from Nkwo-Ogwu had the highest count (5.27±0.48 $\log_{10}$ cfu/g) and Ugba purchased from Orie-Ikpa market had the lowest (5.03±0.48 $\log_{10}$ cfu/g). Generally, mean TAPC and CC were significantly higher than mean EC and FC ($P<$ 0.001).

Bacteria isolated include; *Bacillus* sp (95.00%), *Staphylococcus* sp (80.00%), *Lactobactillus* (90.00%), *Klebsiella* sp (35.00%) and *Escherichia coli* (35.00%). Fungi isolated were *Aspergillus* sp (45.00%), *Penicillium* sp (35.00%) and *Saccharomyces* sp (100.00%) (Table 2). The highest occurrence of *Saccharomyces* was observed in Ugba purchased from all markets. The least occurrence of *E. coli* (20%) and *Klebsiella* sp (20%) were however seen

Table 1. Mean count of isolates from fermented Ugba sold in Mbaise

Location	Mean Count (Log10 Cfu/g)			
	TAPC	CC	EC	FC
A	7.98±0.14	5.88±0.17	4.31±0.06	5.03±0.48
B	7.99±0.09	5.78±0.15	4.34±0.40	5.27±0.48
C	8.03±0.08	5.75±0.15	4.12±0.30	5.18±0.48
D	8.05±0.11	5.82±025	4.25±0.48	5.16±0.48

Key: A- Orie-Ikpa market; B- Nkwo-Ogwu market; C - Orie-Amaigwe market; D- Afor-Ajaala; CFU- Colony forming unit; TAPC- Total aerobic plate count; CC- Coliform count; EC- Escherichia coli count; FC- Fungal count

Table 2. Percentage occurrence of bacteria and fungi isolated from fermented Ugba sold in Mbaise, Imo state

Organism	A (N = 5)	B (N = 5)	C (N = 5)	D (N = 5)	Total (N = 20)
Bacteria					
Staphylococcus sp	4(80.00)	3(60.00)	5(100.00)	4(80.00)	16(80.00)
Bacillus sp	4(80.00)	5(100.00)	5(100.00)	5(100.00)	19(95.00)
Escherichia coli	2(40.00)	3(60.00)	1(20.00)	2(40.00)	7(35.00)
Lactobacillus	3(60.00)	5(100.00)	5(100.00)	5(100.00)	18(90.00)
Klebsiella sp	1(20.00)	3(60.00)	1(20.00)	2(40.00)	7(35.00)
Fungi					
Aspergillus sp	2(40.00)	3(60.00)	2(40.00)	2(40.00)	9(45.00)
Penicillium sp	3(60.00)	1(20.00)	1(20.00)	2(40.00)	7(35.00)
Saccharomyces sp	5(100.00)	5(100.00)	5(100.00)	5(100.00)	20(100.00)

Key: A- Orie-Ikpa market; B- Nkwo-Ogwu market; C - Orie-Amaigwe market; D- Afor-Ajaala; N- Number of samples collected

in Ugba purchased from Orie-Amaigwe and Orie-Ikpa markets respectively.

Table 3 shows the mean proximate analysis result. The analysis carried out yielded the following (%/100 g): crude protein content (29.30), ash content (8.83), crude fibre (5.75), moisture content (33.70), lipid (18.32), Carbohydrate (3.55) and dry mass (66.23). Table 4 shows the conventional test results of isolated bacteria. All isolates fermented the sugars subjected to.

4. DISCUSSION

Analysis on the Ugba samples revealed the association of both bacterial and fungal isolates. Five genera of bacteria, namely- *Bacillus, Escherichia, Lactobacillus, Klebsiella and Staphylococcus,* and three genera of fungi- *Aspergillus, Saccharomyces* and *Penicillium* were isolated.

Food pathogens such as *Clostridum perfringens*, *C. botulinum*, *Salmonella* sp., *Shigella* sp. and *Vibrio* sp. were not isolated in this study. This concurs with previous studies by [9,17]. However, bacteria like *E. coli* and *Staphylococcus aureus* were isolated. These are bacteria capable of causing food-borne infections. This is risky, since no heating process is involved in the preparation of African salad from processed Ugba, thus, ruling out the possible eliminating of these bacteria during preparation. Nevertheless, its processing from oil bean seeds and/or its addition as condiment to soup involves heating, which could eliminate these pathogens. *Bacillus* sp and *Lactobacillus* sp which were isolated in this study are known fermentation agents [4,6,9]. This may explain their presence in the present study.

Table 3. Summary of proximate analysis of fermented Ugba

Composition	Percentage (%/100 g)
Moisture content	33.70
Dry mass	66.23
Ash content	8.83
Crude fibre	5.75
Lipid	18.32
Crude protein	29.30
Carbohydrate	3.55

Table 4. Conventional test result on bacterial isolates

Biochemical test			Bacterial isolate		
Gram reaction	+	+	+	-	-
Morphology	Long rods	Long clustered rods	Clustered cocci	Short rods	Short rods
Motility	+	-	-	+	-
Coagulase	ND	ND	+	-	-
Catalase	+	-	+	+	+
Gelatin liquefaction	+	+	ND	ND	ND
Starch hydrolysis	+	-	+	+	+
Indole	ND	ND	ND	+	-
Voge-proskaeuer	+	ND	+	-	+
Citrate utilization	+	ND	ND	-	+
Oxidase	ND	ND	ND	-	-
H2S production	+	ND	ND	ND	ND
Urease	+	-	-	-	+
Sugar fermentation					
Glucose	AG	AG	AG	G	G
Lactose	A	A	A	+	+
Mannitol	AG	AG	AG	+	+
Sucrose	A	A	AG	+	+
Presumptive isolate	*Bacillus*	*Lactobacillus*	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella*

Key: += Positive, - = Negative, ND= Not Done, A = Acid, G = Gas, AG = Acid and Gas

Result shows that the Ugba samples bought from Nkwo-Ogwu market had significantly higher bacteria, while the Ugba samples from Orie-Ikpa market had higher fungal count as compared to other locations in the market. This could be as a result of unhygienic and unsanitary practices employed in poor handling of Ugba by market sellers. It could also be as a result of moving the products from one place to another in the market which leads to direct contact between the samples and passers-by. Result also showed that total aerobic plate count (TAPC) was higher compared to coliform count, *Escherichia coli* count and fungal count. This is not surprising, as all the microbes isolated in this study are aerobes. *Escherichia coli* count was the lowest. This is an indicator that the risk of contacting *E. coli* enteric infection from the consumption of Ugba could be low in the region. The presence of *Escherichia coli* and *Klebsiella* sp in the Ugba samples could be from contamination of the water that was used during processing. These pose a risk of diarrhoea to potential consumers [4].

Bacillus sp were the highest occurring bacteria from this study. *Bacillus* has severally been reported as the major microorganism that is responsible for the physicochemical and organoleptic features of Ugba [4,6,9]. It is known to be an active fermentation agent, hence, its use as starter culture for modified fermentation of Ugba for modern industrial purposes [18]. It has also been found to persist until the end of the fermentation, with numbers increasing throughout the period of fermentation while the numbers of other microbes decreased after 24 hours of fermentation [9]. This could explain why its presence superceded those of other bacteria.

All 5 samples collected from Orie-Amaigwe market had *Staphylococcus aureus*. *S. aureus* found on the skin and nasal cavity of humans pose a high risk of gastroenteritis due to its production of enterotoxin. Since the bean seeds were boiled for hours before fermentation, both the fermentation agents and other microorganisms isolated could not have originated from the beans. The bacteria were probably introduced through the air, water, utensils, leaves used in wrapping or by handling during the preparatory stages [9,19]. Example *S. aureus* are more commonly associated with the skin and hence are easily disseminated through handling; thus, the organism can gain entry into the Ugba by direct contact with human skin or air droplets from sneezing. Also, addition of salt would selectively favour the growth of *Staphylococcus* which is known to be salt tolerant [20]. *Staphylococcus* has however, previously been reported to be involved in the fermentation of Ugba (their numbers decreasing after 72 hours of fermentation) [9,21,22]. This may account for their presence in the present study.

The fungi isolated from this study *Aspergillus, Penicillium* and *Saccharomyces* sp have been known to produce mycotoxin, which exposes consumers to food intoxication [23]. The local production process of Ugba renders the product vulnerable to contamination by pathogenic microorganisms, both bacteria and fungi. The packaging system used locally (banana leaves (*Musa sapietum* Linn*)* and ororompo (*Mallotus oppositifolius* Mull) leaves) could have allowed the entrance of these extraneous organisms into the product. They could have also been introduced through the air, water and utensils used for processing, or the handler [4,6,7,9].

Proximate analysis showed that Ugba is highly proteinaceous, which is an indicator that the food is naturally prone to high microbial load since microorganisms grow well in proteinaceous environment. The crude protein was observed to be 29.3%. In previous studies, Ugba has been found to contain 36.2-43.89% crude protein which contains the 20 essential amino acids. However, the sulphur containing amino acid content is much lower than those found in other plant proteins [24-26]. The high content of other essential amino acids makes the seeds a potential source of protein [27]. The lower protein content recorded in this study could be due to a slight difference in production processes by different producers in different locations. This study recorded 3.55% of carbohydrate and 18.32% of lipid from processed Ugba. The oil bean seeds have been found to contain 4-17% carbohydrate, 44-47% oil which has been found to be rich in oleic acid [26,28] and linoleic acid [29]. The low concentration of both carbohydrate and lipids seen in this study could be attributed to the production and fermentation processes, as majority of the carbohydrate present in the seeds may be lost due to leaching during the preparatory stages [30] or lipid lost to *Bacillus* sp which produce lipolytic enzymes, with relevant machinery for efficient breakdown of oil and other substrates.

The major problems of Ugba are its poor shelf life often associated with the uncontrolled

fermentation and poor packaging which often allows maggots to grow on the product as a result of eggs laid by flies that gained entry into the wrapped product. There is need to implement the Hazard Analysis of Critical Control Program on local food production centres with objectives of preventing contamination and to safeguard public health [15]. When Ugba slaw is washed and drained for 30 minutes in a basket lined with banana leaves (*Musa sapietum* Linn*)* and wrapped using ororompo (*Mallotus oppositifolius* Mull) leaves, these leaves can be a source of contamination and thus, encourage the growth of microorganisms. These leaves should be kept in hygienic conditions so as to minimize microbial contamination of the product.

5. CONCLUSION

This study recorded no common food pathogen. However, the presence of *Staphylococcus* and *E. coli* indicates that consumption of Ugba sold or processed in less sanitized condition could pose the risk of food poisoning or gastroenteritis to its consumers. Nevertheless, Ugba prepared and sold in a good hygienic environment serves as an adequate staple food as it is highly proteinaceous.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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