

# Microbial Contamination of *Cyperus esculentus* L (tiger nuts) tuber Sold within Calabar Metropolis, Cross River State, Nigeria

## ABSTRACT

The Microbiological quality of wet and dry tiger nut sold in five different locations within Calabar Metropolis was analyzed using Standard Microbiological and Bacteriological Techniques. *S. aureus*, *Bacillus* spp, *Streptococcus* spp, *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 was *Enterococcus* with 1 (3.70). The percentage occurrence of bacterial isolates 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 ke-gag-ent 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 and Etim Edem Park

## 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 [7,8,9]. 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 contribute 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].

This 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.

## METHODOLOGY

20g weight of both Wet (wTNT) and Dry (dTNT) TNT were soaked in 80mL of normal saline with vigorous shaking for 5 minutes to serve as a stock solution. 1mL 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  $44^{\circ}\text{C}$  was added. The Petri dish was swirled gently until the contents were completely mixed. The agar was allowed to set before incubation at  $37^{\circ}\text{C}$  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, (2006). Gram negative oxidase positive rods were further identified using API-kit.

## 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 isolate 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 was *Enterococcus* with 1 (3.70) (Figure 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 diarrhea which may be mild, watery to severe and bloody stool, stomach cramps and or fever. *Bacillus* Spp 6 (22.2) are known as environmental contaminants, its presence may signify incomplete removal of soil particles 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 agents of food spoilage. Although the occurrence rate of *S. aureus*, *Proteus*, *Klebsiella* and *Enterococcus* were low, but should not be neglected in ready to eat food. However, there is potency in any pathogen to recover in  $10^{-5}$  of any diluents [9]. Abrahama and Eustev (2019) isolated and identified similar pathogens in TNT sold in different markets and vendors in Ghana city and Cape coast metropolis in Ghana.

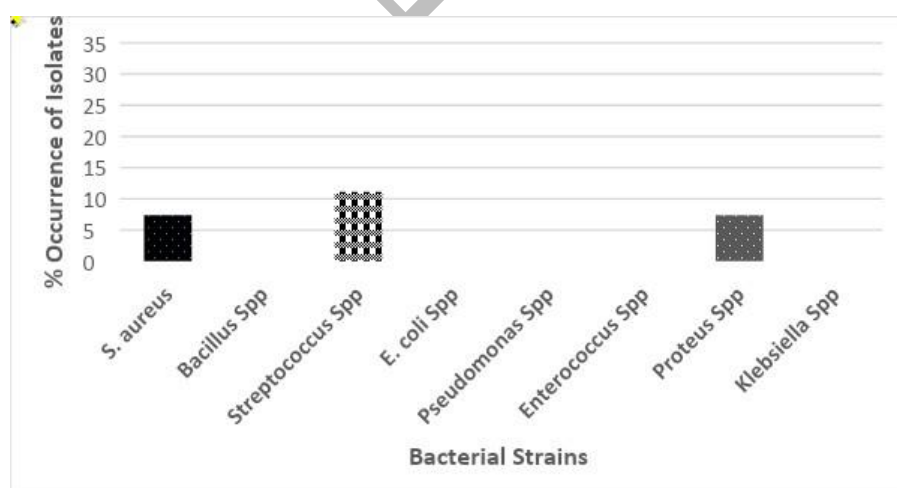
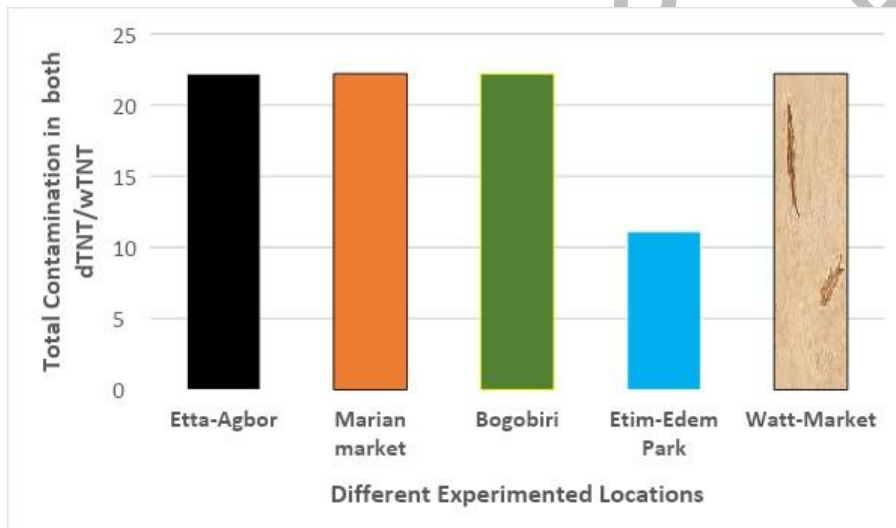


Fig. 1 shows the different strains of microorganisms isolated from tiger nut.

**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</i> spp.	+	-	-	+
<i>Streptococcus</i> spp.	+	-	+	-
<i>E. coli</i>	-	+	-	+
<i>Pseudomonas</i> spp.	-	+	-	+
<i>Enterococcus</i> spp.	+	-	+	-
<i>Proteus</i> spp.	-	+	-	+
<i>Klebsiella</i> spp.	-	+	-	+
<b>Total</b>	<b>4</b>	<b>4</b>	<b>3</b>	<b>5</b>

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).



**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.**

The result of the dry and wet (dTNT and wTNT) tiger nut according to locations showed that Etta-Agbor and Marian had similar percentage occurrence of microbial contaminants for both dTNT and wTNT, Bogobiri and Watt market had 3 (37.5) for both while Etim Edem Park had the least of 1 (12.0), 2 (25.0) for both conditions respectively (Figure 3).

We questioned if water activity played a role in microbial load of the nut. The investigation of the dry (dTNT) and wet (wTNT) showed no significant difference at ( $P = 0.05$ ) in the frequency of occurrence of the organisms. This may be due to shortness in storage period after which it is mounted for sales and during the selling time water is sprinkle intermittently until consumers finally purchase them

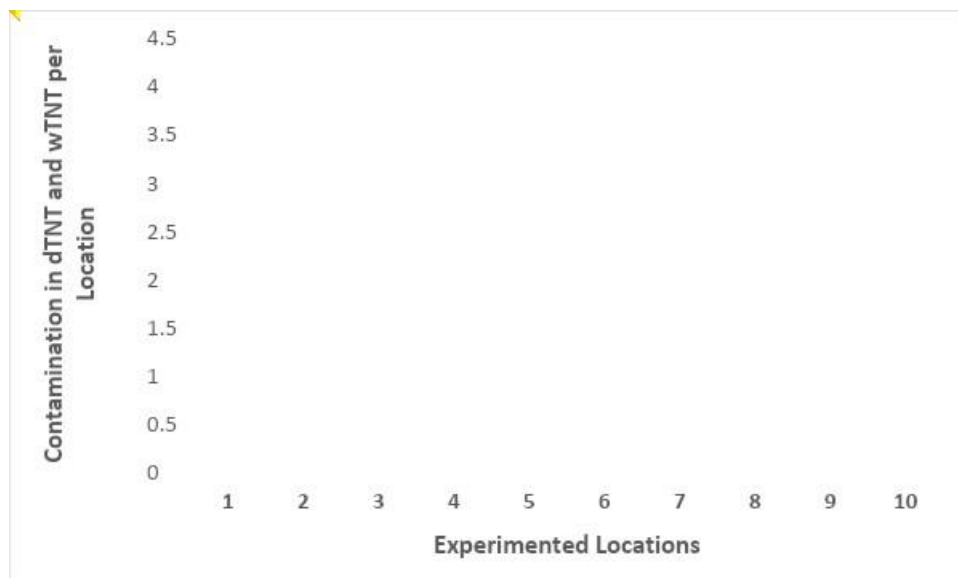


Fig.3: There was no influence of water activity on level of contamination at Bogobiri and Watt market. However, some variations occurred for Etta-Agbor, Marian and Etim Edem Park. Dry tiger nut at Etta-Agbor and wet at Marian market had similar contamination level.

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>	-	-	-	-	+	-	-	-	+	-	2
<i>Bacillus</i> spp	-	+	+	-	-	+	-	+	+	+	6
<i>Streptococcus</i> sp	+	-	-	-	+	+	-	-	-	-	3
<i>E. coli</i>	-	+	+	+	+	-	+	+	+	+	8
<i>Pseudomonas</i> spp	+	-	-	+	-	+	-	-	-	-	3
<i>Enterococcus</i> spp	+	-	-	-	-	-	-	-	-	-	1
<i>Proteus</i>	+	-	-	+	-	-	-	-	-	-	2
<i>Klebsiella</i> spp	-	-	-	+	-	-	-	-	-	+	2
<b>Total</b>	<b>4</b>	<b>2</b>	<b>2</b>	<b>4</b>	<b>3</b>	<b>3</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>3</b>	<b>27</b>

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-resistant 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.

## CONCLUSION

The study reports 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 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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