MICROBIAL EVALUATION OF TIGER NUTS (*Cyperus esculentus L.)* SOLD IN ABA, ABIA STATE, NIGERIA.

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ABSTRACT

The microbial evaluation of tiger nuts (*Cyperus esculentus L.*) sold in Aba metropolis was done using standard microbiological methods. A total of 20 samples drawn randomly from four (4) different traffic-prone areas in Aba metropolis were assessed. Mean aerobic bacterial, coliform and fungal counts (CFU/g) ranged between (4.5×10^3) ±0.55 to (3.3×10^4) ±0.31; (1.9×10^1) ±0.03 to (2.1×10^2) ±0.39 and (0.4×10^2) ±0.07 to (2.3×10^3) ±0.10 respectively. A total of seven bacterial and four fungal isolates were identified to include -bacteria: *Bacillus* species (40%), *Pseudomonas* species (28%), *Staphylococcus* species (23%), *Proteus* species (19%), *Escherichia coli* (7%), *Klebsiella* species (6%), *Streptococcus* species (5%) and fungi: *Aspergillus* species (25%) *Penicillium* species (21%), *Rhizopus* species (15%), *Mucor* species (11%). The high microbial counts reported in this work which are above acceptable thresholds are indications of serious health concern since the tubers are usually consumed without further washing. From the results obtained, it is advisable that an adequate campaign should be organized for the major stakeholders (vendors/ hawkers and consumers) in order to educate them on the inherent risks associated with the crude practices and applicable way forwards to avert food safety issues.

Key words: Tiger nuts**,** Food safety, Contamination, Microbial thresholds.
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Introduction

Tiger nut (*Cyperus esculentus L.*) is a tuber that grows freely and is consumed widely in Nigeria and in various parts of West and East Africa. It has long been recognized as one of the best nutritional crops used to augment diets with its rich iron and calcium contents for body growth and development, since a substantial intake has reduced reported cases of various

health related conditions such as cardiovascular disease, diabetes, cancer, and obesity, and also was found ideal for children, older persons and sportsmen (Ekeanyanwu & Ononogbu, 2010). Tiger nuts with its inherent nutritional and therapeutic advantage could serve as alternative to cassava in baking industry (Ade-Omowaye *et al.*, 2008).

Root crops (tubers) are important sources of nourishment and a vital ingredient in healthy and balanced diets, but they harbor varied loads of microbial contaminations while passing from farm to table. These various microbial contaminations can be contacted before, during or after harvest (Sapers *et al.*, 2006). Fruits and vegetables may become contaminated by infected field-workers, food preparers, consumers, cross contamination, use of contaminated irrigation water, use of inadequate composted manure or contact with contaminated soil. They serve as good source of food borne illness when contaminated with pathogenic microorganisms during harvesting, handling, transporting and display in street markets. Food is generally a fertile ecosystem, in which microorganisms compete for nutrients. Various microorganisms find their way into foods; they are either introduced from the soil in which they were grown, or during harvest, packaging, storage and handling (Nyarko *et al.*, 2011).

It has been revealed that lack of effective antimicrobial treatments at any step from planting to consumption suggested that pathogens introduced at any point may be present on the final food product. Some diverse microbial species associated with tiger nuts include *Bacillus subtilis, Staphylococcus aureus, Aspergillus flavus, Aspergillus niger, Fusarium solani, Saccharomyces cerevisiae, Saccharomyces fubiligera* and *Candida pseudotropicalis (*Adejuyitan, 2011). The presence of pathogenic *Escherichia coli, Streptococcus faecalis* and *Staphylococcus aureus* usually constitute a direct proof of faecal contamination of irrigation water (Cheesbrough, 2010).

In Aba and other major towns across Nigeria, tiger nuts are sold on the streets usually in uncovered wheelbarrows that are exposed to atmospheric elements. They are widely patronized by the populace based on the numerous health benefits, especially among males due to its perceived aphrodisiac property. It is also a favourite snack among children due to its sweet-nutty flavour. The sellers often use bare hands and rusted cups to dispense the nuts into polythene wrappers to consumers, which most times are eaten immediately. There is need to

place emphasis on food safety, such that foods from a source like the street market should be protected from contamination and spoilage during subsequent handling, packaging, and storage while on transit (Chevallier, 2001). Therefore, this study was targeted at evaluating the microbiological quality of tiger nuts sold in Aba, Abia State.

Materials and Methods

Study area

The study area is Aba Metropolis, Abia State, in the South-Eastern Nigeria. The Aba town which has been known as a major commercial centre in Eastern Nigeria is of the Igbo tribe and inhabited by Ngwa people. They are predominantly traders in the popular Ariaria market. They have rich cultural history and Aba town is about 49km away from its state capital city, Umuahia and about 52km away from Port Harcourt city, the capital of Rivers State. The geographical coordinates are 5.1215^0N, and 7.3732^0E. The area is of tropical climatic conditions with rain forest features. The soil type is silt-clay and the weather is typical of rain forest, with an average annual temperature ranging between 25 - 35°C as lowest and highest values, respectively (Oriji, 2011).

Sources of sample

The samples for the study were purchased at random from four (4) different traffic-prone areas in Aba metropolis namely: Osisioma junction, Aba Mega mall, Ehere market and MCC junction.

Sample collection

A total of twenty (20) samples of tiger nuts distributed at 5 grammes (approx.) per sample were bought at random from four (4) different traffic-prone areas in Aba metropolis. The street vendors were located around Osisioma junction, Aba Mega mall, Ehere market and MCC junction. These samples were aseptically packaged in sterile ziploc bags for microbiological analysis. Samples were analyzed in the laboratory within thirty (30) minutes of collection.

Microbiological analysis of samples

Ten fold serial dilutions of samples were done. Spread plate and streaking techniques (Cappucino and Sherman, 2010) were used to enumerate and isolate bacteria and fungi in the samples. Five (5) grammes of each sample was washed in five (5) milliliters sterile distilled

water in a sterile glass container to form stock. Each sample was shaken vigorously during washing to ensure adequate disengagement of microorganisms. One (1) milliliter of each sample was aseptically transferred, mixed and homogenized in nine (9) milliliter 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 replicates using plate count agar for mean aerobic bacteria enumeration and isolation, tergitol agar for coliforms enumeration and isolation, and fortified sabouraud dextrose agar (SDA) for fungal enumeration and 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 Boone *et al*. (2005). The purified fungal isolates were identified on the basis of macroscopic and microscopic characteristics by slide culture technique, and lactophenol staining. The schemes of Barnet and Hunter (2000) and Watanabe (2010) were used for the identification. The plates were incubated at 35±2°C for 72 hours and 24 hours for mean bacterial and coliform counts respectively and 25±2°C for 120 hours for mean fungal counts.

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 aerobic bacterial, coliform and fungal counts of tiger nuts samples sold in four (4) different traffic-prone areas in Aba metropolis are shown in Table 1, 2 and 3 respectively**.** It showed that tiger nuts samples from Osisioma junction had significant mean bacterial counts when compared to other samples, while samples from MCC junction had the least mean counts. Results showed that samples from Osisioma junction took lead in mean bacterial counts ($3.3x10^4$) ±0.31, followed by Aba mega mall samples ($3.0x10^4$) ±0.22, Ehere market samples ($2.8x10^4$) ±0.11 and MCC junction samples ($2.3x10^4$) ±0.21 in that order.

Conversely, there is a shift in trend as mean coliform counts were different compared to that obtained in bacterial counts. Samples from Osisioma junction had the highest in mean coliform counts ($2.1x10^2$) ±0.39), followed by MCC junction ($1.9\ x10^2$) ±0.10, Aba mega mall ($1.8x10^2$)

±0.18, and Ehere market ($1.5x10^2$) ±0.22 with the least counts. Meanwhile, high mean coliform counts were observed mainly among samples drawn from the junctions (Osisioma junction and MCC junction). Mean fungal counts had same pattern with bacterial counts with samples from Osisioma junction as the highest ($2.3x10^3$) ±0.10), followed by Aba mega mall ($1.1x10^3$) ±0.21), Ehere market samples ($1.0x10^3$) ±0.12) and MCC junction ($0.5x10^3$) ±0.40 as the least in mean counts. Figures 1 and 2 showed the prevalence of bacterial and fungal species isolated from tiger nuts samples drawn from different strategic vending locations in Aba metropolis. *Bacillus* and *Aspergillus* species had the highest prevalence (40% & 25%) in samples from Osisioma junction, while *Streptococcus* and *Mucor* species had the least prevalence (3% & 7%) in samples drawn from MCC junction,

Discussion

Some vendors wash the tiger nuts in clean tap water before bagging them for hawking, while others don't wash tiger nuts tubers because they assume consumers usually wash them after purchase prior to eating. Meanwhile, these nuts were sold to consumers with bare hands and rusted cups. Some consumers like the road artisans do not pay attentions to health risks associated with raw consumption of these nuts as they eat these nuts unwashed immediately after purchase. The looks of hawked tiger nuts may be deceitful regarding claims by vendors that they wash them before hawking as they regularly sprinkle water on the surfaces to maintain freshness.

The presence of microorganisms especially in the higher thresholds can be traced to poor hygienic conditions in terms of personal hygiene, handling, storage, vending and dispensing conditions as good hygiene practices were not implemented by vendors/ hawkers (Ukpabi and Ukenye 2015; Nyarko *et al*., 2011). Also, the use of bare hands, rusted cups, contaminated water during washing and regularly sprinkling to wet the nuts surfaces for freshness could be sources of huge contamination. However, most of these hawkers are unaware of the implications of these crude practices, in relation to health hazards and food safety concerns.

The results showed high microbial counts in Tables 1, 2 and 3. The mean aerobic bacterial, coliform and fungal counts are higher in samples purchased at Osisioma junction. The least in mean aerobic bacterial count was recorded with samples from Ehere market, while that of mean coliform and fungal counts recorded least counts with samples from MCC junction. The

high microbial loads in these samples could be linked to heavy environmental and traffic pollutions around the area plus improper handling. This assertion is in agreement with the reports of Ike *et al*. (2015) during the study of microbiological quality evaluation of locally prepared snacks sold in Aba metropolis, Abia State, Nigeria. These high microbial loads, though very high, are within thresholds, but considering microbial replication pattern and timing, some of these samples with very high counts can pose serious health threats in few days (Ike *et al*., 2015).

The presence of coliforms could be traced to poor hygiene and sanitary practices of using bare hands by vendors/ hawkers and is an indication of faecal contamination. *Escherichia coli* has been identified and used as an indicative microorganism for faecal contamination and its presence points to poor hygiene and sanitary practices by vendors/ hawkers (Jay *et al*., 2005). In Figure 1 and 2, *Bacillus* and *Aspergillus* species had the highest prevalence in samples from Osisioma junction, while *Streptococcus* and *Mucor* species had the least prevalence in samples drawn from MCC junction. The prevalence trend for both bacteria and fungi maintained a pattern among the identified microorganisms with *Bacillus* species leading, followed by *Pseudomonas* species, *Staphylococcus* species, Proteus species, *Escherichia coli*, *Klebsiella* species, and *Streptococcus* species in that order for bacteria, while *Aspergillus* species took the lead, followed by *Penicillium*, *Rhizopus*, and *Mucor* species in that order for fungi. *Bacillus* and *Klebsiella* species are known as environmental contaminants, considering rate of exposure of tiger nuts by most vendors/ hawkers, while *Staphylococcus aureus* is known to inhabit the human skin as normal flora and opportunistic during a break or under depressed immunity. *Bacillus* species are known as spore formers, inhabiting the air, water and soil and can withstand harsh weather conditions hence can contaminate vended nuts as the vending activities takes place in busy-crowded environments (Ike *et al*., 2015). The presence of *Aspergillus*, *Penicillium*, *Rhizopus*, and *Mucor* species could be attributed to the surrounding environment (Chukwu *et al*., 2013), dilapidated vending wheel barrow, rusted dispensing cups, and packaging materials. The results of this study are in agreement with the works of Falola *et al*. (2011) during the study of microbiological quality analysis of meat pies sold by street hawkers of Mainland local government area of Lagos, Nigeria.

A close observation on Tables 1-3 and Figures 1-2 showed that Osisioma samples took the lead in microbial count and prevalence when compared with other location samples, as a result of its strategic location to heavy-highway traffic along the expressway and also nearness to Abia State Environmental Protection Agency (ASEPA) dump site. There is statistical significance among different values obtained in the results ($p<0.05$).

Conclusion

The results obtained in this study had revealed high microbial loads among the different samples of tiger nuts drawn from different vending locations, which if left unchecked could pose serious health risks that might lead to food borne outbreaks. Therefore, it is advisable that an adequate campaign should be organized for the major stakeholders (vendors/ hawkers and consumers) in order to educate them on the inherent risks associated with the crude practices and applicable way forwards to avert food safety issues.

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Table 1: Mean Aerobic Bacteria Count

Tiger nut samples (CFU/g)	**Sample sources**			
	Osisioma junction	**Aba mega mall**	**Ehere market**	**MCC junction**
S_1	(3.3 x10^4) ±0.31^a	(3.0 x10^4) ±0.22^b	(2.8 x10^4) ±0.11^c	(2.3 x10^4) ±0.21^d
S_2	(6.1 x10^3) ±0.37^c	(3.5 x10^4) ±0.17^a	(2.5 x10^4) ±0.15^b	(4.5 x10^3) ±0.55^c
S_3	(2.4 x10^4) ±0.15^a	(2.2 x10^4) ±0.09^b	(2.0 x10^4) ±0.27^c	(2.0 x10^4) ±0.09^d
S_4	(1.5 x10^4) ±0.26^b	(1.3 x10^4) ±0.14^d	(1.6 x10^4) ±0.05^a	(1.4 x10^4) ±0.04^c
S_5	(3.0 x10^4) ±0.14^a	(1.8 x10^4) ±0.23^c	(2.0 x10^4) ±0.32^b	(1.6 x10^4) ±0.33^d

Within rows, values with the same letters are not significantly different.
Standards: Aerobic bacteria count (ABC) = ≤ 10^5/g, Coliform count (CC) = < 100/g, Fungal count (FC) = ≤ 10^4/g (PHLS, 2000); (FSANZ, 2001).

Table 2: Mean Coliform Count

Tiger nut samples (CFU/g)	**Sample sources**			
	Osisioma junction	**Aba mega mall**	**Ehere market**	**MCC junction**
S_1	(1.3 x10^2) ±0.50^a	(1.2 x10^2) ±0.03^b	(1.2 x10^2) ±0.37^b	(1.3 x10^2) ±0.01^a

S_2	(1.8×10^2) $\pm 0.19^a$	(1.7×10^2) $\pm 0.04^b$	(1.5×10^2) $\pm 0.22^c$	(2.4×10^1) $\pm 0.25^d$
S_3	$(0.9 \times 10^2) \pm 0.10^b$	$(2.3 \times 10^1) \pm 0.16^d$	$(0.8 \times 10^2) \pm 0.56^c$	$(1.8 \times 10^2) \pm 0.33^a$
S_4	$(2.1 \times 10^2) \pm 0.39^a$	$(1.8 \times 10^2) \pm 0.32^b$	$(1.4 \times 10^2) \pm 0.55^d$	$(1.5 \times 10^2) \pm 0.49^c$
S_5	$(2.0 \times 10^1) \pm 0.14^b$	$(1.8 \times 10^2) \pm 0.18^a$	$(1.9 \times 10^1) \pm 0.03^c$	$(1.9 \times 10^2) \pm 0.10^a$

Within rows, values with the same letters are not significantly different.
Standards: Aerobic bacteria count (ABC) = $\leq 10^5$/g, Coliform count (CC) = < 100/g, Fungal count (FC) = $\leq 10^4$/g (PHLS, 2000); (FSANZ, 2001).

Table 3: Mean Fungal Count

Tiger nut samples (CFU/g)	**Sample sources**			
	Osisioma junction	**Aba mega mall**	**Ehere market**	**MCC junction**
S_1	$(2.3 \times 10^3) \pm 0.10^a$	$(1.4 \times 10^2) \pm 0.18^b$	$(1.0 \times 10^2) \pm 0.03^c$	$(0.5 \times 10^3) \pm 0.40^d$
S_2	$(1.1 \times 10^2) \pm 0.07^a$	$(0.7 \times 10^2) \pm 0.22^b$	$(0.4 \times 10^2) \pm 0.09^d$	$(0.5 \times 10^2) \pm 0.08^c$
S_3	$(0.8 \times 10^2) \pm 0.12^b$	$(1.0 \times 10^3) \pm 0.05^a$	$(0.8 \times 10^3) \pm 0.31^b$	$(1.0 \times 10^2) \pm 0.11^a$
S_4	$(1.0 \times 10^2) \pm 0.06^b$	$(0.4 \times 10^2) \pm 0.07^d$	$(1.1 \times 10^2) \pm 0.06^a$	$(0.9 \times 10^2) \pm 0.32^c$
S_5	$(1.2 \times 10^3) \pm 0.09^a$	$(1.1 \times 10^3) \pm 0.21^b$	$(1.0 \times 10^3) \pm 0.12^d$	$(1.1 \times 10^2) \pm 0.06^c$

Within rows, values with the same letters are not significantly different.
Standards: Aerobic bacteria count (ABC) = $\leq 10^5$/g, Coliform count (CC) = < 100/g, Fungal count (FC) = $\leq 10^4$/g (PHLS, 2000); (FSANZ, 2001)

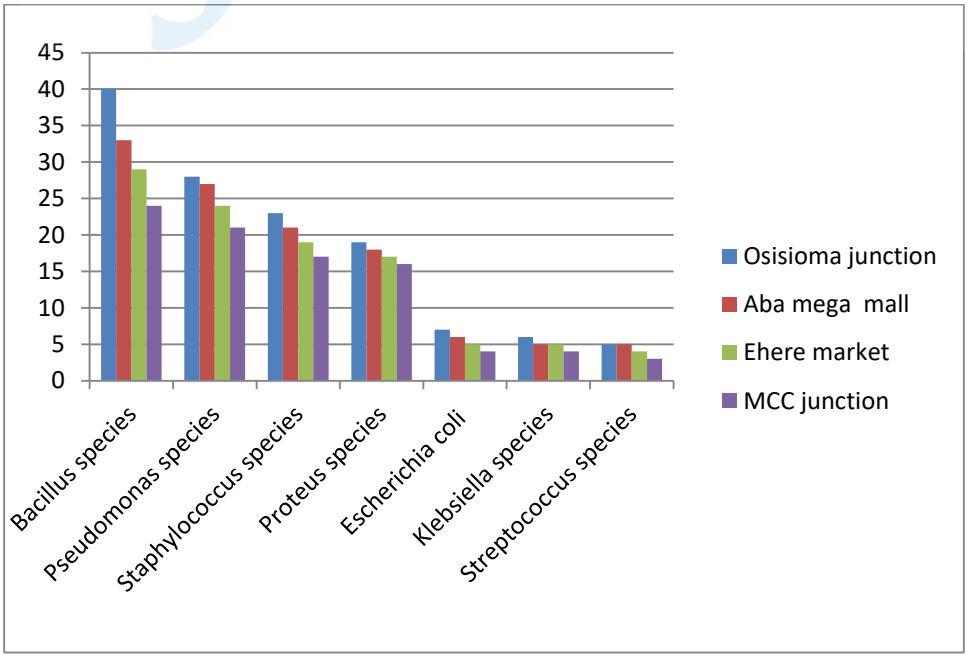

Fig. 1: Percentage prevalence of bacterial isolates from tiger nuts samples

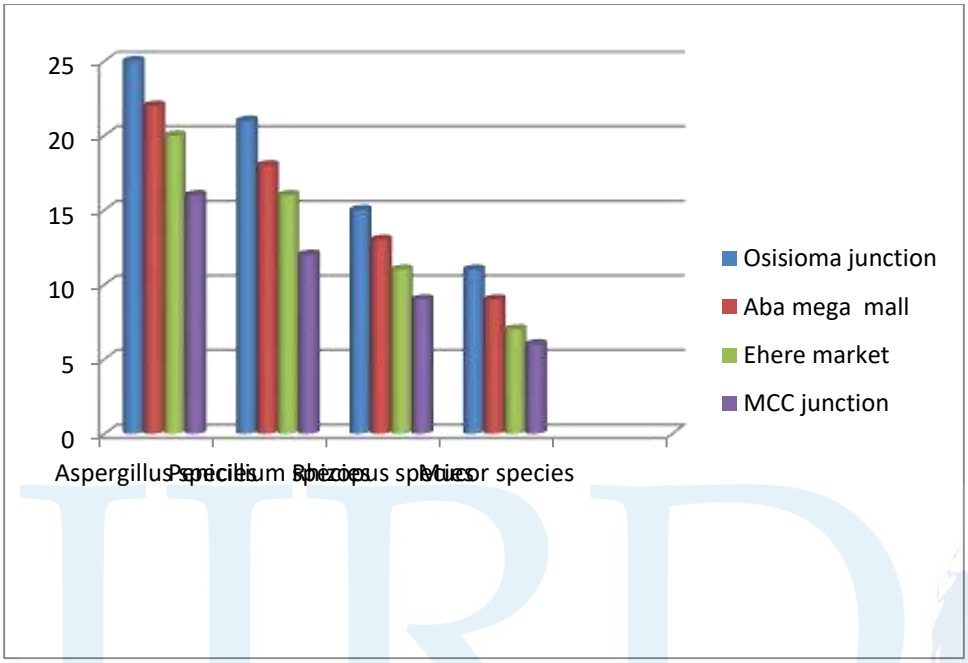

Fig. 2: Percentage prevalence of fungal isolates from tiger nuts samples