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Assessment of microbiological safety of tiger nuts (*Cyperus esculentus* L.) in the Cape Coast Metropolis of Ghana

[1]Hubert. D. Nyarko*, [1,2]Daniel. N. A. Tagoe and [1]Yaw. Aniweh

[1]Department of Laboratory Technology, University of Cape Coast, PMB, Cape Coast, Ghana
[2]Medical Laboratory Section, Department of Laboratory Technology, UCC, Cape Coast, Ghana

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ABSTRACT

The microbiological safety of tiger nut (Cyperus esculentus L.), a popular tuber eaten in the fresh uncooked state in Ghana was assessed from three major markets in the Cape Coast Municipality to ascertain their microbiological safety in relation to bacterial contamination. A study of 24 samples of 8 each from the markets divided into two batches were analyzed by surface-washing in Phosphate Buffered Water (PBS) only and PBS plus 1% Sodium hypochlorite. Serial dilutions were made and required volumes (0.1ml) of test samples were dispensed separately into sterile Petri dishes and pour plated with Plate Count Agar (PCA) for enumeration of colonies. MacConkey and Uriselect agars were used for enteric bacteria and species identifications. Mean Total Viable Count (TVC), Mesophilic Aerophilic Count (MAC), and Coliform Count (CC) on PCA using PBS only ranged between 2.54 $\times 10^5$- 8.22$\times 10^5$; 1.87$\times 10^5$-5.44$\times 10^5$ and 9.44$\times 10^2$ - 3.32$\times 10^3$ CFU/g respectively whilst that of PBS plus 1% Sodium hypochlorite ranged between 1.51$\times 10^2$-1.35$\times 10^3$; 2.52$\times 10^3$-1.06$\times 10^3$ and 4.86$\times 10^1$-8.69$\times 10^1$ CFU/g respectively. There was marked significant differences p-(0.0319; 0.0267; and 0.0104) between PBS only and PBS plus 1% Sodium hypochlorite for TVC, MAC and CC respectively. Seven different bacteria species isolated were made up of Bacillus spp. and Escherichia coli (18.9%) each; Enteroccoccus spp. (16.2%); Pseudononas aeruginosa and Staphylococcus aureus (13.5%) each, Streptococcus spp. (10.8%) and Enterobacter cloacae (8.1%). This study looks at the levels and nature of microbial contaminations of tiger nut retailed in the Cape Coast Metropolis, and the possible means of reducing such contamination.

Keywords: Phosphate buffered Water, Mesophilic aerophilic count, Coliform, Sodium hypochlorite, *Escherichia coli.*

INTRODUCTION

It is a known fact that most fruits and vegetables are important source of nourishment and a vital ingredient in healthy and balanced diets [1], but harbour varied loads of microbial flora while passing from farm to table [2] and microbial contaminations before, during or following harvest [3]. 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 [4]. They as well serve as a good source of food borne illness when contaminated with pathogenic microorganisms during harvesting, handling, transport containers and display in street markets [5]. Food is generally a fertile ecosystem, in which microorganisms vie for nutrients. A variety of microbes find their way onto foods, introduced from the soil in which they were grown, and during harvest, packaging, storage and handling [6].

A study revealed that the 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 [7]. A mixture of microorganisms that have been isolated from exposed tiger nuts included *Bacills subtilis, Staphylococcus aureus, Aspergillus flavus, A. niger, Fusarium solani, Saccharomyces cerevisiae, S. fubiligera* and *Candida pseudotropicalis,* with varied percentage frequencies of occurrence, which rendered the tiger nuts unwholesome, except for the unexposed samples which recorded relatively lower microbial loads [8]. The presence of pathogenic *E. coli, S. faecalis, and S. aureus* usually constitute a direct proof of faecal contamination of irrigation water [9].

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 [10]. It has long been recognised as one of the best nutritional crops used to augment diets, since a substantial intake decreased reported cases of various health related conditions such as cardiovascular disease, diabetes, cancer, and obesity, and also ideal for children, older persons and sportsmen [11], as well as an excellent source of iron and calcium for body growth and development [12]. A quantity (33.33%) of tiger nuts was included in the diet of cockerel starters [13]. Again, tiger nuts with its inherent nutritional and therapeutic advantage, could serve as alternative to cassava in baking industry [14].

In Ghana, tiger nuts are sold on the streets usually in wide open metal basins uncovered and exposed to the environment. It is widely patronized among the populace based on the numerous health benefits, especially among males due to its perceived aphrodisiac property. The sellers often use their bare fingers to dispense the tiger nuts into either old newspapers or polythene wrappers and bought ready to be eaten. According to [15], tiger nuts from the farm to the point of sale are likely to be exposed to all forms of contaminations, including microorganisms. As consumers, however, there is always the need to place emphasis on food safety, such that food from a source like the street market should be protected from contamination and spoilage during subsequent handling, packaging, and storage while in transit.

In spite of the high nutritive value of the tiger nut, its production has been hampered due to the deteriorating effect of some microorganisms on the milk [10]. The present study is therefore, aimed at the microbial safety of tiger nuts on retail in the Cape Coast Metropolis of Ghana to

ascertain the contamination levels and the types of bacterial contaminants, in an attempt to help and protect the general Ghanaian populace.

MATERIALS AND METHODS

Study Area: The study was conducted in two major markets (Kotokuraba and Abura) located in the Cape Coast Metropolis of the Central Region of Ghana between November, 2009 and March, 2010.

Sample Collection and Surface Analysis: A total of 24 samples of 8 each were taken from three major markets in the manner as sold to consumers in the Cape Coast Metropolis of Ghana. The samples were divided into 2 of which 10g of the first batch was surface-washed in 90ml Phosphate Buffered Water (PBS) only. The remaining half (10g) was surface-sterilized in 1% sodium hypochlorite solution for 5 minutes and thereafter rinsed thoroughly with sterile distilled water. 90ml Phosphate Buffered Water (PBS) was then added as in the first batch. The sample was shaken vigorously to ensure adequate contact and effective washing of entire surfaces.

Microbiological Analysis

The aerobic plate count was enumerated using the pour plate technique in a non-selective medium Plate Count Agar (PCA). 1ml of the solution from each sample and from both batches of washed surfaces was used to perform serial dilutions to the 10^{-5} factor. Using a sterile pipette, 0.1ml of each dilution was dispensed separately into sterile Petri dishes and pour plated with Plate Count Agar (PCA). The content was swirled in a clockwise and counter clockwise fashion to ensure thorough mixing and allowed to set. The plates were incubated at 35˚C for 24hrs. This was done in duplicates for each sample. Colonies that grew on the plates were counted to determine the colony forming units.

Bacterial isolation, enumeration and identification: MacConkey and Uriselect agars were used for the detection of enteric bacteria and species identification. Again, 0.1ml of each dilution was dispensed aseptically into separate Petri dishes and pour plated with the selective media. Bacteria colonies were counted on each plate after incubation to determine colony forming units (CFU). Bacteria isolates were further sub cultured to obtain pure cultures. Characterizations of isolates, however, were made by microscopy, gram staining, morphologic examination, oxidation-fermentation tests and other biochemical tests [16]

Statistical Analysis: Mean viable cell counts were compared between PBS only and surface sterilization in 1% sodium hypochlorite solution using the Paired Means Comparison.

RESULTS AND DISCUSSION

Microbial counts on tiger nuts washed with PBS only and 1% sodium hypochlorite sterilization for 5mins followed by PBS washing showed varied microbial loads. The means of TVC, MAC, and CC ranged from 5.17×10^5, 3.51×10^5 and 1.97×10^3 respectively for NS compared with the SS, which had 6.89×10^2, 5.64×10^2 and 6.95×10^1 respectively. The microbial counts of non-sterilised (NS) tiger nuts, which were treated with PBS, indicate unacceptable levels of all forms of contamination with a record high of 10^6 cfu/g. This high microbial load supports the assertion

that total aerobic microorganism number as 10^5-10^{10} cfu/g and coliform counts of 10^6 - 10^{10}cfu/g on raw vegetables used in salads mixture [5]. This is expected since chlorinated water in distribution has been found to be contaminated and even sachet water which is considered more purified was found to contain bacteria counts ranging between 2.8 X 10^3 and 5.9 X 10^5 cfu/mL [17]. The high microbial loads of the non-sterilised tiger nut samples investigated, which were unsatisfactory/unacceptable, contradicts findings of the vast majority (99.5%) of uncooked ready-to-eat organic vegetables sampled at retail in the UK, that were of acceptable microbiological quality according to published guidelines of Public Health Laboratory Services, (PHLS, 2000) [7].

There was marked reduction in microbial load after surface sterilization (SS), which was significant ($P<0.05$) (Table 1). The SS tiger nuts, however, had a relatively low mean colony counts of 10^2, a much more acceptable bacterial load which compared favourably with microbial loads isolated from some supermarkets [18]. It is also in conformity with the study conducted on chemical intervention of shrimp and prawn, using different sanitizers including calcium hypochlorite solution, which yielded remarkable reduction of *E. coli* and aerobic plate count as compared to the water washing that served as control [19].

The relatively high levels of coliform contaminants of *E. coli* (18.9%) and *Enterococcus spp.* (16.2%) are reliably proofs of faecal contamination of the tiger nuts suggesting the use of poor water sources for irrigation, which includes water from drains in developing countries including Ghana. Furthermore, the high presence of mesophilic aerophile *Bacillus spp*. (18.9%) confirms contamination by food-borne pathogens in the soil environment in which the tiger nuts grow. The presence of *Bacillus spp*., *S. aureus* and *P. aeruginosa* have been implicated in food spoilage and food-borne diseases, hence, are of considerable interest in food hygiene [6]. The ingestion of contaminated tiger nuts, particularly with the aforementioned pathogen is likely to pose health risk to the numerous consumers. This intends buttresses the assertion that intake of contaminated food can cause diarrhoeal-associated illnesses with bacteria as one of the major causes of food-borne contamination [20]. Therefore, to reduce microbial load from pre-market sources, the handling processes, preservation and most importantly irrigation sources must be checked for possible sources of contamination regularly. Additionally, the hygiene and retail methods of the sellers must be considered since Manu *et al*, (1999) reported high levels of contamination of sellers with enteric pathogens including *E. coli*. These and many other contributions, point to the fact that the use of sanitizers as a measure to reduce microbial contamination on food surfaces after cleaning must be encouraged.

The isolation of microorganisms on the tiger nuts showed a total of 7 different bacteria with varied frequencies of occurrence. The most predominantly encountered species were *E. coli* and *Bacillus spp*., which had 18.9% each. Others include *Enterococcus spp*. (16.2%), *S. aureus* and *P. aeruginosa* (13.5%) each, and *Streptococcus spp.* (10.8%). *Enterobacter cloacae* was the least frequent isolate (8.1%) (Table 2). Similar observations of varied incidences of microbial isolates, which rendered tiger nuts milk unpalatable and unsafe for human consumption, were recorded by Onovo and Ogaraku (2007).

Table 1: Descriptive Statistics and Paired Mean Comparison of Total Viable Count (TVC), Mesophilic Aerophile Count (MAC) and Coliform Count (CC) analysis of sample between [a]Non-sterile (NS) and [b]Surface Sterilized (SS) sample

Statistics	*TVC NS cfu/g*	*TVC SS cfu/g*	*MAC NS cfu/g*	*MAC SS cfu/g*	*CC NS cfu/g*	*CC SS cfu/g*
No. of Samples	24	24	24	24	24	24
Minimum	1.5×10^{5}	0.0	1.2×10^{3}	0.0	3.0×10^{5}	0.0
Maximum	5.0×10^{6}	7.0×10^{3}	3.3×10^{5}	6.3×10^{3}	1.5×10^{4}	2.0×10^{2}
Mean	5.17×10^{5}	6.89×10^{2}	3.51×10^{5}	5.64×10^{2}	1.97×10^{3}	6.95×10^{1}
***P*-value (*P*<0.05)**	0.0319		0.0267		0.0104	

a) Sample washed in Phosphate Buffer Saline Only
b) Sample surface sterilized in 1% sodium hypochlorite for 5mins and then washed with Phosphate Buffer Saline.

Table 2 Bacteria species and their frequency of occurrence on samples

Organism	*Frequency*	*%*
Bacillus spp.	7	18.9
Escherichia coli	7	18.9
Enterococcus spp.	6	16.2
Staphylococcus spp.	5	13.5
Pseudomonas aeruginosa	5	13.5
Streptococcus spp.	4	10.8
Enterobacter cloacae	3	8.1
Total	37	100

CONCLUSION

The work has shown that raw market retailed tiger nuts (non-sterile) are contaminated with high bacteria loads that are implicated in both food spoilage and food-borne diseases. However, surface sterilization of the tiger nuts has the potential of reducing the contamination and its attendant health implications.

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