



Microbiological Evaluation of Ready-to-Drink Tigernut Drinks Sold within Port Harcourt Metropolis, Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author FSI designed the study and performed the statistical analysis. Author GKB wrote the protocol. Author NM wrote the first draft of the manuscript. Authors GKB and FSI managed the analyses of the study. Authors NM and GKB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Tigernut drink are made from tigernut tubers (*Cyperus esculentus* L.) and rich in nutrients. This drink is locally produced and widely consumed in Nigeria irrespective of social status. This study is aimed at evaluating the microbial quality and physicochemical property of tigernut drinks sold within Port Harcourt metropolis.

Methodology: Thirty (30) samples of freshly prepared and packaged tigernut drinks were randomly purchased from different vendors in five locations of Port Harcourt metropolis (Agip Estate, Abuja Campus (Uniport), Choba, Mile 1 and Mile 2 Markets). The samples were analyzed using standard microbiological and physicochemical methods. SPSS (Statistical Package for the Social Sciences) was used to analyze the data.

Results: Results obtained showed that the pH of the samples ranged from 4.2 to 4.6 while the total heterotrophic bacterial count ranged from 6.54-6.74 log₁₀ CFU/mL. Total fungal count of tigernut drinks ranged from 6.0-6.2 log₁₀ CFU/mL. A total of nine (9) bacterial genera namely

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Staphylococcus sp. (37.3%), *Escherichia* sp. (21.3%), *Salmonella* sp. (12%), *Pseudomonas* sp. (12%), *Klebsiella* sp. (4%), *Bacillus* sp. (4%), *Micrococcus* sp. (4%), *Enterobacter* sp. (2.7%) and *Corynebacterium* sp. (2.7%) were isolated from the samples. Six (6) fungal genera were also encountered in the drink sampled which include *Rhizopus* sp. (1.4%), *Saccharomyces* sp. (4.4%), *Aspergillus* sp. (30.9%), *Fusarium* sp. (26.5%), *Penicillium* sp. (30.9%) and *Candida* sp. (5.9%). The result revealed that *Staphylococcus* sp. had the highest percentage of occurrence (37.3%) followed by *E. coli* (21.3%), while *Enterobacter* sp. (2.7%) and *Corynebacterium* sp. (2.7%) recorded the least. Among the fungal isolates, *Aspergillus* sp. and *Penicillium* sp. had the highest percentage of occurrence (30.9%) whereas *Rhizopus* sp. had the least (1.4%). The results of this study revealed that all the samples from the five (5) locations were heavily contaminated with pathogenic microorganisms and found not suitable for human consumption based on the standard recommended by National Agency for Food and Drug Administration and Control (NAFDAC). NAFDAC stipulated that mesophilic aerobic count of locally prepared beverages should be < 5.0 log₁₀ CFU/mL.

Conclusion: The huge contamination recorded in all the samples irrespective of the location could be linked to poor hygienic levels during processing. Therefore, good manufacturing practices, public health enlightenment campaign and strict regulations from relevant agencies are recommended to avoid foodborne infections, diseases and possible deaths which could result from consumption of such contaminated tigernut drinks.

Keywords: Tigernut drinks; contamination; microbial quality; food safety; Port Harcourt.

1. INTRODUCTION

Tigernuts (*Cyperus esculentus* L.) is a perennial plant which has scaly rhizomes at its base which give rise to hard spherical tubers. Rush nut, yellow nut-grass, Zulu nut, chufa, water grass, Earth almond and edible rush are common names given to tigernut which is actually a tuber and not a nut [1,2]. The usefulness of tigernut tubers obtained from tigernut plant which include preparation of tigernut drink was comprehensively reported by [3]. Many people prefer tigernut drink to industrially-produced beverages such as 'Coca-cola', 'Fanta', 'Pepsi' etc because it is home-made, cheap, natural and the raw materials are sourced locally [4]. Tigernut drink is rich in energy, fat, starch, glucose, fibre and protein [5]. However, most soft drinks lack fibre, protein, minerals, vitamins and other essential nutrients. Instead, it contains refined cane sugars or corn syrups which is linked to obesity in children which could lead to type 2 diabetes, osteoporosis and very weak bones. Excess consumption of soft drinks over a long period could cause cancer, gallstones and teeth enamel erosion because of toxic substances such as benzene and phosphoric acid contained in most soft drinks. Interestingly, tigernut drink could be beneficial in managing and controlling type 2 diabetes since it contains natural sugar [6]. It has been opined nutrition-related disorders could be prevented by consuming beverages [7]. Tigernut drink also known as vegetable milk from tigernut tubers comes in different varieties. They

are natural tigernut milk, pasteurized tigernut milk, sterilized tigernut milk, ultra-high temperature tigernut milk and concentrated and condensed tigernut milk. Any of these products is recommended for those who experience milk allergies such as galactosemia and lactose intolerance [8]. A non-alcoholic drink obtained from tigernut tubers widely eaten raw by children, older persons and sportsmen in Nigeria, parts of West Africa and East Africa is known as tigernut beverage [9,10]. In Northern Nigeria, it is popularly known as 'kunu-aya' [11]. This product is white in colour, refreshing when it is chilled and consumed both in wet and dry season [12]. Some researchers refer this product as a milk substitute (phyto milk) or vegetable milk because it is obtained from plants such as tigernut, soybean, bambara nut and baobab which have a high protein content [8].

Non-alcoholic tigernut drink is rich in nutrients which encourages growth of microorganisms. It has a short shelf life which could be attributed to poor hygienic practices during preparation, packaging, storage and distribution which predisposes the product to microbial contamination [13]. Studies have shown that non-alcoholic drinks prepared at home using traditional methods or commercially by industries are predisposed to microbial contamination [14]. According to a study carried out by [15], no sample of commercially prepared tigernut beverage contained viable microorganisms but *Escherichia coli*, *Bacillus* spp., *Shigella* sp.,

yeasts and moulds were isolated from home-made tigernut beverage. Pandukur [12] reported the presence of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* sp. from tigernut beverage (kunu aya) sold in the University of Jos community, Plateau state, Nigeria.

Thousands of residents of Port Harcourt metropolis, consume local beverages especially tigernut drinks daily primarily to quench thirst as well as providing stimulatory effects rather than its food value. Currently, tigernut drinks are commonly produced and vended in various villages and cities due to its high demand by unsuspecting customers and less complication in its processing steps. The unregulated production of this drink has led to uncertainty in strict adherence to food safety protocols during the process chain which could result to food borne illnesses and diseases. Hence, there is urgent need to evaluate the microbiological quality of locally produced tigernut drinks vended in some parts of Port Harcourt metropolis. Therefore, the study was aimed at assessment of microbiological and physicochemical property of tigernuts sold within Port Harcourt metropolis, Rivers state, Nigeria.

2. MATERIALS AND METHODS

Thirty (30) freshly prepared and packaged tigernut drinks were randomly purchased from vendors in five (5) locations in Port Harcourt metropolis. The five locations were assigned different codes AGTN, ABTN, CHTN, M1TN and M2TN which represent Agip Estate, Abuja Campus (Uniport), Choba market, Mile 1 market and Mile 2 market, respectively. These locations can be found between Latitude 4°45'N and Latitude 4°55'N, and Longitude 6°55'E and Longitude 7°05'E [16].

2.1 Determination of pH of the Tigernut Drinks

The pH of the tigernut drinks was determined directly using a Pye Unikam pH meter at room temperature (28±2°C) after using standard buffers 4.0 and 7.0 pH to calibrate the pH meter.

2.2 Serial Dilution

Ten-fold serial dilution was carried out by transferring 1 ml of each sample of tigernut drink into a test tube containing 9 ml of peptone water using sterile pipette and mixed to obtain dilution 10⁻¹. One milliliter (1 ml) of dilution (10⁻¹) was then transferred into another test tube (10⁻²) containing 9 ml of peptone water. Using separate

1 ml pipette, these transfers were repeated until dilution 10⁻⁵ was achieved.

2.3 Microbiological Analysis

2.3.1 Total heterotrophic bacterial count of the tigernut drinks

Total heterotrophic bacterial counts involved the use of nutrient agar. Isolation of bacterial species involved the use of MacConkey agar, mannitol salt agar, Salmonella-Shigella agar and thiosulphate citrate bile-salt sucrose agar prepared according to manufacturers' instructions [17]. Aliquot of 0.1 ml of the 10⁻³ and 10⁻⁴ dilutions of samples of tigernut drink were inoculated in triplicates using sterile hockey stick i.e. spread plate technique as adopted by [18]. The culture plates were incubated at 37°C for 48 h. Thereafter, the mean counts of bacterial colonies for each sample in triplicate were taken and bacterial population were obtained and the results were expressed in colony forming units per milliliter (CFU/mL).

2.3.2 Isolation and maintenance of pure culture

Each bacterial colony was picked using a sterilized inoculating needle and streaked as a primary inoculant on nutrient agar plate and nutrient agar slant in Bijoux bottles. The culture plates were incubated at 37°C for 24 h whereas the slants were maintained inside the refrigerator at 4°C.

2.3.3 Characterization and identification of bacterial isolates

Bacterial isolates were characterized and presumptively identified based on their cultural and morphological characteristics followed by battery of biochemical tests and Gram staining using the methods described by [19,20,21]. The biochemical tests are indole, citrate utilization, Methyl Red (MR), Voges-Proskauer (VP), starch hydrolysis, hydrogen sulphide production, Triple Sugar Iron agar (TSI), motility, catalase and coagulase. Identification of bacterial isolates was accomplished by comparing the characteristics of the culture with known characteristics using the determinative schemes of [22].

2.3.4 Total fungal count of the tigernut drinks

An aliquot (0.1 ml) of the dilutions 10⁻³ and 10⁻⁴ of the tigernut drink samples were transferred aseptically into freshly prepared potato dextrose

agar (PDA) in triplicates in well labeled Petri dishes and incubated at room temperature ($28 \pm 2^\circ\text{C}$) for 5 days. Fungal growth on the culture plates were counted and average readings per sample was recorded accordingly. Fungal population was obtained and the results were expressed in colony forming units per milliliter (CFU/mL).

2.3.5 Identification of fungal isolates from the tigernut drinks

The fungal isolates were characterized and identified based on colonial morphology and microscopic characteristics. The microscopic morphology of the fungal isolates was determined by viewing their mycelia under the microscope using x40 objectives with lactophenol cotton blue stain. The morphology of the fungal isolates under the microscope was compared with the descriptions of [22] and [23].

2.4 Statistical Analysis

Microbial analysis was carried out in triplicates and the results obtained were expressed as Mean \pm Standard error of mean. SPSS (Statistical Package for the Social Sciences) was used to analyze the data. Both bacterial and fungal counts were converted to base-10 logarithm using the number of colony forming units per mL of tigernut milk drink samples (\log_{10} CFU/mL).

3. RESULTS AND DISCUSSION

Fig. 1 depicts the pH of tigernut drinks sampled from different locations. The results show that the pH of all the samples ranged from 4.2 to 4.6. The tigernut drink obtained from Agip Estate had the highest pH 4.6 whereas a similar product from Mile 1 market had the lowest pH 4.2. The pH of tigernut drinks also known as kunu-aya from five locations in Port Harcourt metropolis were within the range 4.2-4.6. Our result is in agreement with a similar study carried out by [11] which reported 3.5-4.5 as the pH of kunu aya samples sold in a university campus in Nigeria. They attributed acidic property of the drink to presence of particular species of lactic acid bacteria namely *Lactobacillus leichmanni* and *L. fermentum* which is involved in fermentation process. Low pH of the tigernut drinks might have favoured fungal growth and acidophilic bacteria deterioration. Since the tigernut drinks were acidic, it is an indication that spoilage had already commenced before the products were purchased from the

vendors irrespective of the fact that low pH generally inhibits the growth of pathogenic microorganisms.

The total heterotrophic bacterial counts (THBC) of the tigernut drinks from different locations is shown in Fig. 2. The results indicated that all the tigernut drink from different location were significantly contaminated. The total heterotrophic bacteria count of the sampled tigernut drinks ranged from 6.54 – 6.74 \log_{10} CFU/mL. The results obtained indicated that tigernut drink from Agip Estate had the highest THBC (6.74 \log_{10} CFU/mL) while samples from Mile 1 market had the lowest THBC (6.54 \log_{10} CFU/mL). The study revealed that total heterotrophic bacterial count of samples from Agip Estate, Mile 2 market, Abuja campus (Uniport), Choba market and Mile 1 market was 6.74, 6.73, 6.64, 6.60 and 6.54 \log_{10} CFU/mL, respectively.

All the samples of tigernut drink from the locations are not suitable for human consumption based on the standard recommended by National Agency for Food and Drug Administration and Control (NAFDAC) which stipulate that mesophilic aerobic count of locally prepared beverages should not exceed 5.0 \log_{10} CFU/mL [19]. According to [24], any milk sample containing 3.7 \log_{10} CFU/mL of bacteria is good for consumption, 4.00-5.60 \log_{10} CFU/mL is fairly good, 6.30 \log_{10} CFU/mL is manageable but when it is above 7.30 \log_{10} CFU/mL, it is bad for consumption. Since tigernut drink is a vegetable milk, it is manageable to consume the products purchased from the five locations. According to [25], total aerobic bacterial count of kunu-aya sold at Umaru Musa Yar'adua university campus, Katsina was within the range 4.34-6.15 \log_{10} CFU/mL. Result from this study is not in agreement with [9] reported lower bacterial count of kunu-aya obtained from four cardinal points of University of Maiduguri which range from 4.5-5.0 \log_{10} CFU/mL. Similarly, total fungal count of kunu-aya consumed by students of Kaduna State University which ranged from 2.26-2.86 \log_{10} CFU/mL as reported by [11] is lower than total fungal count (TFC) of tigernut drinks sampled from five locations within Port Harcourt metropolis.

The study revealed that total heterotrophic bacterial count of samples from Agip Estate, Mile 2 market, Abuja campus (Uniport), Choba market and Mile 1 market was 6.74, 6.73, 6.64, 6.60 and 6.54 \log_{10} CFU/mL, respectively. All the samples

of tigernut drink from the locations are not suitable for human consumption based on the standard recommended by National Agency for Food and Drug Administration and Control (NAFDAC) which stipulate that mesophilic aerobic count of locally prepared beverages should not exceed $5.0 \log_{10}$ CFU/mL [19]. According to [24], any milk sample containing $3.7 \log_{10}$ CFU/mL of bacteria is good for consumption, $4.00-5.60 \log_{10}$ CFU/mL is fairly good, $6.30 \log_{10}$ CFU/mL is manageable but when it is above $7.30 \log_{10}$ CFU/mL, it is bad for consumption. Since tigernut drink is a vegetable milk, it is manageable to consume the products purchased from the five locations. According to [25], total aerobic bacterial count of kunu-aya sold at Umaru Musa Yar'adua university campus, Katsina was within the range $4.34-6.15 \log_{10}$ CFU/mL. Result from this study is not in agreement with [9] which reported lower bacterial count of kunu-aya obtained from four cardinal points of University of Maiduguri which range from $4.5-5.0 \log_{10}$ CFU/mL.

The results show that TFC of $6.2 \log_{10}$ CFU/mL obtained in tigernut drink from Agip Estate was the highest while samples from other locations were within the range of $6.0-6.05 \log_{10}$ CFU/mL. Higher microbial load reported in this study compared with similar studies carried out by [11] and [25] could be attributed to tropical temperature which encourages growth and multiplication of bacteria, poor hygienic practices during preparation of the tigernut drinks, poor storage condition, use of untreated water and reusing of unsterilized plastic bottles as well as other utensils by local producers to package tigernut drink [4,9,12]. Due to poor shelf life of tigernut drink, between 2-24 h after the product has been prepared, it must be consumed especially when it is left in an environment which has a temperature within the range of $40-100^{\circ}\text{C}$ [11]. Since tigernut drink is preferable to consumers when chilled, dropping ice into the drink could contaminate it because ice is a potential source of microbial contamination upon melting [19]. Many hawkers/vendors of home-made tigernut drink in Port Harcourt metropolis may not offer consumers tigernut drinks that always meet this requirement due to inability to finish selling the drinks produced in a day and coupled with epileptic public power supply and non-addition of preservatives to the product. A study carried out by [4] revealed that laboratory prepared kunu-aya had fewer bacterial isolates and lower bacterial count than locally produced indigenous kunu-aya. Their results show that

Bacillus subtilis, *Streptococcus* sp. and *Shigella* sp. were absent in kunu-aya prepared in the laboratory but present in the samples bought from vendors.

The total fungal counts (TFC) of sampled tigernut drinks from different locations is shown in Fig. 3. The results indicated that the TFC of tigernut drinks was within the range of $6 \log_{10}$ CFU/mL to $6.2 \log_{10}$ CFU/mL. Highest TFC ($6.2 \log_{10}$ CFU/mL) was recorded from tigernut drinks from Agip Estate while the lowest TFC ($6 \log_{10}$ CFU/mL) was obtained in samples from Mile 1 market. Similarly, total fungal count of kunu-aya consumed by students of Kaduna State University which ranged from $2.26-2.86 \log_{10}$ CFU/mL as reported by [11] is lower than total fungal count (TFC) of tigernut drinks sampled from five locations within Port Harcourt metropolis.

Table 1 depicts the cultural and morphological characteristics of bacterial isolates from tigernut drinks. Biochemical characteristics of bacterial isolates from tigernut drinks is shown in Table 2. These results revealed that a total of nine (9) bacterial genera were isolated from tigernut drinks sold in the sampled locations. They include *Escherichia* sp., *Klebsiella* sp., *Pseudomonas* sp., *Bacillus* sp., *Micrococcus* sp., *Staphylococcus* sp., *Enterobacter* sp., *Corynebacterium* sp. and *Salmonella* sp. Table 3 depicts the colonial characterization and morphological identification of fungal isolates from tigernut drinks. A total of six (6) fungal genera namely *Rhizopus* sp., *Saccharomyces* sp., *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp. and *Candida* sp. were isolated from the tigernut drinks. Since tigernut is the main raw material used for preparation of tigernut drink, microbial contamination of tigernut tubers could influence the microbial quality of tigernut drink [26]. Majority of home-made tigernut tubers buy raw tigernut tubers from hawkers/vendors within their locality [27]. A study carried out by [28] reported that tigernut tubers sold in a city in Ghana were contaminated by *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas* spp., enterococci and staphylococci. Similarly, [10] reported the presence of bacteria namely *Bacillus* sp. (40%), *Escherichia coli* (7%), *Klebsiella* spp. (6%) and *Streptococcus* spp. (5%) and fungal genera namely *Aspergillus* spp. (25%), *Penicillium* spp. (21%), *Rhizopus* spp. (15%) and *Mucor* spp. (11%) from vendors/hawkers of tigernut tubers in Aba metropolis. Therefore, tigernut tubers purchased from vendors/hawkers should be

decontaminated by proper washing and surface sterilization before processing it into tigernut drink in order to drastically reduce microbial contamination of the drink. [29] examined several

ways tigernut tubers and tigernut-derived products could be contaminated and proffered various strategies the level of contamination could be reduced.

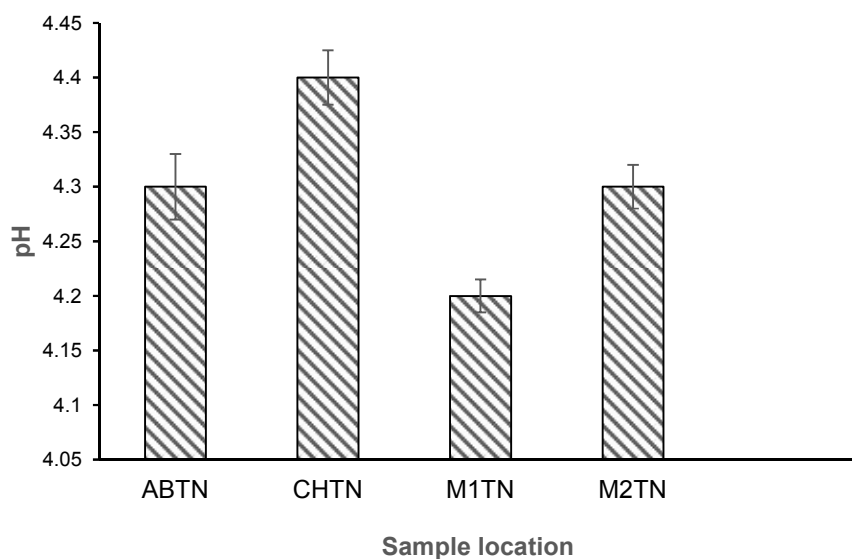


Fig. 1. pH of tigernut drinks sampled from different locations

Key: AGTN - Agip Estate, ABTN - Abuja Campus (Uniport), CHTN - Choba market, M1TN - Mile 1 market, M2TN - Mile 2 market

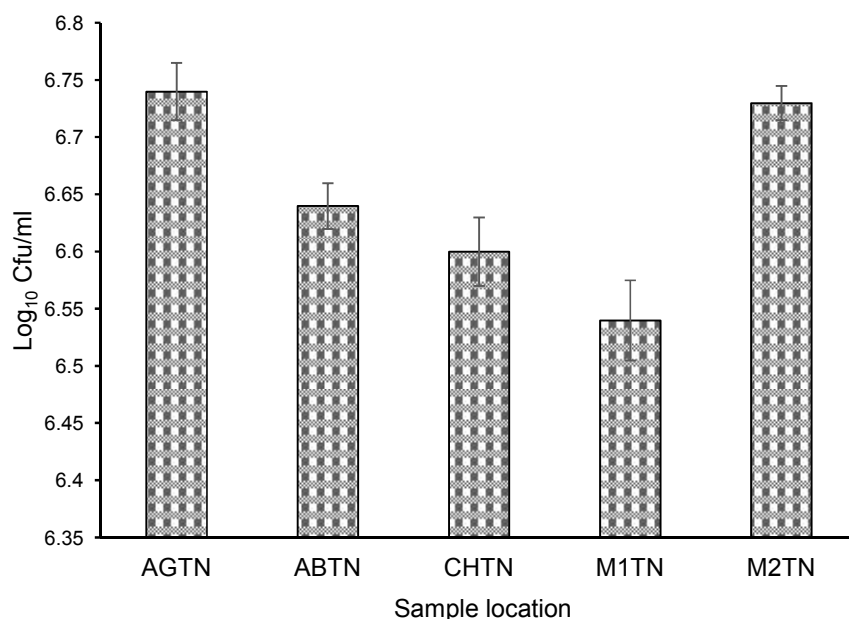


Fig. 2. Total heterotrophic bacterial count of tigernut drinks sampled from different locations

Key: AGTN - Agip Estate, ABTN - Abuja Campus (Uniport), CHTN - Choba market, M1TN - Mile 1 market, M2TN - Mile 2 market

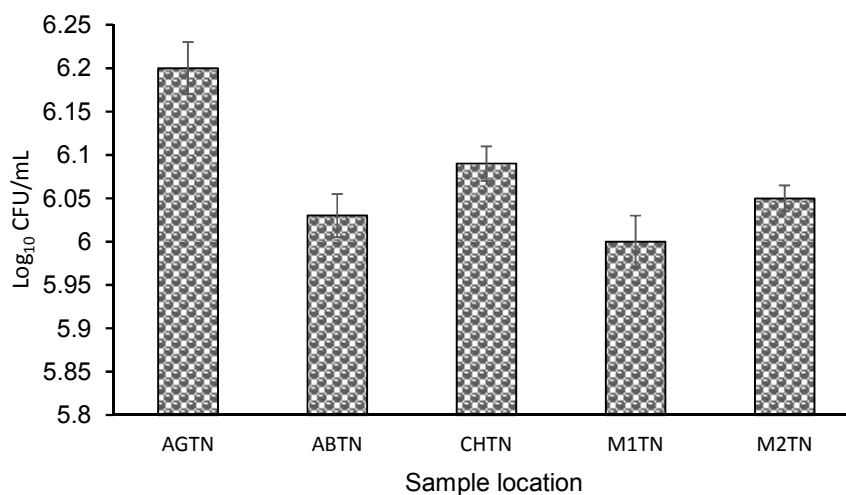


Fig. 3. Total fungal counts of tignut drinks sampled from different locations

Key: AGTN - Agip Estate, ABTN - Abuja Campus (Uniport), CHTN - Choba market, M1TN - Mile 1 market, M2TN - Mile 2 market

Table 1. Cultural and morphological characteristics of bacterial isolates from tignut drinks

Isolate code	Size	Opacity	Edge	Surface	Elevation
4	2 mm	Opaque	Entire	Mucoid	Raised
6	2 mm	Opaque	Entire	Mucoid	Raised
7	1 mm	Opaque	Entire	Mucoid	Raised
5	2 mm	Opaque	Entire	Mucoid	Flat
8	3 mm	Opaque	Entire	Mucoid	Raised
1	1 mm	Opaque	Entire	Mucoid	Raised
3	2 mm	Opaque	Entire	Mucoid	Raised
2	3 mm	Opaque	Serrated	Dry	Raised
9	2 mm	Opaque	Smooth	Mucoid	Raised

Table 2. Biochemical characteristics of bacterial isolates from tignut drinks

Isolate	Shape	Gram reaction	Citrate	Glucose	Oxidase	Lactose	TSI slant	Agar butt	H ₂ S	Gas	MR	VP	Indole	Catalase	Motility	Coagulase	Probable Organism
1	Rod	-	-	+	-	+	A	A	-	-	+	-	-	+	+	-	<i>Escherichia</i> sp.
2	Rod	-	+	+	-	+	A	A	-	+	-	+	-	+	-	-	<i>Klebsiella</i> sp.
3	Rod	-	+	+	-	+	B	B	-	-	-	+	+	+	-	-	<i>Pseudomonas</i> sp.
4	Rod	+	+	+	-	-	B	A	-	+	+	-	-	+	+	-	<i>Bacillus</i> sp.
5	Cocci	+	-	+	-	+	B	A	-	-	+	+	-	+	+	-	<i>Micrococcus</i> sp.
6	Cocci	+	+	+	-	+	A	A	-	-	+	+	-	+	-	-	<i>Staphylococcus</i> sp.
7	Rod	-	+	+	-	+	B	A	+	+	+	-	-	+	+	-	<i>Enterobacter</i> sp.
8	Rod	-	-	-	+	-	B	B	-	-	-	-	+	+	-	-	<i>Corynebacterium</i> sp.
9	Rod	-	+	+	-	-	B	A	-	+	+	-	+	+	+	-	<i>Salmonella</i> sp.

Key: MR - Methyl Red; VP - Voges-Proskauer; TSI - Triple Sugar Iron; A - acid; B - base

Table 3. Colonial characterization and morphological identification of Fungal isolates from tigernut drinks

Isolate code	Colonial features	Morphology	Probable organism
1	Whitish colonies growing rapidly and filling the petri dish with dense cottony mycelium and becoming brownish-black with age	Non-septate mycelia. Sporangiospores are smooth walled. Sporangia and columella are subglobose. Sporangiospores are ovoid in shape	<i>Rhizopus</i> sp.
2	Dense white budding colony separated by a brown line	Non septate hyphae with large sporangia heads having numerous sporangiospores	<i>Saccharomyces</i> sp.
3	Bluish green colonies with a suede like surface	Conidia heads are large, globose, dark-brown and biseriate, conidia are globose and rough walled. Conidiospores are smooth walled	<i>Aspergillus</i> sp.
4	Black colonies with white edges	Conidia heads are large, globose, dark-brown and biseriate, conidia are globose and rough walled. Conidiospores are smooth walled	<i>Aspergillus</i> sp.
5	Flat, creamy and mucoidy colonies	Branched conidiospores with smooth conidia but with a rough wall	<i>Fusarium</i> sp.
6	Green and velvety	Colonies are smooth and ellipsoidal. Conidiospores are smooth and short. Mycelia are arranged irregularly with branches of various lengths	<i>Penicillium</i> sp.
7	Pink and cottony colonies	Microconidia are ovoid in shape. Microconidia are borne on phialides on branched conidiospores, septate fusiform, slightly curved and pointed at both ends is present	<i>Fusarium</i> sp.
8	Yellowish and powdery colonies	Circular spore, no hyphae and conidia	<i>Candida</i> sp.

Fig. 4 depicts the frequency of occurrence of bacterial isolates from tigernut drinks of each location. The bacteria which had the highest percentage occurrence was *Bacillus* sp. (44%) isolated from tigernut drink obtained from Mile 1 market. In terms of bacteria with the lowest percentage occurrence in the tigernut drinks involved *Salmonella* sp. (6.3%) and *Bacillus* sp. (6.3%) from Abuja campus (Uniport); *Micrococcus* sp. (6.3%) and *Escherichia* sp. (6.3%) from Mile 1 market. The percentage frequency of occurrence of bacterial isolates from tigernut drinks shows that *Staphylococcus* sp. (43%), *Escherichia coli* (22%), *Corynebacterium*

sp. (14%), *Salmonella* sp. (14%) and *Micrococcus* sp. (7%) was associated with the drink sampled from Agip Estate. As for tigernut drink sampled from Choba market, it was *Pseudomonas* spp. (33%), *Escherichia* sp. (27%), *Staphylococcus* sp. (27%) and *Salmonella* sp. (13%). The percentage frequency of occurrence of bacterial isolates from tigernut drinks sampled from Abuja campus (Uniport) shows the following: *Salmonella* sp. (6%), *Staphylococcus* sp. (31%), *Escherichia* sp. (19%), *Klebsiella* sp. (13%), *Bacillus* sp. (6%) and *Pseudomonas* spp. (25%). In terms of percentage occurrence, *Staphylococcus* sp.

(19%), *Bacillus* sp. (44%), *Klebsiella* sp. (13%), *Micrococcus* sp. (6%), *Enterobacter* sp. (13%) and *Escherichia* sp. (6%) was associated with tigernut milks from Mile 1 market. The percentage occurrence of bacterial isolates from tigernut drinks shows that *Enterobacter* sp. (7%), *Staphylococcus* sp. (43%), *Salmonella* sp. (29%) and *Escherichia coli* (21%) was associated with the drink sampled from Mile 2 market.

The presence of pathogenic bacteria in the tigernut drinks in high population and sometimes irrespective of the population renders the drink unfit for human consumption. In a related study, [12] identified the presence of *Escherichia coli*, *Salmonella* sp. and *Staphylococcus aureus* in tigernut drink (kunu-aya) sold in the University of Jos community, Plateau state, Nigeria. Another study carried out by [26] reported the percentage occurrence of bacterial isolates from kunu-aya sold in Wukari, Nigeria which are *Klebsiella* spp. (60%), *Bacillus* spp. (60%), *Staphylococcus aureus* (70%), *Citrobacter* spp. (10%), *Salmonella* spp. (10%), *Proteus* spp. (20%), *Escherichia coli* (40%), *Pseudomonas* spp. (20%), *Shigella* spp. (10%), *Micrococcus* spp. (10%) and *Enterococcus* spp. (20%). The presence of these bacterial isolates in tigernut drink have serious public health implications in the sense that *Staphylococcus aureus* is implicated in several diseases in humans such as furuncles, carbuncles, erysipelas, folliculitis, cellulitis, scalded skin syndrome (toxemia), staphylococcal food poisoning and meningitis. Although, *Staphylococcus aureus* is part of normal flora of human body, it is also associated with food spoilage. Most of the tigernut drinks evaluated in this study were contaminated with *Salmonella* sp. This bacterium has been implicated in bacterial food poisoning, systemic fever and enteric fever. The presence of *Salmonella* sp. in the tigernut drinks except the samples from Mile 1 market and Agip Estate is a thing of concern because of its ability to survive refrigeration temperature during storage of the drink [12]. *Salmonella enteritidis* is a pathogen implicated in salmonellosis which is a classic food-borne disease. Severe cases of salmonellosis could lead to death [19]. *Klebsiella* spp., *Enterobacter*, *Pseudomonas*, Enterococci and *Staphylococcus* sp. are implicated in recurrent infections and structural abnormalities of the urinary tract [28]. The presence of coliforms such as *Escherichia coli* in non-alcoholic beverages is an indication of faecal contamination from humans, domestic pets, water, dirty utensils and environment. *E. coli*

constitute normal flora of intestine of vertebrates and human. It is implicated in gastroenteritis, diarrhea and urinary tract infection [30]. *Bacillus* spp. is a common contaminant of food which could be traced to food handlers, environment and post process contamination. This pathogen is known to cause *Bacillus* food borne intoxication [26]. Bacteremia/septicemia and endocarditis could be caused by some species of *Bacillus* [31].

The percentage occurrence of bacterial isolates in all the tigernut drink samples is presented in Fig. 5. As shown in the Fig. *Staphylococcus* sp. had the highest percentage occurrence (37.3%) followed by *Escherichia coli* (21.3%), *Salmonella* sp. (12%) and *Pseudomonas* sp. (12%) while the least were *Corynebacterium* sp. and *Enterobacter* sp. which had (2.7%).

Considering bacterial isolates from all samples, our study shows that *Staphylococcus aureus* recorded the highest frequency of occurrence (37.3%) followed by *Escherichia coli* (21.3%). This result partly agrees with [4] which reported that *S. aureus* (27.27%) and *E. coli* (27.27%) accounted for highest incidence of bacterial isolates from samples of tigernut drink (kunu aya). Highest frequency of occurrence of *S. aureus* in the tigernut drinks could be as a result of poor hygienic practices of the personnel handling the product which contaminated it with the bacterium known to be a normal flora of the skin, palms, hair, nose and mucus membrane. Twelve percent (12%) frequency of occurrence of *Salmonella* sp. and *Pseudomonas* sp. in the tigernut drinks poses a threat to public health. The source of *Salmonella* sp. in the tigernut drinks could be from humans and animal's body since this bacterium does not exist in other habitats. Transmission of *Salmonella* sp. which is an enteric pathogen is by consuming water or food contaminated with faeces or urine of human and animal carriers [12]. Sources of *Pseudomonas* sp. which contaminated the drinks could be from soil and water which inhabits the bacterium. The same percentage occurrence (4%) of *Klebsiella* sp., *Micrococcus* sp. and *Bacillus* sp. in the tigernut drinks was reported and could be attributed to unhygienic handling of the product in a dirty environment. The percentage occurrence of *Enterobacter* sp. and *Corynebacterium* sp. from all the tigernut drinks showed the same result (2.7%). The presence of *Enterobacter* sp. which is a fecal coliform could be linked to unhygienic environment and poor personal hygiene of those handling the products.

Corynebacterium sp. which is part of human microbiota is generally considered as a non-pathogenic bacterium until recently when it was linked to infections in immunocompromised patients. This bacterium found in soil, animal

feces, fruits and vegetables is implicated in food spoilage and contamination [32]. The presence of *Corynebacterium* sp. in tigernut drink could be traced to contamination of tigernut tubers with soil and animal feces in the field.

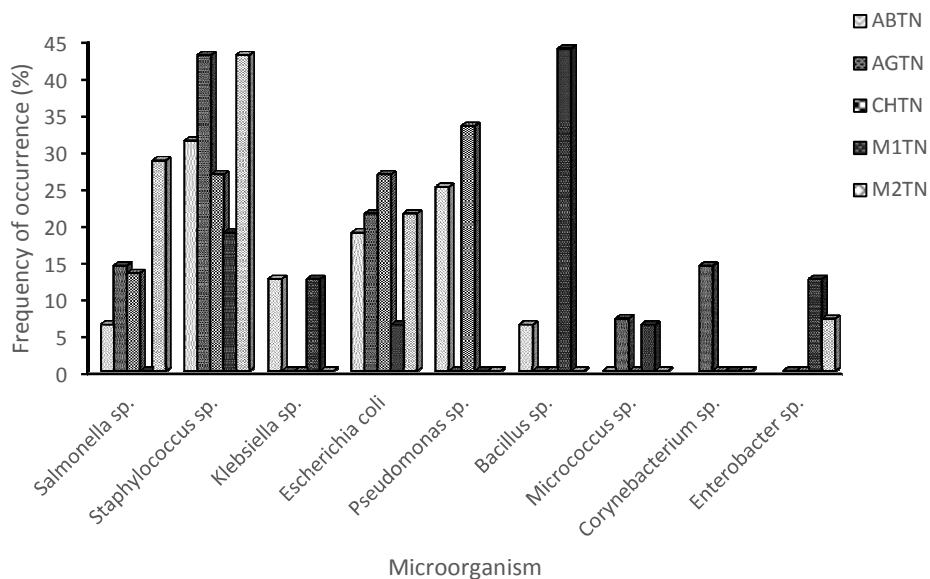


Fig. 4. Frequency of occurrence of bacterial isolates from tigernut drinks

Key: AGTN - Agip Estate, ABTN - Abuja Campus (Uniport), CHTN - Choba market, M1TN - Mile 1 market, M2TN - Mile 2 market

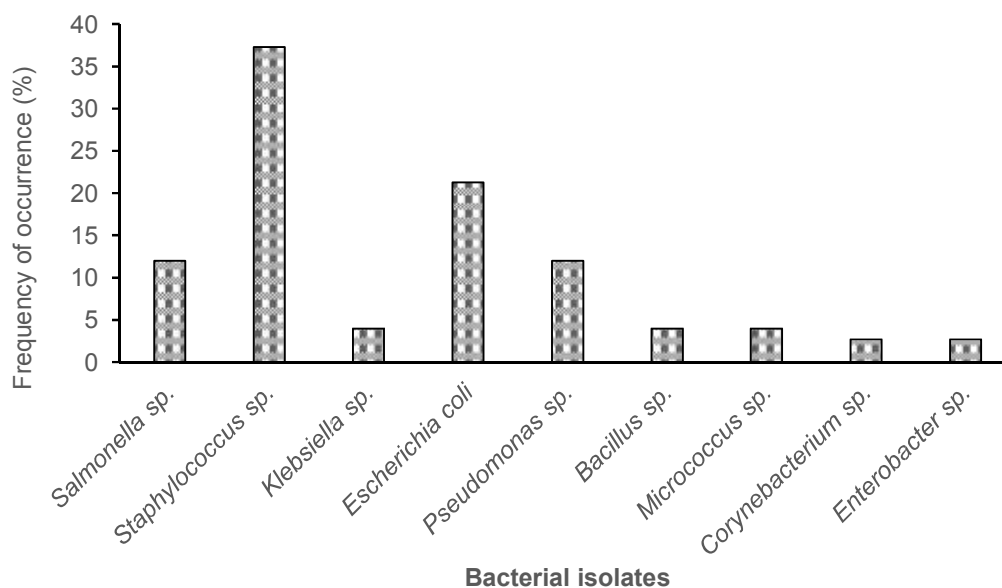


Fig. 5. Percentage occurrence of bacterial isolates in all the samples

The frequency of occurrence of fungal isolates from tigernut drinks of each location is depicted in Fig. 6. The result revealed that *Penicillium* sp. (46%) had highest percentage occurrence and was recorded in tigernut drinks from Agip Estate whereas *Saccharomyces* sp. had the lowest percentage occurrence (6%) which was obtained from Mile 1 market.

results presented in this Fig. indicated that *Penicillium* sp. and *Aspergillus* sp. had the highest percentage occurrence (30.9%), followed by *Fusarium* sp. with percentage occurrence of 26.5%. *Rhizopus* sp. had the lowest percentage occurrence (1.4%).

Fig. 7 shows the percentage occurrence of fungal isolates in all the tigernut drinks. The

The fungal isolates from all the tigernut drinks which recorded highest frequency of occurrence were *Penicillium* sp. (30.9%) and *Aspergillus* sp. (30.9%). This result is similar with research

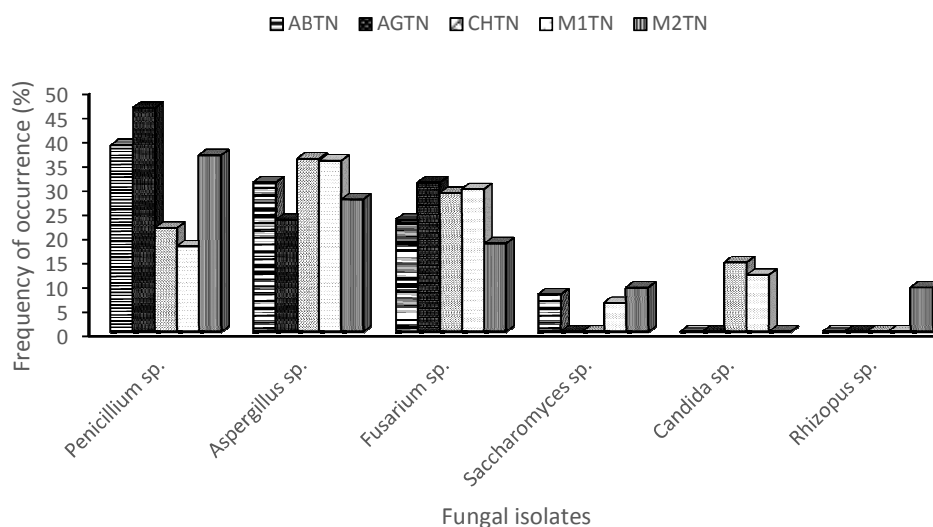


Fig. 6. Frequency of occurrence of fungal isolates from tigernut drinks
 Key: AGTN - Agip Estate, ABTN - Abuja Campus (Uniport), CHTN - Choba market, M1TN - Mile 1 market, M2TN - Mile 2 market

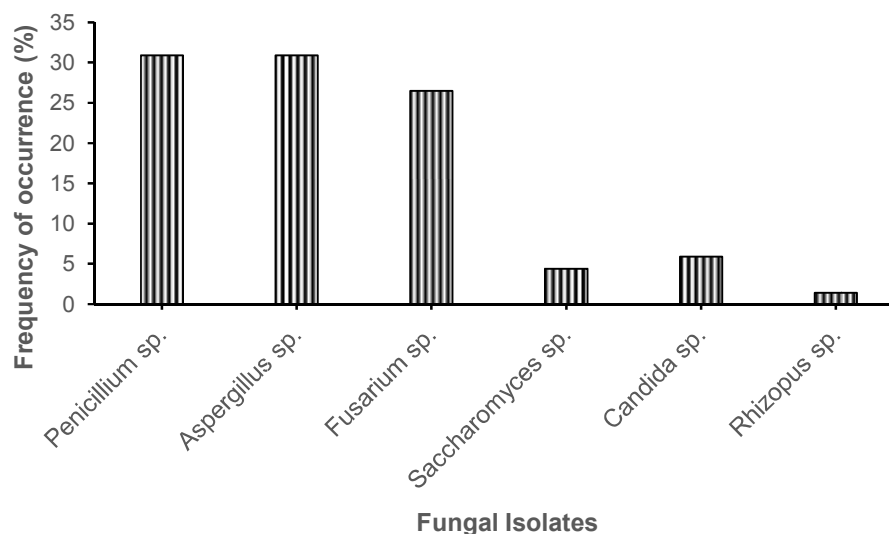


Fig. 7. Percentage occurrence of fungal isolates in all the samples

findings of [7] in a related study which reported *Aspergillus niger* (30.6%), *A. flavus* (22.6%), *Penicillium* sp. (27.4%) and *Saccharomyces cerevisiae* (19.4%) as percentage occurrence of fungal isolates from fresh samples of tigernut drink (kunu aya) obtained from four sites in Dutse, Jigawa state. Percentage occurrence of other fungal isolates from the tigernut drinks indicate that *Fusarium* sp., *Candida* sp., *Saccharomyces* sp. and *Rhizopus* sp. were 26.5, 5.9, 4.4 and 1.4%, respectively. The sources of the fungal isolates in the tigernut drinks could be from air, dust, packaging material and processing environment. In another related study, [9] reported *Candida albicans* (12.5%), *Saccharomyces cerevisiae* (37.5%) and *Rhizopus oryzae* (25.0%) as percentage occurrence of fungal isolates obtained from samples of kunu aya sold in different locations in University of Maiduguri, Nigeria.

The presence of pathogenic fungi in the tigernut drinks is of public health importance. *Penicillium* sp. (39%), *Aspergillus* sp. (31%), *Fusarium* sp. (23%), *Saccharomyces* sp. (8%) is the percentage occurrence of fungal isolates from Abuja campus (Uniport). The percentage occurrence of fungal isolates from tigernut drinks shows that *Penicillium* sp. (46%), *Aspergillus* sp. (23%) and *Fusarium* sp. (31%) were associated with the drink sampled from Agip Estate. *Candida* sp. (14%), *Aspergillus* sp. (36%), *Fusarium* sp. (29%) and *Penicillium* sp. (21%) isolated from tigernut drinks purchased from Choba market depicts the percentage occurrence of the fungal isolates. The percentage occurrence of fungal isolates from tigernut drinks obtained from Mile 1 market shows that *Saccharomyces* sp. (6%), *Penicillium* sp. (18%), *Candida* sp. (12%), *Aspergillus* sp. (35%) and *Fusarium* sp. (29%). As for tigernut drink sampled from Mile 2 market, the percentage occurrence of fungal genera was *Saccharomyces* sp. (9%), *Aspergillus* sp. (27%), *Penicillium* sp. (36%), *Rhizopus* sp. (9%) and *Fusarium* sp. (18%). In a related study, [7] isolated *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* spp. and *Saccharomyces cerevisiae* from samples of tigernut milk drink (kunu-aya) obtained from different locations in Dutse, Jigawa state. This result partially agrees with findings from this study. A wide range of temperature and pH could support fungal growth which can release mycotoxins responsible for mycotoxicosis in humans [5]. Consumption of tigernut drink containing mycotoxins could be hazardous to health as a result of

bioaccumulation of mycotoxins in the human body [7]. Certain food products such as corn, rice etc could be contaminated by mycotoxins released by *Penicillium*, *Fusarium* and *Aspergillus* species [31]. The presence of yeast in the samples of tigernut drink could add to the aroma, taste and flavour of the drink whereas spoilage of the product will definitely occur as a result of presence of moulds such as *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp. and *Rhizopus* spp. irrespective of their population in the product [30]. Moniliformin are toxins produced by *Fusarium* sp. Citrinin and cyclopiazonic acid are *Penicillium* toxins which could cause kidney disorder and Kodua poisoning, respectively [31]. *Aspergillus niger* has less tendency to cause human disease but other *Aspergillus* sp. could cause aspergillosis which is a serious lung disease. However, people could suffer pain in the ear, temporary hearing loss, and in severe cases, the ear canal and tympanic membranes could be damaged as a result of otomycosis commonly associated with *Aspergillus niger*. Humans could experience symptoms such as pulmonary oedema, vomiting, abdominal pain, convulsions, coma and death as a result of acute aflatoxicosis caused by *Aspergillus* sp. [31]. Those who have compromised immune system could suffer infection caused *Saccharomyces cerevisiae* which is an opportunistic pathogen. In healthy individuals, rarely will *S. cerevisiae* cause infection in any area it has colonized. The presence of *S. cerevisiae* in the vagina is linked to 1% of all vaginal yeast infections which manifest symptoms identical with yeast infections associated with *Candida albicans*. Allergy, asthma and some respiratory problems could be linked with citromycetin produced by *Penicillium glabrum*.

According to National Agency for Food and Drug Administration and Control (NAFDAC), *Escherichia coli* should not be present in all food samples [12,19]. Food Drug and Administration (FDA) states that any food containing *Bacillus* sp. above 10⁶ CFU/mL is an indication of active growth and proliferation which poses a potential hazard if the product is consumed [27]. Hence, all the sampled tigernut drinks from the various locations were not safe for consumption.

4. CONCLUSION

The result of the present study revealed that all the tigernut drinks sampled, harboured various types of pathogenic microorganisms. The total heterotrophic bacterial count and total fungal

count ranged from 6.54 to 6.74 log₁₀ CFU/mL and 6.0 to 6.2 log₁₀ CFU/mL, respectively. The study indicated that a total of nine (9) bacterial genera namely *Escherichia* sp. (21.3%), *Klebsiella* sp. (4%), *Pseudomonas* spp. (12%), *Bacillus* sp. (4%), *Staphylococcus* sp. (37.3%), *Enterobacter* sp. (2.7%), *Micrococcus* sp. (4%), *Corynebacterium* sp. (2.7%) and *Salmonella* sp. (12%) were isolated from tigernut drinks sampled from five locations within Port Harcourt metropolis. Six (6) fungal genera were equally encountered which include *Rhizopus* sp. (1.4%), *Saccharomyces* sp. (4.4%), *Aspergillus* sp. (30.9%), *Fusarium* sp. (26.5%), *Penicillium* sp. (30.9%) and *Candida* sp. (5.9%) The tigernut drinks were acidic and did not meet the requirements recommended by National Agency for Food and Drug Administration and Control (NAFDAC) in terms of mesophilic aerobic count. Specifically, *Penicillium* sp. and *Bacillus* sp. isolated from Agip Estate and Mile 1 market had the highest percentage of occurrence of 46% and 44%, respectively. Therefore, strict hygienic practices during production, handling and distribution of tigernut drinks, use of sterilized plastic containers, natural antimicrobial preservatives, potable water and proper storage conditions should be enforced by relevant regulatory bodies to maintain the general public health from consuming tigernut drink contaminated with pathogenic microorganisms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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