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Evaluation of Microbial Pathogens and Effect of Time on the Quality of Fermented African Oil Bean Seed

Mbah-Omeje Kelechi,Nkechinyere, PhD

Senior Lecturer, Department of Applied Microbiology and Brewing, Enugu State University of Science and Technology, Nigeria. *kelechimbahomeje@gmail.com*

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Abstract

The African oil bean seed (ugba) is a fermented food kept and sold for a period of time by vendors. The study was carried out to determine the presence of pathogens in the african oil bean seed (ugba) and determine the effect of time on the samples. A total of 12 samples were obtained from vendors in three different markets within Enugu metropolis, Enugu state, Nigeria. The already fermented samples were further kept for 4 days and their microbial count determined. Cultures were done on macconkey agar, potato dextrose agar, salmonella shigella agar, eosin methylene blue agar and nutrient agar. The isolates were characterized and identified by standard microbiological methods and antibiotic susceptibility pattern were identified by disk diffusion method. The values for the microbial counts were carried out in triplicate and results reported as mean ± standard error, the data obtained were subjected to one-way ANOVA using statistical package for social science (SPSS) for windows evaluation. There was statistical difference at $p<0.05$ between the microbial count from the first to the fourth day in the samples from the various markets. The values ranged from (1.61 ± 0.03) in Garriki, (1.37 ± 0.02) in Mayor and (1.52 ± 0.08) in Agbani market in the first day while values of the fourth day were; (1.45 ± 0.09) in Garriki, (8.60 ± 0.28) in Mayor and (1.29 ± 0.03) in Agbani market. Organoleptic changes in texture and colour were seen to be a factor of time due to storage. *E.coli*, *Salmonella sp* and *Bacillus sp* were isolated from samples in all the market. A decline in growth of *E.coli and Salmonella sp* by the fourth day of storage was observed for all samples.From the results, ugba

spoilage is primarily a result of the continued activity of fermentative organism; *Bacillus sp* and other spoilage organisms; *E. coli* and *Salmonella sp*.

Keywords: Microbial spoilage, African bean seed, organoleptic change, fermentation, micooganisms

1. Introduction

Ugba is a fermented product from African oil bean seed *Pentaclethra macrophylla.* a woody plant predominant in the rain forest areas of West and Central Africa belonging to the family Leguminosae, sub-family[6]. Ugba is of primary importance, as cheap and easily available source of plant protein in developing countries of the world and Africa in particular. Fermented seeds are not just palatable but serve as a delicacy amongst consuming regions where it is consumed and garnished with other vegetables or stables. Consumption of ugba seeds could pose as a means of addressing the prevailing Protein Energy Malnutrition (PEM) in developing countries [2].

African oil bean seeds are oval, flat and black in colour. The seeds are composed of 35-52% oil, 17-22% protein and 12-23% carbohydrates[12]. Unprocessed seeds are bitter and possess anti-nutritional factors amongst which are pancine, cyanide, oxalates, saponin, phytic acid, phytate and tannins[1]. Processing of these seeds entails boiling, removal from pod, cutting into slices, further boiling, wrapping in banana/plantain leaves and fermentation. Thermal treatment induces a resultant rise in nutrient bioavailability and seed digestibility. Processing ugba seeds drastically reduces the level of anti-nutritional compounds mentioned while increasing iron, calcium, potassium, thiamine and riboflavin levels[4].

Preparation of ugba is by mixed fermentation carried out spontaneously by a number of microorganisms. Microorganisms isolated from fermented ugba include *Micrococcus sp.*, *Lactobacilli*, *Staphylococcus sp.*, *Leuconostoc mesenteroides*, *Proteus* and *Escherichia coli* [5,9,11] The major problem with the fermented oil bean seed ugba is the restricted availability due to its very short shelf life. Under room temperature, fermented ugba spoils within three to four days. Spoilage is identified with increased softness[2], color change, off flavour and sliminess[9] and production of pungent ammonical odor [13].

Extending the shelf life of ugba has been a case of interest to many researchers. Reports on strategies to extend the shelf life of fermented ugba include treatment with varying concentrations of sodium chloride[13], preservation in high density polyethylene sachets and aluminum foil wraps[14], canning within tomato puree, brine solution or refined groundnut oil [1] and use of starter cultures to shorten period of fermentation[9]. The deterrent in the methods reported so far include the cost of implementation, no remarkable difference in shelf life extension and lack of ease in method application making it difficult or impossible for local commercial dealers to implement such methods.

A key selling point to using fermented oil bean seed (ugba) to address Protein Energy Malnutrition (PEM) issues is the ease of process adoption by local producers. Advocating for commercialization of this process though commended may advertently lead to a hike in product prize making this rich source of protein unavailable to the impoverished that are direly in need of the nutrients, which this can provide. A concise knowledge of the spoilage associations of fermented oil bean seed will enable the identification of the chief sources of spoilage and therefore help to identify simpler techniques to address spoilage problems.

The aim of the study is to determine microbial spoilage pattern of ugba sold at the part of the market and determine the antibiotic susceptibility pattern of the isolates.

II. Materials and Methods

Media

The media used in the study include MacConkey Agar, Salmonella Shigella Agar, Nutrient Agar and Potato Dextrose Agar and were purchased from Oxoid. They were prepared according to manufacturer's instruction.

Sample Collection

A total of 12 different fermented ugba were purchased from vendors in Agbani, Mayor and Gariki market, Enugu State, Nigeria. The samples were freshly fermented for three days according to the dealers to ensure uniformity of results. The samples were put in sterile polyethylene bags and taken to Enugu State University of Science and Technology microbiology laboratory for analysis; they were kept for four days and checked daily.

Preparation of Sample

5g of each ugba sample was taken aseptically with sterile forceps and blended with 45ml of distilled water. Serial dilutions of $10^1 – 10^{10}$ were made by pipetting serially 1ml of the solution into a sterile test tube containing 9ml of sterile water. The test tubes were labeled appropriately as diluted.

Antimicrobial Susceptibility Test for Bacterial Isolates

The commercially available antibiotics Kirby-Bauer disk diffusion method for gram positive and gram negative organisms were used for the potentially pathogenic organisms isolated from ugba. The sub-cultured plates were observed and isolated bacteria colony was suspended into growth media and standardized through a turbidity test (McFarland's standard). The standardized suspension was then inoculated onto the solidified agar plate and the antibiotic treated paper is tapped on the inoculated plate. The disc containing the antibiotic was allowed to diffuse through

the solidified agar, resulting in the formation of an inhibition zone after 24hrs incubation at 37ºC. Thereafter, the size of the inhibition zone formed around the paper disc was measured.

Antifungal Susceptibility Test

200g of the antifungal drug (ketoconazole) was dissolved in 5ml of distilled water given a concentration of 40mg/ml and was taken as the stock solution. Double fold dilution was further carried out by transferring 2m from the stock to five different test tubes containing 2ml of distilled water giving a concentration of 40, 20, 10, 5 and 2.5ml respectively. 10 ml of distilled water was pipetted into a sterile test tube and a loopful of 48hr colony of the organism *Aspergillus candidus* was transferred into the tube and agitated. 5ml of peptone was transferred into three different test tubes, 0.1ml of the test organism was added into the test tube and 0.1ml of different concentration of the drug (ketoconazole) was added respectively. The test tube was then incubated at room temperature for 24hrs. After 24hrs, Potato Dextrose Agar (PDA) was prepared and allowed to cool at 45ºC. 0.1ml of the mixture containing the test organism, peptone water and ketoconazole was introduced into petridish before pouring the agar. It was swirled in a clockwise direction and incubated for 7 days.

III. Results

Table 1: Effect of time on the storage quality of fermented ugba

Time	Color	Texture
24 (hrs)	light brown	soft
48 (hrs)	light brown	soft and slimy
72 (hrs)	brown	soft and slimy
96 (hrs)	brown	soft and very slimy

The samples showed organoleptic changes with varied changes in color. It also became very slimy and soft by the fourth day.

Table 2: Effect of time on the microbial population of fermented ugba from different markets.

Time (hrs)	Cfu/ml from ugba in Agbani	Cfu/ml from ugba in Gariki market	Cfu/ml from ugba in Mayor market

	market		
24	1.52 ± 0.08^{a}	1.61 ± 0.03^{a}	1.37 ± 0.02^{a}
48	1.04 ± 0.02^{b}	1.15 ± 0.02^{b}	1.04 ± 0.01^{a}
72	1.37 ± 0.03^{ac}	1.17 ± 0.01^{b}	1.02 ± 0.01^{a}
96	1.29 ± 0.03^{c}	1.45 ± 0.09^{c}	8.60 ± 0.28^{b}

The result represents Mean ± Standard Error. Mean values with different alphabetic superscript differ significantly within each column based on LSD analysis at $p < 0.05$ ($n = 3$).

Table 3: Occurrence of bacterial isolates from fermented ugba

Organisms identified	**Total number of sample**	**Number of samples positive**	**Percentage (%)**
Bacillus spp	12	12	100%
Salmonella spp	12	10	83%
Escherichia coli	12	8	66%

A total of twelve (12) bacteria genera were isolated from various samples collected from three different markets (Table 3). Further characterization revealed these organisms to be *Bacillus sp*, *Salmonella sp* and *Escherichia coli*. Out of the 12 samples of African oil bean seed analyzed, *Bacillus sp* was highest in prevalence at 100%, *Salmonella sp* at 100% and *Escherichia coli* 66%.

Table 4: Growth and morphological characteristics of bacterial isolates on different media

Organisms	**Media**	**Texture**

Bacillus spp	Nutrient agar	smooth, white, slightly raised colonies
Escherichia coli	Macconkey agar	large raised, non mucoid pinkish colonies
Escherichia coli	Eosin methylene blue	blue-black color with green metallic sheen
Salmonella spp	Salmonella shigella agar	pink and black colonies

The isolates showed varied results in their growth and morphological characteristics on different media.

Table 5: Biochemical and microscopic test of bacteria isolates from fermented ugba

Biochemical reactions	***E. coli***	***Bacillus sp***	***Salmonella sp***
Cell morphology	rod shaped	Dry opaque	Rod shaped
Color	pinkish	colorless	Pinkish
Gram staining	Gram -ve	Gram +ve	Gram +ve
Catalase	+	+	+
Oxidase	-	+	-
Lactose	AG	AG	AG
Maltose	A	AG	AG
Glucose	Gas	AG	AG
Fructose	A	AG	AG
Citrate	-	-	-
Microscopic	Gram –ve rod	Gram +ve rod	Gram –ve rod
Urease	-	-	-

Bacillus sp were Gram positive rods while *Escherichia coli* and *Salmonella sp* were Gram negative rods. The isolates showed varied results in their biochemical and sugar fermentation test.

Key

A = Acid production after sugar fermentation

G = Gas production after sugar fermentation

AG = Acid and gas production

Table 6: Morphological characteristics and microscopic examination of fungal isolates from fermented ugba

Organisms identified	**Media**	**Microscopy**

Aspergillus candidus	PDA	white colonies typically globose Conidial heads producing globose or Sub-globose, smooth, thin-walled conidia

Aspergillus candidus were the only fungi isolated from ugba and were white on PDA plate.

Table 7: Antifungal susceptibility pattern for fungal isolates from fermented ugba

Organisms isolated	**Antifungal profile of ketoconazole**		
	Sensitive	**Intermediate**	**Resistant**
Aspergillus candidus	40mg	20mg	10mg
(n=1)	1(100%)	-	-

Aspergillus candidus showed sensitivity at 40mg.

Table 8: Antibiotic susceptibility test for Gram positive isolates from fermented ugba

Microbial agent	***Bacillus sp* (n=5)**		
	Sensitive	**Intermediate**	**Resistant**
Pefloxacin	4(80%)	1(20%)	-
Gentamycin	5(100%)	-	-
Ampliclox	-	1(20%)	4(80%)
ZinnaceF	-	-	5(100%)
Amoxicillin	2(40%)	-	3(60%)
Roceptin	1(20%)	4(80%)	-
Ciprofloxacin	5(100%)	-	-
Septrin	2(40%)	3(60%)	-
Erythromycin	4(80%)	1(20%)	-
Streptomycin	2(40%)	3(60%)	-

The isolates were sensitive to most of the antimicrobial agents

Table 9: Antibiotics susceptibility test of Gram negative isolates from fermented ugba

Microbial agent	***Escherichia coli* (n-2)**			***Salmonella sp* (n-4)**		
	Sensitive	**Intermediate**	**Resistant**	**Sensitive**	**Intermediate**	**Resistant**
Augmentin	1(50%)	-	1(50%)	2(50%)	-	2(50%)
Gentamycin	2(100%)	-	-	3(75%)	1(25%)	-
Pefloxacin	2(100%)	-	-	4(100%)	-	-
Chloramphenicol	1(50%)	-	1(50%)	-	2(50%)	2(50%)
Sparfloxacin	1(50%)	1(50%)	-	4(50%)	-	-
Amoxicillin	1(50%)	1(50%)	1(50%)	2(50%)	2(50%)	-
Ciprofloxacin	1(50%)	1(50%)	-	4(100%)	-	-
Tarivid	2(100%)	-	-	3(75%)	1(25%)	-
Septrin	1(50%)	-	1(50%)	2(50%)	-	2(50%)
Streptomycin	1(50%)	-	1(50%)	2(50%)	-	2(50%)

The bacterial isolates showed sensitivity to most of the drugs.

IV. DISCUSSION

From the study, *Escherichia coli*, *Bacillus sp* and *Salmonella sp* were isolated and this correlated with earlier reports of [10]. From the results, the samples showed organoleptic changes and evidence of microbial succession as keeping time of ugba increased. Freshly fermented ugba was light brown in color and soft but as the keeping period increased there was a darkening of color to brown and dark brown and a change in texture, with the product becoming extremely soft. The values of the microbial counts were carried out in triplicate and results reported as mean ± standard error, the data obtained were subjected to one-way ANOVA using statistical package for social science (SPSS) for windows evaluation. The increase observed in microbial load possibly caused a resultant increase in cellular activities leading to unsolicited organoleptic change in product quality. This is in agreement with the report by [9] who postulated that spontaneous activities of microorganisms present in ugba lead to short shelf-life of product. Other researchers observed *Bacillus sp* to have been implicated in all the reported cases of ugba fermentation, either when fermented spontaneously or inoculated specifically as starter cultures [4,8.10,11]. From the results, the product softened as growth of *Bacillus sp* increased and this corresponds with the report by [11] that *B. subtilis* was responsible for softening of ugba during fermentation. Deterioration of ugba quality was also witnessed in form of sliminess, a common occurrence in spoilage of food with high residual moisture content and also the presence of proteolytic organisms. This is in agreement with the report of [8] whose result further confirmed

that continuous activities of these organisms are actually responsible for the spoilage of ugba. From results, it showed that amongst all samples from various markets, colony forming units of *Salmonella sp* and *Escherichia coli* reduced drastically with increase in keeping time. This is an indication that these organisms were introduced into the food as microbial contaminants this is in agreement with [11,8].

The population of *Bacillus sp* increased with keeping time for all samples from various markets. *Bacillus sp* is known to play key roles in ugba fermentation. The fundamental problem associated with the indigenous fermented ugba include: short shelf life arising from uncontrollable fermentation and vulnerability of the product to contamination by pathogenic microorganism during production and storage. The increase in population of *Bacillus sp* shows that the organism is the key fermenting organism of ugba. Spoilage is most likely a result of unstopped fermentation by *Bacillus sp* which are not killed or inactivated after ugba production [11,1,2]. *Aspergillus candidus* a fungi was also implicated, and it is the causative organism of spoilage in ugba.

In addition to the health implications of the survival of these food indicator bacteria in the fermented African oil bean seed, their antibiotics resistance is also of great concern. According to [7,15,16], antibiotics resistance in food borne pathogens is a reality and recovery of strains of resistant food borne pathogens to a variety of antimicrobials has become a major health concern. In this study, the isolates were sensitive to most of the test agents.

V. Conclusion

From the results, *Bacillus sp* was observed to be the key fermentative organism of ugba. Spoilage was noted to be due to continuous activities of *Bacillus sp*, after desired fermentation was reached. Other organisms isolated from ugba are likely contaminants exposed to the food after boiling, prior to fermentation through the air, water, leaves or poor aseptic measures during handling.

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