



Microbial Contamination of *Cyperus esculentus* L (Tiger Nuts) Tuber Sold within Calabar Metropolis, Cross River State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The Microbiological quality of wet and dry tiger nuts sold in five different locations within Calabar Metropolis was analyzed using Standard Microbiological and Bacteriological Techniques. *S. aureus*, *Bacillus* sp, *Streptococcus* sp, *E. coli*, *Pseudomonas*, *Enterococcus*, *Proteus* and *Klebsiella* species were microbial strains isolated from dry and wet tiger nut samples. *E. coli* was the most frequent isolates occurring 8(29.6) followed by *Bacillus* 6(22.2), *Streptococcus* and *Pseudomonas* occurred 3(11.1), *S. aureus*, *Proteus* and *Klebsiella* also had similar % occurrence of 2(7.41) while the least

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was *Enterococcus* with 1(3.70). The percentage occurrence of bacterial isolated from wet tiger nut was 14 (28.0) and 13 (26.0) from dry tiger nut. Four locations had similar 6(75.0) rate of isolates compare to Etim Edem Park with 2 (25.0). The general overview concludes that the microbial threshold in the wet tiger nut was higher 14 (28.0) than that of dry tiger nut with 13 (26.0). Association of microbial pathogens with food crops endangers human lives and are key agent to food related infections and possible outbreaks.

Keywords: *Cyperus esculentus L (tiger nuts); Microbial isolates; wet T. nut (wTNT); dry T. nut (dTNT); watt market; Marian market; Etta Agbor; Bogobiri; Etim Edem Park.*

1. INTRODUCTION

Cyperus esculentus L (tiger nuts), belongs to grass family *Cyperaceae*. It is a perennial monocotyledonous plant with tough erect fibrous root growing to about six inches depth into the soil [1,2]. The plant can house 50- 250 tubers with weigh of about 2 – 26g per tuber [3]. They come in different commercialized varieties such as yellowish brown, dark brown-black etc., and are sold in open containers as wet (with little amount of water) or dry nut [4].

In Nigeria especially in Cross River State, it is widely consumed for its nutritional composition, some of which include; calcium, chromium, cobalt, iron, manganese, phosphorus, potassium, selenium, sodium, zinc, carbohydrate, protein, lipids and vitamins C, E etc. [5,6]. Literature according to Ndubuisi, 2009 reported that the nut has the ability to prevent against heart attack, Thrombosis, absence of cholesterol among others [7-9]. Despite the nutritional values, the nut is subject to scarcity in the nearest future due to susceptibility of the nut to bacterial contamination.

Increase in the world population continues and availability of non-contaminated food to guarantee food safety is becoming more challenging. There is no balance in the growing population and Agricultural/food products especially in some rural areas where children are suffering from mal-nutrition [10,11].

These contributes to low immunity and susceptibility to infections, thus, maximum utilization of agricultural products including tiger nut tubers that are nutritional healthy are to be fully explored [1].

The research set out to evaluate the microbial load of ready- to -eat commercialized tiger nut at different conditions (wet and dry) and locations with the objective of making the research data an information template to the public on the

consequences of consuming a low quality or improperly handled Agricultural/food products thereby mitigating the occurrence of food poison in the society.

2. METHODOLOGY

20g weight of both Wet (wTNT) and Dry (dTNT) TNT were soaked in 80 mL of normal saline with vigorous shaking for 5 minutes to serve as a stock solution. 1 mL was taken from the stock solution to perform a ten-fold serial dilution down to 10^{-10} . Pour plating method was the culturing technique used in this study. Nutrient and MacConkey agar (Liofilchem® s.r.l., Italy) were media used. 1mL amount of 10^5 dilution of each suspension was placed in a clean sterile Petri dish; then, 20 mL of already molten Nutrient or MacConkey agar at about 44°C was added. The Petri dish was swirled gently until the contents were completely mix. The agar was allowed to set before incubation at 37°C for 24 hours in a humidified incubator.

After 24 hours of incubation, plates were examined for growth and the emergent colonies counted and recorded. Thereafter, discrete colonies were isolated after three successive sub-culturing and purification on Nutrient agar. Colonies were characterized by standard bacteriological techniques as described by Cheesbrough, [12]. Gram negative oxidase positive rods were further identified using API-kit.

3. RESULTS AND DISCUSSION

S. aureus, Bacillus Spp, Streptococcus Spp, E. coli, Pseudomonas, Enterococcus, Proteus and Klebsiella species were prominent organisms isolated from dry (dTNT) and wet (wTNT) tiger nut samples purchased from different locations. Their corresponding percentage occurrence were; 7.41, 22.2, 11.1, 29.6, 11.1, 3.70, and 7.41 respectively. *E. coli* was the most frequent isolates occurring 8(29.6) followed by *Bacillus* 6(22.2), *Streptococcus* and *Pseudomonas*

occurred 3(11.1), *S. aureus*, *Proteus* and *Klebsiella* also had similar % occurrence of 2(7.41) 1(3.70) (Fig. 1).

High prevalence rate 8(29.6) of *E. coli* is a reliable proof of fecal contamination of the nut. Its presence points to poor hygiene and sanitary practices by Hawkers. This organism can cause diarrhoea which may be mild, watery to severe and bloody stool, stomach cramps and or fever. *Bacillus spp* 6(22.2) are known as environmental contaminant, its presence may signify incomplete removal of soil particle that accompanied the nut during harvest. It may lead to food poisoning outbreak and its associated negative impact. The occurrence of *Streptococcus* and *Pseudomonas* in T. nut (ready to eat food) is not healthy for consumers as they have been reported among causative agent of food spoilage. Although the occurrence rate of *S. aureus*, *Proteus*, *Klebsiella* neglected in ready to eat food. However, there is potency in any pathogen to recover in 10⁻⁵ of any diluents [9]. Abraaham and Eustevo (2019) isolated and identified similar pathogens in TNT sold in different while the least was *Enterococcus* with and *Enterococcus* were low, but should not be markets and vendors in Ghana city and Cape coast metropolis in Ghana.

Five locations (Etta-Agbor, Marian market, Bogobiri, Watt market and Etim Edem Park) were considered in this study. Four locations except Etim Edem Park had similar level of contamination. The major influencing factor here may be the population of the different locations, the four locations are market while the park is a transient setting where people board their fare to other locations (Fig. 2).

The result of the dry and wet (dTNT and We questioned if water activity played a role in wTNT) tiger nut according to locations microbial load of the nut. The investigation of the showed that Etta-Agbor and Marian had dry (dTNT) and wet (wTNT) showed no similar percentage occurrence of microbial significant difference at ($P = 0.05$) in the contaminants for both dTNT and wTNT, frequency of occurrence of the organisms. This Bogobiri and Watt market had 3(37.5) may be due to shortness in storage period after for both while Etim Edem Park had the least of which it is mounted for sales and during the 1(12.0), 2(25.0) for both conditions respectively selling time water is sprinkle intermittently until (Fig. 3). consumers finally purchase them.

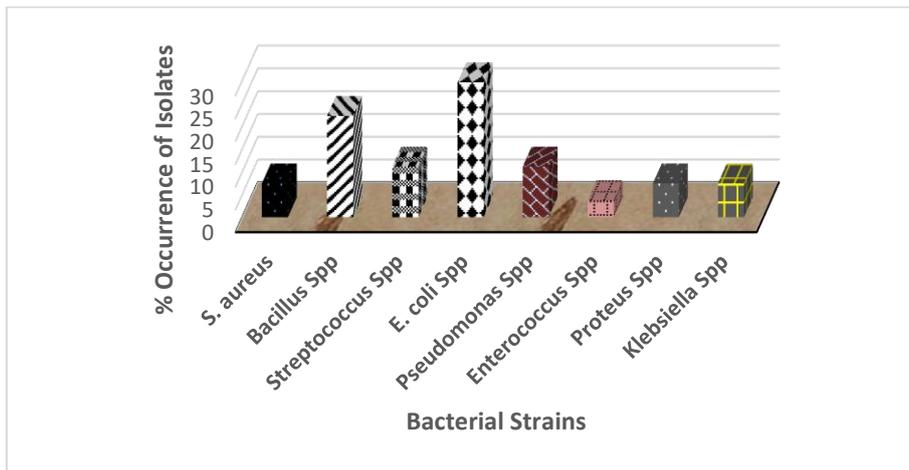


Fig. 1. Different strains of microorganisms isolated from tiger nut

Table 1. Classification of Isolated Organisms Based on Gram Reaction

Bacterial Isolates	Gram Positive (+ve)	Gram negative (-ve)	Cocci	Rods
<i>S. aureus</i>	+	-	+	-
<i>Bacillus spp.</i>	+	-	-	+
<i>Streptococcus spp.</i>	+	-	+	-
<i>E. coli</i>	-	+	-	+
<i>Pseudomonas spp.</i>	-	+	-	+
<i>Enterococcus spp.</i>	+	-	+	-
<i>Proteus spp.</i>	-	+	-	+
<i>Klebsiella spp.</i>	-	+	-	+
Total	4	4	3	5

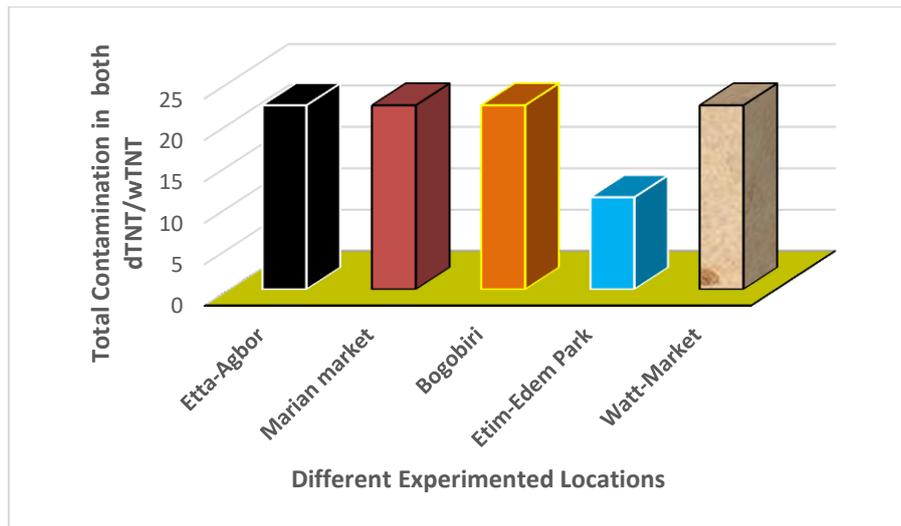


Fig. 2. The level of contamination of tiger nut was evaluated based on percentage occurrence of microbial isolates per experimented location. Etim Edem Park was the least contaminated location

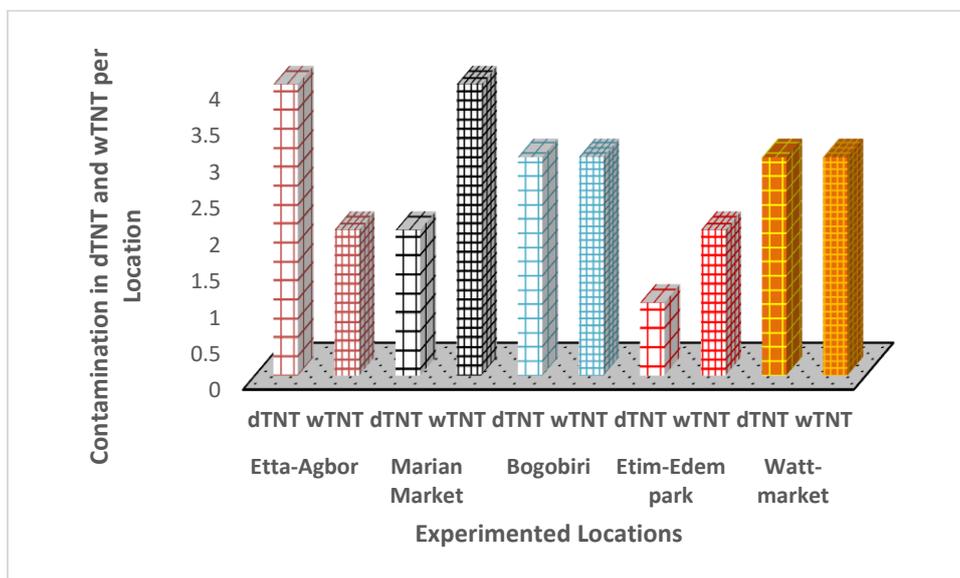


Fig. 3. There was no influence of water activity on level of contamination at Bogobiri and Watt market. However, some variations occurred for Etta-Agbor, Marian and Etim Edem Park. Dry tiger nut at Etta-Agbor and wet at Marian market had similar contamination level

The possible route of tiger nut contamination is numerous ranging from contamination of irrigated water, post-harvest processes, unhygienic handling by commercial vendors among others. However, the research focus more on the microbial load of the ready-to-eat tiger nut tuber sold at different locations. The presence of pathogenic microbes in ready-to-eat tiger nut as reported in the present research is a

conspicuous public health threat to consumers. Thus, decontamination of irrigation water, cultivation of disease resistance nut and observation of personal hygiene during harvest and post-harvest as well as proper washing of the nut before consumption are better remedial measures to eliminate some pathogens and mitigate food related infections and outbreaks [13].

Table 2. Distribution of Isolates from Dry (dTNT) and Wet (wTNT) Tiger Nut Tuber based on locations

Bacterial Isolates	Etta-Agbor		Marian		Bogobiri		Etim Edem Park		Watt Market		Frequency of Occ.
	dTNT	wTNT	dTNT	wTNT	dTNT	wTNT	dTNT	wTNT	dTNT	wTNT	
<i>S. aureus</i>	-	-	-		+	-	-	-	+	-	6
<i>Bacillus spp</i>	-	+	+	-	-	+	-	+	+	+	3
<i>Streptococcus sp</i>	+				+	+					8
<i>E. coli</i>		+	+	+	+		+	+	+	+	3
<i>Pseudomonas spp</i>	+	-	-	+	-	+	-	-	-	-	1
<i>Enterococcus spp</i>	+	-	-		-	-	-	-	-	-	2
<i>Proteus</i>	+	-	-	+	-	-	-	-	-	-	2
<i>Klebsiella spp</i>	-	-	-	+	-	-	-	-	-	+	27
Total	4	2	2	4	3	3	1	2	3	3	2

4. CONCLUSION

The study report that the presence of these microbes (*S. aureus*, *Bacillus Spp*, *Streptococcus Spp*, *E. coli*, *Pseudomonas*, *Enterococcus*, *Proteus* and *Klebsiella* species) in ready-to-eat tiger nut is a potential threat to the public and a pointer to food related infections and possible outbreak. The level of population of a particular location is an influencing factor to the level of contamination. All experimented locations except Etim Edem Park was the only location with the least level of microbial contamination which is not scientifically negligible. Contamination based on the water contents (wet and dry) of the nut was statistically insignificant. The reported information intensifies the health consequences of improperly handled ready-to-eat foods as a result of negligent of personal hygienic measures such as proper washing of public purchased ready-to-eat food products before final consumption. This calls for regular educational awareness outreaches by Government, Non-Governmental organizations and Researchers in both rural and urban settings.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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