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Evaluation of saline treatments on bacteriological quality of Tiger Nut (*Cyperus esculentus L.*) for consumption post purchase

Nana Ewusi Arkoh, Priscilla Cecilia Amenya, George Aboagye*

Department of Nutrition and Dietetics, School of Allied Health Sciences, University of Health and Allied Sciences, Volta Region, Ghana.

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ABSTRACT

Food-borne diseases are usually caused by ingestion of contaminated food. One of such foods susceptible to microbial contamination is Tiger nut (*Cyperus esculentus L.*). We evaluated the bacteriological quality of Tiger nuts under various consumer treatment conditions post purchase.Three tiger nut samples each from 7 vendors were obtained by convenient sampling in Ho, the capital of the Volta Region of Ghana. The samples were subjected to vigorous washes in 1%, 2% and 3% sterile saline with tap water as control. The resulting liquids were analysed for microbial load and species using specific media and standard microbial methods. Data obtained from the study were analysed by ANOVA, and significant differences among means were defined at $p<0.05$. The mean bacterial count for the fresh tiger nut in tap water ranged from 1.95×10^6 to 1.59×10^7 cfu/mL whilst that in tap water only (control), 1%, 2% and 3% saline solutions recorded a range of 7.08×10^5 to 1.18×10^7, 7.31×10^5 to 1.34×10^7, 4.0×10^4 to 1.13×10^7 and 1.11×10^5 to 9.62×10^6 cfu/mL respectively. The higher bacterial load from the tiger nut in the sterile tap water was above the acceptable reference limit of 10^5 cfu/mL by the National Administration for Food Drugs and Control (NAFDAC). The mean bacterial count of *Escherichia coli* and *Staphylococcus aureus* in all the 21 samples was 6.15 $\log_{10}$ and 6.18 $\log_{10}$ cfu/mL respectively, all of which exceeded the acceptable range from 10^2 to 10^3 cfu/mL by the International Commission on Microbiological Specifications for Foods (ICMSF).To improve the bacteriological quality of tiger nut, proper farming practices, handling and treatment regimens under hygienic conditions should be employed to reduce or eliminate the occurrence of contaminants to ensure the protection and safety of the consumer.

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1. Introduction

Due to urbanization, patronage of food has increased and has led to the blooming of food businesses (1). The provision of ready-to-eat foods by vendors has made food accessibility more convenient for people who are unable to cook at home. However, due to the various processes by which food is prepared, the chance for contamination may be increased (1). Food is generally an ecosystem where microorganisms

*Corresponding author. Tel.: +233202993078
E-mail address: gaboagye@uhas.edu.gh

compete for nutrients, therefore, making it a habitat for microorganisms (2). The World Health Organization (WHO) has stated that diseases that result from consuming contaminated food are the leading health problems currently in the world (3). Tiger nut (*Cyperus esculentus L.*) is a tuber that is grown in the soil with dimensions ranging from 6 to 10 mm and occurs in different varieties with a sweet flavor when eaten (4). Tiger nut grows freely and is consumed across various parts of West and East Africa (4). In Nigeria, 3 varieties are known and are the black, brown and yellow types of which the yellow and brown varieties are readily available due to their bigger size, fleshier body, and attractive color (5). The tuber contains the essential amino acid lysine and has 22.5% and 33.8% of oil and carbohydrates respectively (6). Tiger nut is mostly consumed for its medicinal and nutritional benefits (7) as well as its aphrodisiac properties employed by people of the Middle East (8). Tiger nut is found all around the world but primarily in the Eastern hemisphere. They also grow wild and free, from Southern Europe to Madagascar to Africa to some places in India and beyond. Per the business outlet, the agronomy of tiger nuts started in Egypt, from where they spread to other parts of North Africa, and eventually crossed the Strait of Gibraltar, to the Iberian Peninsula. In Ghana, the yellow and black varieties are common and are cultivated in many parts of the country such as Bodwease, Techiman and Kwahu. Nonetheless, among all these areas, Aduamoa in the Kwahu South District is recognized as the production hub of tiger nuts, indeed producing the sweetest and best variant in the country (9) In the Volta region, tiger nut is grown in places such as Hohoe, Jasikan, Biakoye, Ho, Kadjebi, Adaklu-Anyigbe, Akatsi, South Tongu and North Tongu. Although tiger nut has a lot of uses, it is underutilized (10). Due to the variations of treatment of the nut exhibited by the populace following purhase i.e. washing with different water sources including saline, calls for the evaluation of the treatments identified on the microbial quality of the nuts in order to provide answers to the food safety risks this could pose tothe highly patronizing consumers worldwide.

2. Materials and Methods

2.1. Study area

The study was conducted in the Ho Municipality of the Volta Region with a total population of 177,281. The municipality is located between latitudes 6˚20′′N and 6˚55′′N and longitudes 0˚12′E and 0˚55′E. The municipality shares boundaries with the Adaklu and Agotime-Ziope Districts to the South, Ho West District to the North and West and the Republic of Togo to the East. Its total land area is 2,361 square kilometers thus representing 11.5 percent of the region's total land area (11). Generally, the mean monthly temperature in the Municipality ranges between 22 and 32˚C with an annual mean temperature range between 16.5 and 37.8˚C. In effect, temperatures are generally high throughout the year promoting good farming practices and concomitant microbial proliferation along the food chain.

2.2. Sampling sites

Samples were collected randomly from 7 tiger nut vendors in the municipality.

2.3. Survey

A total of 21 tiger nut samples were obtained for the study i.e., 3 samples each from 7 vendors in the Ho market by convenience sampling.

2.4. Study design

An experimental study with empirical data collection was employed to determine the bacteriological quality of the tiger nuts based on various treatment practices exhibited by consumers after purchase.

2.5. Sample size determination

Using a population size (N) of 44, and the Hotjar sample size calculator, at a confidence interval of 95% and a marginal error of 5%, the sample size (n_o) = 40 which was adjusted for smaller populations to 21 samples using the Cochrane formula below:

$$n = \frac{no}{1 + [(no - 1)/N]}$$

Where:

n = sample size (newly adjusted)

n_o = sample size

N = population size

$$n = \frac{40}{1 + [(40 - 1)/44]}$$

n = ~ 21

2.6. Sampling technique

A convenience sampling technique was employed to obtain the samples.

2.6.1. Sample collection and preparation

A total of 21 tiger nut samples were obtained for the study i.e., 3 samples each from 7 vendors. Each set of samples collected from the vendors was kept in a sterile nylon container and transported to the microbiology laboratory of the University of Health and Allied Sciences for analysis. Each sample was subdivided into four groups (5 g each). The tiger nuts were soaked in 45 ml of tap water, 1%, 2% and 3% saline respectively, in a sterile bottle for 1 h 30 min. After this duration, the tiger nuts were washed by shaking the bottles vigorously.

2.7. Microbiological analysis

The aerobic bacterial species were enumerated using the pour plate technique in a non-selective nutrient agar. One-millilitre of the various stock solutions from each sample was diluted to 10^{-6}. Using a sterile pipette, 1 mL of the dilutions 10^{-4} and 10^{-5} were dispensed separately into sterile petri dishes and pour-plated with nutrient agar (Biomark Laboratories). The content was swirled both clockwise and anti-clockwise to ensure thorough mixing and allowed to set. The plates were incubated at 37°C for 24 to 48 h. The resulting colonies were counted to determine the colony-forming units per millilitre.

2.8. Bacterial isolation, enumeration and identification

The detection and identification of bacterial species were carried out using 2 primary media i.e. Mannitol Salt agar (Oxoid, CM0085) and MacConkey agar (Biomark Laboratories). Then also, 1 mL of dilutions 10^{-4} and 10^{-5} was dispensed aseptically into separate petri dishes, and pour-plated with the selective media. Bacterial colonies were counted on each plate after incubation to determine colony-forming units per millilitre.

2.9. Statistical analysis

The data collected were captured on excel spreadsheet and analyzed using Statistical Package for the Social Sciences (SPSS) software version 22. This was followed by analysis of variance (ANOVA). Differences among means were separated and their significance was

determined at $p<0.05$. The means were compared with both national and international standards.

The results obtained are presented in Tables 1 to 6 below.

3. Results

Table 1. Mean Log_{10} cfu/mL of bacteria in 21 Tiger nut samples isolated on different growth media

SAMPLE	NA	MCA	MSA
		Log_{10} cfu/mL	
S1	6.39	6.18	6.45
S2	6.71	6.40	6.40
S3	6.48	7.03	6.81
S4	6.43	6.27	6.62
S5	6.70	6.54	6.64
S6	6.63	6.25	6.28
S7	6.97	6.90	6.54
S8	7.10	6.38	6.7
S9	6.66	6.77	6.72
S10	6.73	6.22	6.71
S11	6.67	6.61	6.61
S12	6.51	6.11	6.06
S13	6.22	5.94	5.85
S14	6.10	5.38	5.81
S15	6.31	5.85	5.76
S16	6.67	6.61	6.61
S17	6.33	5.92	3.96
S18	5.54	5.41	5.88
S19	6.23	5.74	5.58
S20	6.31	5.12	5.98
S21	6.49	5.68	5.92
MINIMUM	5.54	5.16	3.96
MAXIMUM	7.10	7.03	6.81
MEAN	6.49±0.33	6.16±0.51	6.18±0.64

KEY: **NA-** Nutrient Agar, **MCA-** MacConkey Agar, **MSA-** Mannitol Salt Agar

The mean log_{10} cfu/mL for the 4^{th} and 5^{th} dilutions isolated from nutrient (NA), MacConkey agar (MCA) and Mannitol Salt agar (MSA) are presented in Table 1 above. From sample 1 to 21, a range of 5.54 to 7.10, 5.16 to 7.03 and 3.96 to 6.81 mean log_{10} cfu/mL were recorded for NA, MCA and MSA respectively.

The mean log_{10} cfu/mL for the 4^{th} and 5^{th} dilutions isolated on the different media for tiger nut treated under tap water are presented in Table 2. For all the 21 samples, a range of 6.51 to 7.26, 6.13 to 7.35 and 6.10 to 6.85 mean log_{10} cfu/mL were recorded for NA, MCA and MSA respectively with the lowest range from MSA and the highest range from NA.

Table 2. Mean Log_{10} cfu/mL of bacteria in 21 tiger nut samples washed in tap water only and isolated on different growth media

SAMPLE	NA	MCA	MSA
		Log_{10} cfu/mL	
S1	6.82	6.32	6.22
S2	6.62	6.36	6.37
S3	6.65	7.35	6.68
S4	6.69	6.34	6.85
S5	6.93	6.7	6.8
S6	6.61	6.48	6.37
S7	7.26	6.9	6.71
S8	7.15	6.89	6.54
S9	6.83	7.02	6.82
S10	6.95	6.23	6.82
S11	6.92	6.75	6.75
S12	6.85	6.85	6.49
S13	6.51	6.42	6.47
S14	6.55	6.19	6.1
S15	6.51	6.35	6.16
S16	6.92	6.75	6.75
S17	6.69	6.39	6.53
S18	6.78	6.31	6.41
S19	6.61	6.41	6.36
S20	6.8	6.13	6.44
S21	6.85	6.15	6.15
MINIMUM	6.51	6.13	6.10
MAXIMUM	7.26	7.35	6.85
MEAN	6.79±0.20	6.54±0.33	6.51±0.24

KEY: **NA-** Nutrient Agar, **MCA-** MacConkey Agar, **MSA-** Mannitol Salt Agar

Table 3. Mean Log_{10} cfu/mL of bacteria in 21 Tiger nut samples washed in 1% saline only and isolated on different growth media

SAMPLE	NA	MCA	MSA
		Log_{10} cfu/mL	
S1	6.4	6.34	6.32
S2	6.54	6.59	6.41
S3	6.38	7.25	6.77
S4	7.13	6.36	6.68
S5	6.84	6.62	6.71
S6	6.54	6.53	6.41
S7	7.19	6.85	6.71
S8	7.04	6.09	6.82
S9	6.77	7.01	6.82
S10	6.83	6.07	6.79
S11	6.85	6.7	6.7
S12	6.88	6.4	6.27
S13	6.52	5.57	5.91
S14	6.23	5.81	6.17
S15	6.49	5.97	6.03
S16	6.85	6.7	6.7
S17	6.35	6.74	5.32
S18	6.42	5.86	6.38
S19	6.23	5.98	5.69
S20	6.23	5.45	6.06
S21	6.41	5.85	6.08
MINIMUM	6.23	5.45	5.32
MAXIMUM	7.19	7.25	6.82
MEAN	6.62±0.30	6.32±0.48	6.37±0.41

KEY: **NA-** Nutrient Agar, **MCA-** MacConkey Agar, **MSA-** Mannitol Salt Agar

The mean log_{10} cfu/mL for the same dilution series as before, and treated under 1% saline solution are presented in Table 3. For all the 21 samples, a range of 6.23 to 7.19, 5.4 to 7.25 and 5.32 to 6.82 mean log_{10} cfu/mL were recorded for NA, MCA and MSA respectively with the lowest range from MSA and the highest range from NA.

The mean log_{10} cfu/mL recorded for 2% saline solution are presented in Table 4. and a range of 2.27 to 7.7.18, 5.04 to 7.04 and 4.0 to 6.87 mean log cfu/mL were recorded for NA, MCA and MSA respectively with the lowest range from MSA and the highest from MCA.

Table 4. Mean Log_{10} cfu/mL of bacteria in 21 Tiger nut samples washed in 2% saline only and isolated on different growth media

SAMPLE	NA	MCA	MSA
		Log_{10} cfu/mL	
S1	6.03	5.85	6.85
S2	6.61	6.4	6.31
S3	6.45	6.93	6.8
S4	5.28	6.18	6.63
S5	6.75	6.46	6.6
S6	6.61	6.22	6.13
S7	6.99	7.04	6.63
S8	7.18	6.25	6.87
S9	6.71	6.97	6.71
S10	6.7	6.34	6.67
S11	6.74	6.6	6.6
S12	6.58	5.99	5.95
S13	6.57	6.08	5.72
S14	6.00	5.20	5.78
S15	6.33	5.77	5.80
S16	6.74	6.60	6.60
S17	6.25	5.60	4.00
S18	2.27	5.48	5.69
S19	6.15	5.56	5.40
S20	6.11	5.04	5.83
S21	6.32	5.54	5.88
MINIMUM	2.27	5.04	4.00
MAXIMUM	7.18	7.04	6.87
MEAN	6.26±0.44	6.10±0.57	6.16±0.68

KEY: **NA-** Nutrient Agar, **MC-** MacConkey Agar, **MSA-** Mannitol Salt Agar

Table 5. Mean Log_{10} cfu/mL of bacteria in 21 Tiger nut samples washed in 3% saline only and isolated on different growth media

SAMPLE	NA	MCA	MSA
		Log_{10} cfu/mL	
S1	6.45	6.21	6.39
S2	7.11	6.24	6.51
S3	6.41	6.59	6.99
S4	6.5	6.19	6.32
S5	6.56	6.35	6.44
S6	6.71	5.76	6.2
S7	6.67	6.79	6.11
S8	7.07	6.27	6.57
S9	6.53	6.08	6.52
S10	6.54	6.22	6.56
S11	6.52	6.38	6.38
S12	5.88	5.18	5.52
S13	5.52	5.7	5.28
S14	5.86	4.3	5.18
S15	6.01	5.32	5.04
S16	6.52	6.38	6.38
S17	5.52	4.95	0.00
S18	5.99	4.00	5.04
S19	6.04	5.00	4.85
S20	5.94	4.00	5.58
S21	6.21	5.18	5.58
MINIMUM	5.52	4.00	0.00
MAXIMUM	7.11	6.79	6.99
MEAN	6.31±1.00	5.67±0.85	5.69±1.45

KEY: **NA-** Nutrient Agar, **MCA-** MacConkey Agar, **MSA-** Mannitol Salt Agar

Table 6. Mean Log_{10} cfu/mL of bacteria in tap water only and isolated on nutrient agar

SAMPLE	NA (Log_{10} cfu/mL)
S1	6.24
S2	6.67
S3	6.53
S4	6.54
S5	6.4
S6	6.67
S7	6.74
S8	7.07
S9	6.47
S10	6.62
S11	6.34
S12	6.35
S13	5.99
S14	5.85
S15	6.21
S16	6.34
S17	6.86
S18	6.25
S19	6.13
S20	6.46
S21	6.65
MINIMUM	5.85
MAXIMUM	7.07
MEAN	6.45±0.29

KEY: **NA-** Nutrient Agar

The mean log_{10} cfu/mL isolated on the different media for tiger nut treated under 3% saline solution are presented in Table 5. For all the 21 samples, a range of 5.52 to 7.11, 4.0 to 6.79 and 0.00 to 6.99 for NA, MCA and MSA respectively with the lowest range from MSA and the highest range from NA . The mean log_{10} cfu/mL of bacteria obtained from tap water only (control) are presented in Table 6, with counts ranging from 5.85 to 7.07 log_{10} cfu/mL. Generally, the mean count in NA for both tiger nuts washed in tap water and tap water only (control).

showed a statistical significance difference ($p<0.05$; 0.000071). Also, the mean count for NA for both tiger nuts washed in tap water and in 1% saline solution recorded a significant difference ($p<0.05$; 0.047). Also, the results for both tiger nuts washed in tap water and tiger nut in 2% saline solution, showed a significant difference ($p<0.05$; 0.022). Furthermore, the mean count for NA for both tiger nuts washed in tap water and those washed in 3% saline solution showed a significant difference ($p<0.05$; 0.000058). However, the mean count for NA between tiger nuts washed in 1%, 2% and 3% saline solution showed no significant difference ($p<0.05$; 0.152907).

4. Discussion

With a tremendous growth of the food industry due to changes in lifestyle, high food availability, consumption and handling practices have increased the chance of food contamination (1). Gastroenteritis is one of the complications that arise from microbial contamination of foods (12). In general, the mean bacterial count for the fresh tiger nuts washed in tap water (1.95×10^6–1.59×10^7 cfu/mL) was higher compared to the mean bacterial count obtained for tap water only (7.08×10^5–1.18×10^7 cfu/mL), tiger nut washed in 1% saline (7.31×10^5–1.34×10^7 cfu/mL), 2% saline (4.0×10^4–1.13×10^7 cfu/mL) and 3% saline (1.11×10^5–9.62×10^6 cfu/mL). The bacterial load from the tiger nuts in tap water (1.95×10^6–1.59×10^7 cfu/mL) was unacceptable beyond 10^5 cfu/mL (13). The reason for the lower mean bacterial count of tiger nut in the 1%, 2% and 3% saline solutions may be due to the salt content which conferred antimicrobial activity by lowering the water activity of the medium. Thus, media caused efflux of water from the cytoplasm of the bacterial cells through their semipermeable membrane. As cells must maintain a suitable level of cytoplasmic water for effective functioning of cellular components, they achieve and maintain homeostasis by the active accumulation of ions, uptake or synthesis of compatible solutes. The energy expended in these activities reduces growth rate and eventually prevents growth. (14). The tap water only showed the presence of bacteria with counts in the range of 7.01×10^5-7.62×10^6 which correlated with findings by (15) who also reported the presence of coliforms. In their study, the concentrations of total heterotrophic bacteria were beyond the WHO recommendations between 1.24×10^4 and 5.4×10^5 cfu/mL.

In comparing the mean bacterial count for tiger nut in the three different saline solutions in NA, the mean bacterial count in 1% saline (6.23 $\log_{10}$ cfu/mL–7.19 $\log_{10}$ cfu/mL) was the highest followed by 3% saline (5.52 $\log_{10}$ cfu/mL–7.11 $\log_{10}$ cfu/mL) and then 2% saline (2.27 $\log_{10}$ cfu/mL–7.18 $\log_{10}$ cfu/mL). Also, in comparing the mean bacterial count for tiger nut in the three different saline solutions from MCA, the mean bacterial count in 1% saline solution (5.45 $\log_{10}$ cfu/mL – 7.25 $\log_{10}$ cfu/mL) was the highest followed by 2% saline (5.04 $\log_{10}$ cfu/mL–7.04 $\log_{10}$ cfu/mL) and then 3% saline solution (4.00 $\log_{10}$ cfu/mL–6.79 $\log_{10}$ cfu/mL).

Also, comparing the mean bacterial count for tiger nut in the three different saline solutions from MSA, the mean bacterial count in 1% saline (5.32 $\log_{10}$ cfu/mL–6.82 $\log_{10}$ cfu/mL) was the highest followed by 2% saline (4.00 $\log_{10}$ cfu/mL–6.87 $\log_{10}$ cfu/mL) and then 3% saline (0.00 $\log_{10}$ cfu/mL–6.99 $\log_{10}$ cfu/mL). The results above clearly showed that mostly, there was a decrease

in the mean bacterial count as the percentage of saline concentration increased from 1% to 3% in the three media. This finding concurs with a study conducted in 2016 even though on different substrates, the study focused on the "effect of salt reduction on the microbial composition and quality characteristics of sliced roast beef turkey". Four different salt concentrations were used for the study in a decreasing order of 2.5%, 2.0%, 1.5% and 1%. The results showed that 2.5% had the lowest mean aerobic count (5.29 $\log_{10}$ cfu/g) followed by 2.0% (6.23 $\log_{10}$ cfu/g), 1.5% (6.25 $\log_{10}$ cfu/g) and 1% (6.26 $\log_{10}$ cfu/g) (16). The mean bacterial count of *Escherichia coli* on MCA in all the 21 samples was 6.15 $\log_{10}$ cfu/mL which exceeded the acceptable limit of 10^2–10^3 cfu/mL) (17,18). The relatively high level of *Escherichia coli* is a reliable proof of faecal contamination of the tiger nuts suggesting the possible use of contaminated water sources for irrigation, which might have included water from drains, as practiced in some societies among developing countries including Ghana. The mean bacterial count of *Staphylococcus aureus* on the MSA in all the 21 samples was 6.18 $\log_{10}$ cfu/mL which also exceeded the acceptable limit of 10^2–10^3 cfu/mL. *Staphylococcus aureus* has been implicated in food spoilage and food-borne diseases, hence, the organism is of considerable interest in food hygiene. The ingestion of contaminated tiger nuts, particularly with the aforementioned pathogen poses health risks to the numerous consumers. This buttresses the assertion that the intake of contaminated food can cause diarrheal-associated illnesses with bacteria as one of the major causes of food-borne contamination (19).

5. Conclusion

The study provided a general overview of the bacterial quality of tiger nuts washed in tap water, 1%, 2% and 3% saline solutions. The study found that the microbial load for tiger nut in tap water was higher than that recorded for tap water only, followed by the 1%, 2% and 3% saline solutions respectively. The study also showed that most of the tiger nut samples were contaminated with high bacteria and species load that are implicated in both food spoilage and food-borne diseases. However, treating tiger nuts with saline solutions before consumption could reduce the bacterial load. To improve the microbial quality of tiger nut, proper farming methods, handling practices and treatment under hygienic conditions should be employed to reduce the occurrence of microorganisms, in order to promote the health of consumers.

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Authorship contribution

NEA, PA, and GA developed the research concept and designed the methodology, data analyses, interpretation and preparation of the manuscript. NEA carried out the sample collection and laboratory work, whilst PA and GA provided critical comments on the methodology and reviewed the manuscript. All authors read and approved the final manuscript before submission for publication.

Declaration of competing interest

The authors declare no competing interest from conception to finalisation of the study.

Data availability

Additional information or data related to the study shall be made available on request.

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Ethical approval and consent to participate

The study did not involve any human or animal testing. Ethical review was sought from the University of Health and Allied Sciences, Research and Ethics Committee (UHAS-REC) of the Institute of Health Research. Following approval of the research, the Institute issued the certificate number UHAS-REC A.1 [87] 22-23. The tiger nuts were purchased from vendors by convenience sampling, mimicking similar instances of patronage by the general public, whilst handling and hygienic practices were determined by empirical observations.

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