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BACTERIOLOGICAL QUALITY OF PROCESSED AFRICAN OIL BEAN (*Pentaclethra macrophylla*) SEED "UGBA" SOLD IN UMULOLO AND IHUBE COMMUNITIES IN OKIGWE, IMO STATE, NIGERIA.

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ABSTRACT

The study investigated the bacteriological quality of processed African oil bean (*Pentaclethra macrophylla*) seed "ugba" sold in Umulolo and Ihube communities in Okigwe, Imo State, Nigeria. A total of fourty five (45) samples of locally processed, packaged and sold African oil bean seed (ugba) were randomly purchased from three different markets and analyzed by standard microbiological methods to determine the colony forming units per gram of samples. Bacteriological loads obtained from the three different markets studied showed that total heterotrophic bacterial counts ranges from (2.7±0.22) $x10^2$ CFU/g to (4.6±0.64) $x10^3$ CFU/g, and coliform count ranges from (0.4±0.40) $x10^1$ CFU/g to (1.6±0.68) $x10^1$ CFU/g. Temperature values of samples studied ranges from 36±0.76 to 37±0.25, pH values ranges from 6.4±0.20 to 6.6±0.41, colour of samples ranges from light brown to dark brown and texture of samples ranges from soft to hard textured. Nine bacterial species were isolated and they include: *Bacillus* species, *Proteus* species, *Micrococcus* species, *Staphylococcus* species, *Pseudomonas* species, *Lactobacillus* species, *Leuconostoc mesenteroides, Escherichia coli, and Klebsiella* species. The bacterial loads were high in all fermented ugba samples from the three different markets, but obtained results were within thresholds. Poor hygiene and food safety practices had been indicted as reason for the high bioloads and number of isolated species. Therefore, there is need for processors to observe food safety principles and also for consumers to maintain optimal storage conditions to reduce both contamination and geometric replication of inherent spoilage organisms, so as to extend the shelf life of the fermented ugba.

Key words: Snacks**,** Food safety, GMP, GHP, HACCP, Microbial thresholds.

Introduction

There are various plant seeds that are fermented and used as food in some rural and urban parts of Nigeria, among which is 'ugba' from African oil bean (*Pentaclethra macrophylla*). African oil bean seed also known as "ugba" and "ukpaka" in Igbo language is a popular food delicacy in Nigeria especially among Igbo ethnic group. It is a fermented product rich in protein and is obtained by a solid state fermentation of the boiled, shredded seed of African oil bean tree (*Pentaclethra macrophylla* Benth). It is an essential food item for various traditional delicacies where it is mixed with slices of boiled stock fish (ugba and okporoko). Ugba as a source of protein in developing countries of the world and Africa in particular is of primary importance because it's cheap and available. Fermented seeds are not just palatable but serve as a delicacy amongst consuming regions where it is consumed and garnished with other vegetables or staples. Consumption of fermented ugba seeds could bridge the prevailing protein energy malnutrition (PEM) – marasmus in developing countries [7].

African oil bean tree (*Pentaclethra macrophylla* Benth) is a woody plant predominant in the rain forest areas of West and Central Africa belonging to the family Leguminosaea, sub-family Mimosoidae [13]. The seeds are oval, flat and black to grey in colour. The seeds are composed of 35-52% oil, 17-22% protein and 12-23% carbohydrates [18]. Unprocessed seeds are bitter and possess anti-nutritional factors amongst which are pancine, cyanide, oxalates, saponin, phytic acid, phytate and tannins [8]. Processing of these seeds involves boiling, removal from pod, shredding/ cutting into slices, further boiling, sieving, 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 levels of the anti-nutritional compounds mentioned while increasing iron, calcium, potassium, thiamine and riboflavin levels [9].

Fermentation of African oil bean seed involves the traditional technique of natural fermentation with microbial flora of substrate composition. The major aim of fermentation is to extend shelf life, inhibit spoilage and pathogenic microorganisms, imparts desirable sensory qualities, with improved nutritional value and digestibility. The capacity to preserve food is directly related to the level of technological development. The slow progress in upgrading traditional food processing and preservation techniques in West Africa have contributed to food and nutrition insecurity in the sub-region. Traditional technologies of food processing and preservation are part of the peoples' culture and could date back to thousands of years ago, unlike the automated machine-processed and other

modern technology industries. The traditional methods and their imbibed technologies have long preceded any scientific invention. Indeed, simple, low-cost, traditional food processing techniques are the bedrock of small-scale food processing enterprises in West Africa and their contributions to the economy are enormous.

Diverse groups of bacteria comprising species of *Bacillus*, *Micrococcus*, *Leuconostoc*, *Staphylococcus* and *Enterobacteriaceae* have been reported by various authors [10]; [17]; [1]; [19] as contributing to the individual fermentations. The fermentation of ugba is by mixed fermentation carried out spontaneously through proteolysis by a number of microorganisms such as *Micrococcus* species., *Lactobacillus* species, *Staphylococcus* species., *Leuconostoc mesenteroides*, *Proteus* species and *Escherichia coli* [14]; [12]; [16]. Published studies on the microbiology of the fermentation of African oil bean seeds have identified *Bacillus* species as the major microorganisms responsible for its fermentation. Also, [22] noted that *Bacillus* and *Proteus* species are proteolytic, so they dominate during the fermentation process and therefore are responsible for the observed increase in free amino acids (FAA) that are always recorded during production of the product. The major problem with the fermented oil bean seed (ugba) is the restricted availability due to its very short shelf life.

A major important value in the use of fermented oil bean seed (ugba) is in addressing protein energy malnutrition (PEM) issues and the ease of its adoption by local producers. In spite of the improved nutritional value and its role in bridging the prevailing protein energy malnutrition (PEM) – marasmus among the vast populace of developing countries, there have been cases of associated health hazards emanating from its consumption. This could be traced to poor processing practices, packaging, handling and storage of the fermented ugba. Therefore, this study is targeted to evaluate the microbial quality of processed African oil bean seed (ugba) sold in Umulolo and Ihube communities in Okigwe, Imo State, Nigeria.

Materials and Methods.

Study area.

The study area is Umulolo and its sister community – Ihube, all in Okigwe, Imo State, in the South-East Geopolitical zone of Nigeria. The people of Umulolo and Ihube communities are known as predominant farmers with rich cultural history. They are of the Igbo tribe and are located within the following geographical coordinates; 5.1167^0N, and 7.3667^0E. The area is of tropical climatic conditions with rain forest features. The soil type is silt-clay with rocky bedrock for a purified aquifer

and the weather is typical of rain forest, with an average annual temperature ranging between 26 - 35°C as lowest and highest values respectively. They are known as major producers of yam, cassava, maize, palm oil, stone escavation and many others.

Sources of sample.

The samples for the study were purchased from major markets in the communities namely: Afor Umulolo, Ekeukwu Ihube and Umulolo junction (former Toll gate at Enugu-Port-Harcourt Express).

Sample collection and preparation.

A total of fourty five (45) samples of locally processed, packaged and sold African oil bean seed (ugba) were randomly purchased on the market days from the markets: Afor Umulolo, Ekeukwu Ihube and Umulolo junction. Randomly, samples of the processed ugba were purchased, labeled, packaged in sterile ziploc bags and transported in ice cool bags to the laboratory. The samples collected were for microbiological, physicochemical and organoleptic analysis. In the laboratory, samples were aseptically unpacked, analyzed within thirty (30) minutes of collection for both microbiological and physicochemical analysis. The sample portions for organoleptic analysis were aseptically removed and conducted by panelist.

Microbiological analysis of samples:

Ten fold serial dilutions of samples were done. Spread plate and streaking culturing techniques according to [4] were used to enumerate and isolate bacteria in the samples. One (1) gram of each processed and fermented ugba samples was aseptically blended and homogenized in 10 ml of sterile distilled water (10^{-1} dilution). Serial dilutions of the homogenates were made to 10^{-2} and 10^{-3} and each dilution was plated in replicate using Plate count agar for total heterotrophic bacteria count and isolation of bacterial isolates and Tergitol medium for coliform count and isolation and Mannitol salt agar for Staphylococci isolation. Pure cultures of bacterial isolates were identified using cultural, morphological and biochemical characterization. Identification of the bacteria to genera level was based on the schemes of [3]. The plates were incubated at 35 ± 2°C for 72 hours ± 2 hours and 24 hours ± 2 hours for total heterotrophic bacterial count and coliform count respectively.

Physico-chemical studies

The physicochemical parameters measured include pH and temperature. They were determined using methods of [25].

Data analysis

Data obtained from this research work were analysed using ANOVA. Descriptive statistics in form of means and standard deviation and Duncan post hoc were also used to assess the data. The analyses were done using SPSS 16.

Results

The mean total heterotrophic bacteria counts and physicochemical values of ugba samples from the three (3) markets are shown in Table 1. Ugba samples from Afor Umulolo market had the highest count {(3.7±0.50) $x10^4$ CFU/g}, followed by Ekeukwu Ihube market {(4.6±0.64) $x10^3$ CFU/g }, with the least count recorded with samples from Umulolo Junction market {(2.7±0.22) $x10^3$ CFU/g}. Samples from Afor Umulolo and Ekeukwu Ihube markets had same temperature range (37^0C ±0.25 and 37^0C ±0.08 respectively), while that from Umulolo Junction market was less by a degree difference (36^0C ±0.76). The pH of fermented ugba samples followed similar trend with that of bacterial loads. Afor Umulolo market samples had the highest pH value (6.6±0.41), followed by Ekeukwu Ihube market samples (6.5±0.07), and with the least value recorded with samples from Umulolo Junction market (6.4±0.20) Table 1.

Table 2 showed the mean coliform and organoleptic results of ugba samples from the three (3) markets. Results showed that recorded counts had different trend with that obtained in total heterotrophic bacteria count. Ugba samples from Afor Umulolo market had highest count {(1.6±0.68) $x10^1$ CFU/g}, followed by Ekeukwu Ihube market {(0.4±0.40) $x10^1$ CFU/g}, with the least count recorded with samples from Umulolo junction market {(1.2±0.18) $x10^1$ CFU/g}. Organoleptic results under colour and texture for the ugba samples from the three markets ranges from light brown to dark brown and soft textured to hard textured as shown in Table 2. The values obtained between the various market samples, when compared were statistically significant ($p<0.05$).

Discussion

Ugba processing involves a traditional method with mixed bacteria fermentation and has involved proteolysis as the main activity leading to pronounced increase of free amino acids (FAA) such as lysine [21]; [15]. The activities of these microorganisms enhance the organoleptic properties of the consumed ugba delicacy. Fermentation process as earlier stated has a major aim of extending shelf life, inhibiting spoilage, and pathogenic microorganisms and imparting desirable sensory qualities with improved nutritional value and digestibility.

Several bacterial species were isolated in the work, and they include: *Bacillus* species, *Proteus* species, *Micrococcus* species, *Staphylococcus* species, *Pseudomonas* species, *Lactobacillus* species, *Leuconostoc mesenteroides, Escherichia coli, and Klebsiella* species. It is worthy to note that some of these isolates in this study are in agreement with the works of [24]; [6]; [23]; [22]; [5]; [2]; [20] and [19].

Most of the isolated organisms in this study are not majorly true fermenters of ugba but could be involved in the fermentation process with some others as contaminants. Bacillus species had been identified as the main microorganisms responsible for the fermentation of African oil bean seeds [15]; [21]. Similarly, during the study of microbial and oganoleptic changes associated with 'Ugba' stored at ambient temperature by [22], they noted that *Bacillus* and *Proteus* species are proteolytic, so they dominate during the fermentation process and therefore are responsible for the observed increase in free amino acids (FAA) that were always recorded during production of the product. *Staphylococcus* species exists as opportunistic-natural flora of the human skin. Poor hygiene practices during processing have been identified as the major factor responsible for its introduction into the fermented ugba. *Escherichia coli* is not a fermentative organism for ugba processing, rather an indicative organism for faecal contamination. Poor hygiene/ sanitary practices could introduce it into the fermented ugba during processing and packaging. *Escherichia coli* has been known for its role in food safety as food safety index organism [11].

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. In fact, it is through the activities of *Bacillus* species during fermentation of ugba that the desirable attributes of fermentation is

achieved. Although, mixed fermentation has always been the practice with traditional fermentation of ugba. This attributed to the isolation of other bacterial species in this study.

In the results obtained from Table 1 and 2, there was higher bacterial loads in fermented ugba samples from Afor Umulolo, while higher coliform counts was observed in fermented ugba samples from Umulolo junction. In Table 3, *Bacillus* species had the highest in prevalence for the three markets, followed by *Proteus, Micrococcus, Pseudomonas*, *Staphylococcus* and *Lactobacillus* species in that order, while the least prevalence was observed with *Escherichia coli*, followed by *Leuconostoc mesenteroides*, and *Klebsiella* species. The results obtained in this study were in agreement with that of [24]. Although, the various bacterial loads were high in all fermented ugba samples from the three different markets, but obtained counts/ values were within thresholds. This has shown that the ugba samples sold in these markets were safe, but post-processing conditions like storage conditions have to be improved and maintained at optimal temperatures in order to reduce the geometric replication of inherent organisms, which in turn could pose serious health risks at higher thresholds. Also, it is advised that adequate food safety practices should be observed by local processors in order to reduce the evidenced high bacterial loads observed in this study. There is statistical significance among different values obtained in the results ($p<0.05$).

Conclusion

Ugba/ Ugbaka is a product of mixed fermentation, and with the observed high bacterial loads, there is need for processors to observe food safety principles and also for consumers to maintain optimal storage conditions to reduce both contamination and geometric replication of inherent spoilage organisms, so as to extend the shelf life of fermented ugba.

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Table 1: Mean Total Heterotrophic Bacteria Count and Physicochemical Results

Markets	Sample parameters		
	Bacterial loads	**Temperature (^{0}C)**	**pH**
Afor Umulolo (CFU/g)	$(3.7\pm0.50^{a}) \times 10^{4}$	37 ± 0.25^{a}	6.6 ± 0.41^{a}
Ekeukwu Ihube (CFU/g)	$(4.6\pm0.64^{ab}) \times 10^{3}$	37 ± 0.08^{a}	6.5 ± 0.07^{ab}
Umulolo Junction (CFU/g)	$(2.7\pm0.22^{b}) \times 10^{3}$	36 ± 0.76^{ab}	6.4 ± 0.20^{bc}

Within columns, values with the same letters are not significantly different. Standards: Total heterotrophic bacteria count (TABC) = $\leq 10^5$/g, Coliform count (CC) = < 100/g (FSANZ, 2001; PHLS, 2000).

Table 2: Mean Coliform Count and Organoleptic Results

Markets	Sample parameters		
	Coliform counts	**Colour**	**Texture**
Afor Umulolo	$(1.6\pm0.68^{a}) \times 10^{1}$	Light brown	Hard

(CFU/g)			
Ekeukwu Ihube	$(0.4\pm0.40^{a}) \times 10^{1}$	Light brown	Soft
(CFU/g)			
Umulolo Express	$(1.2\pm0.18^{a}) \times 10^{1}$	Dark brown	Soft
Junction (CFU/g)			

Within columns, values with the same letters are not significantly different. Standards: Total heterotrophic bacteria count (TABC) = $\leq 10^{5}$/g, Coliform count (CC) = < 100/g (FSANZ, 2001; PHLS, 2000).

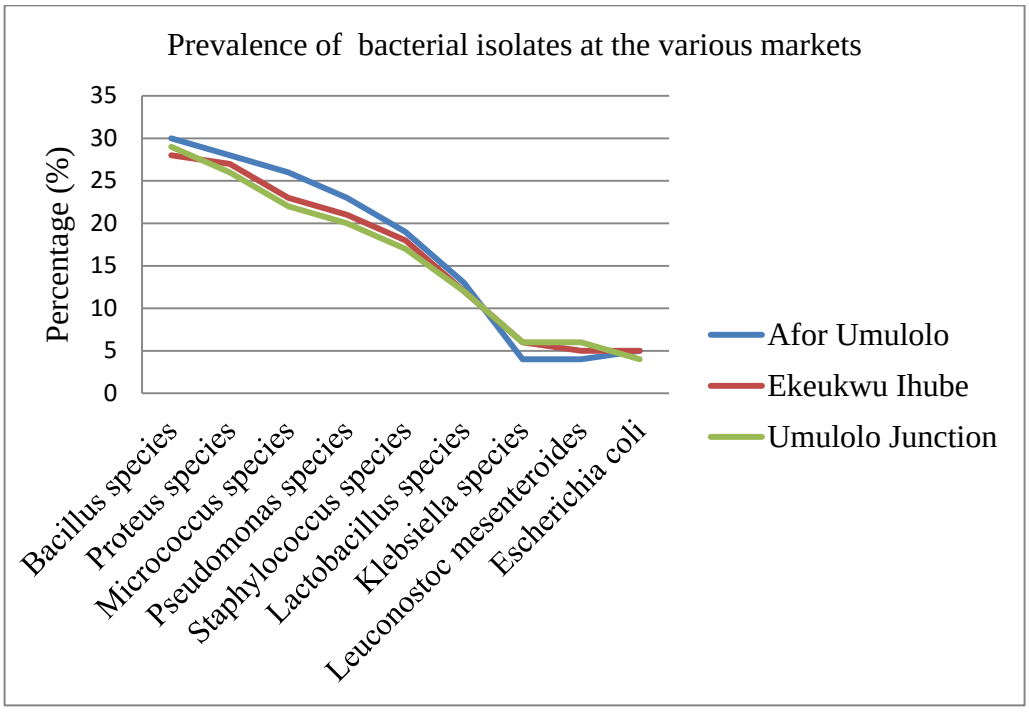

Figure 1: Percentage prevalence of bacterial isolates from samples at various markets