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 tigernutssoldinfivedifferentlocationswithinCalabar Metropolis wasanalyzedusingStandardMicrobiologicalandBacteriologicalTechniques.*S.aureus, Bacillus*sp,*Streptococcus*sp,*E.coli,Pseudomonas,Enterococcus,ProteusandKlebsiellaspecies* weremicrobialstrainsisolated from dry and wet tiger nut samples. *E. coli* was the most frequent isolates occurring8(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*with1(3.70).Thepercentageoccurrenceofbacterialisolated from wet tiger nut was 14 (28.0)and13(26.0)fromdrytigernut.Fourlocationshadsimilar6(75.0)rateofisolatescompareto EtimEdemPark with2(25.0). The general overview concludes that the microbial threshold in the wet tigernutwas higher 14 (28.0) than that of dry tiger nut with 13 (26.0). Association of microbial pathogens with food cropsendangershumanlivesandarekegagenttofoodrelatedinfectionsandpossible outbreaks.

Keywords:CyperusesculentusL(tigernuts),Microbialisolates,wetT.nut(wTNT), dry T. nut (dTNT), Watt market, Marian Market, Etta Agbor, Bogobiri and Etim Edem Park

INTRODUCTION

Cyperusesculentus L (tiger nuts), belongs to grass family *Cyperaceae.* It is a perennial monocotyledonousplantwithtougherectfibrousrootgrowingtoaboutsixinchesdepthinto the soil [1, 2].Theplantcanhouse50-250tuberswith weigh of about 2 – 26g per tuber [3]. They come in different commercialized varietiessuch as yellowish brown, dark brown-black etc., and are sold in open containers as wet (with little amount of water) or dry nut [4].

InNigeriaespeciallyinCrossRiverState,itiswidelyconsumedforitsnutritionalcomposition,some of whichinclude;calcium,chromium,cobalt,iron,manganese,phosphorus,potassium,selenium, sodium, zinc,carbohydrate,protein,lipidsandvitamins C, E etc.[5,6]. Literature according to Ndubuisi, 2009 reportedthatthenuthastheabilitytopreventagainstheartattack, Thrombosis, absence of cholesterol amongothers[7,8,9].Despitethenutritionalvalues,thenutissubjecttoscarcityinthe nearest future due to susceptibility of the nut to bacterial contamination.

Increaseintheworldpopulationcontinuesandavailabilityofnon-contaminated food to guarantee food safety is becoming more challenging. There is no balance inthe growing population and Agricultural/foodproductsespeciallyinsomeruralareaswherechildrenaresufferingfrom mal-nutrition [10,11].Thesecontributestolow 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].

Theresearchsetouttoevaluatethe 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 tothe public on the consequences of consuming a low quality or improperlyhandled Agricultural/food products thereby mitigating the occurrence of food poison in the society.

METHODOLOGY

20gweightofbothWet(wTNT)andDry(dTNT)TNTweresoakedin80mLofnormalsalinewith vigorousshakingfor5minutestoserveasastocksolution. 1mLwastakenfrom the stock solution to performaten-foldserialdilutiondownto10^{-10}. Pour plating method was the culturing technique used in thisstudy.NutrientandMacConkeyagar(Liofilchem®s.r.l.,Italy) were media used. 1mL amount of 10^{-5} dilutionofeachsuspensionwasplacedinacleansterile Petri dish; then, 20 mL of already molten NutrientorMacConkeyagaratabout44°Cwasadded.ThePetridishwasswirled gently until the contentswerecompletelymix.Theagarwasallowedtosetbeforeincubationat37°Cfor24 hours in a humidified incubator.

After24 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 onNutrientagar.Colonieswerecharacterizedbystandard bacteriological techniques as described by Cheesbrough, (2006). Gram negative oxidase positive rods were further identified using API-kit.

RESULTS AND DISCUSSION

S. aureus, BacillusSpp, Streptococcus Spp, E. coli, Pseudomonas, Enterococcus, Proteus and Klebsiella species were prominent organisms isolated from dry (dTNT) and wet (wTNT) tiger nut samplespurchasedfromdifferentlocations.Theircorrespondingpercentageoccurrencewere;7.41, 22.2,11.1,29.6,11.1,3.70,and7.41respectively.*E.coli*wasthemostfrequentisolatesoccurring 8(29.6) followedby*Bacillus*6(22.2),*Streptococcus*and*Pseudomonas*occurred3(11.1),*S.aureus, Proteus*and*Klebsiella*alsohadsimilar%occurrenceof2(7.41)whiletheleastwas*Enterococcus*with 1(3.70) (**Figure 1)**.

Highprevalencerate8(29.6)of*E.coli*isareliableproofoffecalcontaminationofthenut. Its presence pointstopoorhygieneandsanitarypracticesbyHawkers.Thisorganismcancausediarrhoeawhich maybemild,waterytosevereandbloodystool,stomachcrampsand or fever. *Bacillus* Spp 6(22.2) are knownasenvironmentalcontaminant,its presence may signify incomplete removal of soil particle that accompaniedthenutduringharvest.Itmaylead to food poisoning outbreak and its associated negative impact.Theoccurrenceof*Streptococcus*and*Pseudomonas*inT.nut(readytoeatfood)isnot healthy for consumers as theyhavebeenreportedamongcausativeagentoffoodspoilage.Althoughthe occurrence rate of *S. aureus, Proteus, Klebsiella* and *Enterococcus* were low, but should not be neglectedinreadytoeatfood.However,thereispotencyinanypathogentorecoverin10^{-5} ofany diluents[9]. AbraahamandEustevo *(*2019)isolatedandidentifiedsimilarpathogensinTNTsoldin different markets and vendors in Ghana city and Cape coast metropolis in Ghana.

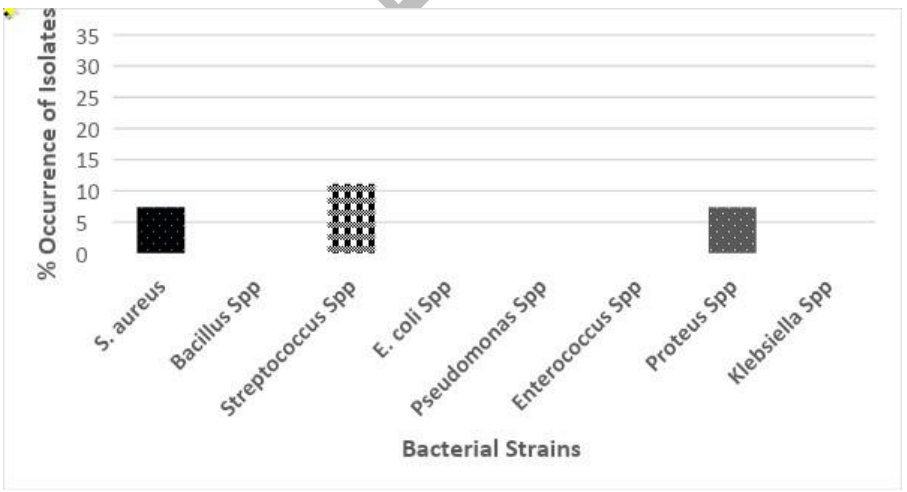

Fig. 1 shows the different strains of microorganisms isolated from tiger nut.

Table 1: Classification of Isolated Organisms Based on Gram Reaction

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

Fivelocations(Etta-Agbor,Marianmarket,Bogobiri, Watt market and Etim Edem Park) were considered in thisstudy.FourlocationsexceptEtimEdemParkhadsimilarlevelofcontamination.Themajor influencingfactorheremaybethepopulationofthedifferentlocations,thefourlocationsaremarket while the park is a transient setting where people board their fare to other locations (Fig 2).

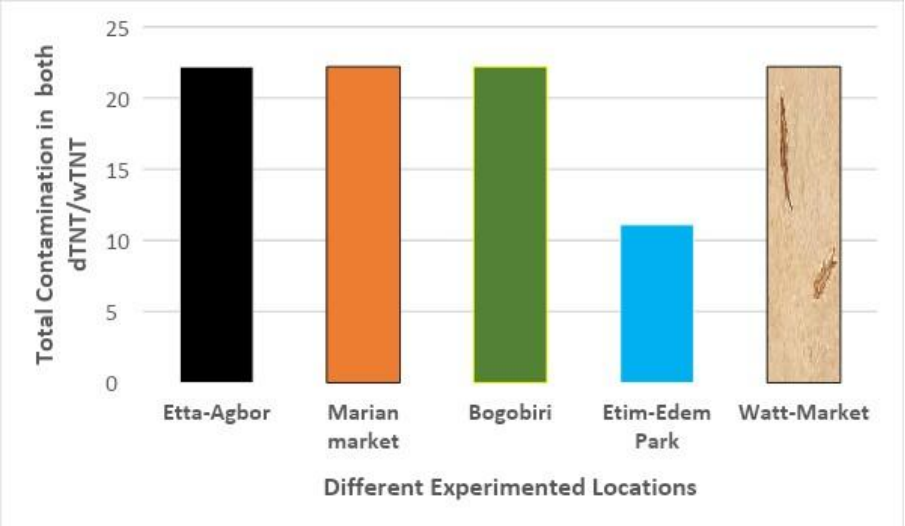

Fig.2:Thelevelofcontaminationoftigernutwasevaluated based on percentage occurrence of microbialisolatesperexperimentedlocation.Etim Edem Park was the least contaminated location.

Theresultofthedryandwet(dTNTandwTNT)tiger nut according to locations showed that Etta-Agbor andMarianhadsimilarpercentageoccurrenceofmicrobialcontaminants for both dTNT and wTNT, BogobiriandWattmarket 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).

Wequestionedifwateractivityplayedaroleinmicrobialloadofthenut. The investigation of the dry (dTNT)andwet(wTNT)showednosignificantdifferenceat (P = 0.05) in the frequency of occurrence of theorganisms.Thismay 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

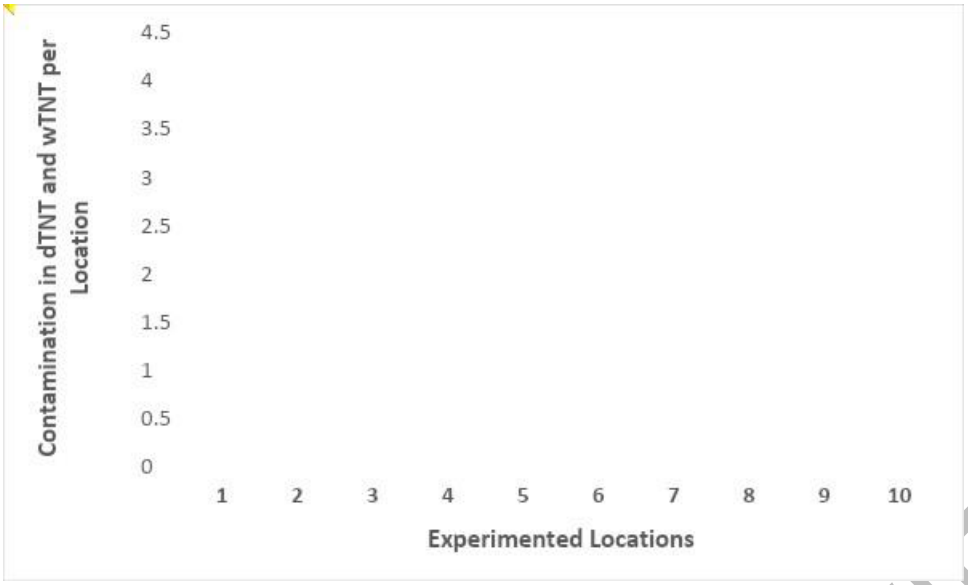

Fig.3:Therewasnoinfluenceofwateractivityon level of contamination at Bogobiri and Watt market.However,somevariations occurred for Etta-Agbor, Marian and Etim Edem Park. Dry tiger nut at Etta-Agbor and wet at Marian market had similar contamination level.

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	
S. aureus	-	-	-		+	-	-	-	+	-	**2**
Bacillus spp	-	+	+	-	-	+	-	+	+	+	**6**
Streptococcus sp	+				+	+					**3**
E. coli	+	+	+		+		+	+	+	+	**8**
Pseudomonas spp	+	-	-	+	-	+	-	-	-	-	**3**
Enterococcus spp	+	-	-		-	-	-	-	-	-	**1**
Proteus	+	-	-	+	-	-	-	-	-	-	**2**
Klebsiella spp	-	-	-	+	-	-	-	-	-	+	**2**
Total	**4**	**2**	**2**	**4**	**3**	**3**	**1**	**2**	**3**	**3**	**27**

The possible rout of tiger nut contamination is numerous ranging from contamination of irrigated water, post-harvestprocesses,unhygienichandling by commercial vendors among others. However, the researchfocusmoreonthemicrobialloadoftheready-to-eattigernut tuber sold at different locations. Thepresenceofpathogenicmicrobesinready-to-eattigernutasreportedinthe present research is a conspicuouspublichealth threat to consumers. Thus, decontamination of irrigation water, cultivation of diseaseresistancenutandobservationofpersonalhygieneduringharvestandpost-harvestaswell as proper washingof the nut before consumption are better remedial measures to eliminate some pathogens and mitigate food related infections and outbreaks.

CONCLUSION

Thestudyreportthatthe 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 threattothe public and a pointer to food related infections and possible outbreak. The level of population ofaparticularlocation is an influencing factor to the level of contamination. All experimented locations exceptEtimEdemParkwastheonly location with the least level of microbial contamination which is not scientifically negligible. Contamination based on the water contents (wet and dry) ofthenutwas statistically insignificant.Thereportedinformationintensifiesthehealthconsequencesofimproperly handled ready-to-eat foodsasaresultofnegligentofpersonalhygienicmeasuressuchasproper washing of public purchased ready-to-eat food products before final consumption. This calls for regular educationalawarenessoutreachesbyGovernment,Non-GovernmentalorganizationsandResearchers in both rural and urban settings.

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