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Prevalence Of Intestinal Bacteria Species in Home Made Tiger Nut Drink Sold in Abakaliki

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Abstract
Tiger nut drinks are non-alcoholic drinks, locally produced and widely consumed in Nigeria irrespective of social status. This study evaluated locally produced tiger nut drinks sold in Abakaliki for bacteria species associated with the intestine as well as the nutritional stabilization using potato extract. One hundred and sixty (160) freshly prepared and packaged tiger nut drinks were randomly purchased from five different locations in Abakaliki and transported to the laboratory in ice parked cooler for analysis. The samples were analyzed using standard microbiological techniques. The results revealed the presence of; Lactobacillus plantarum, Lactobacillus modestisalitorans, Escherichia fergusonii80%) bacteria isolates. Out of the 213 bacteria isolates obtained from various tiger nut drinks, Lactobacillus plantarum was the most dominant 100 (46.9%) species. This was followed by Escherichia fergusonni 63 (29.6%) and Lactobacillus modestisalitoran 20(9.4%) isolates. Serratia marcescens 15 (7.0%), Salmonella spp 13 (6.1%) and Shigella spp 2(0.9%) were isolated. The findings of this study confirm that tiger nut drinks prepared under good hygienic condition contain probiotics and safe from intestinal pathogens.

Keywords: *tiger nut, intestinal bacteria species*

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I. Introduction

Tiger nuts (*Cyperus esculentus*) are an ancient food that have been consumed for thousands of years (Oke *et al.,*2019). They are believed to have originated from Egypt, and were used as a food source by ancient civilizations in Africa, the Middle East, and the Mediterranean. Tiger nuts were also consumed by prehistoric humans, as evidenced by the discovery of tiger nut remains in archaeological sites in Spain that date back more than 8,000 years (Oke *et al.,*2019). Tiger nuts have long been valued for their nutritional properties. Most growers of tiger nuts are the West African countries such as Nigeria, Senegal and Ghana. It can be eaten dry raw or roasted, it can also be transformed into tiger nuts milk. Tiger nuts are a good source of fiber, vitamins, and minerals, including potassium, magnesium, and iron (Musa and Hamza, 2013). They are also rich in healthy fats, such as oleic acid, which is believed to have potential health benefits, such as reducing inflammation and improving heart health (Sánchez-Zapata *et al*., 2012). Tiger nuts or yellow nuts edge, are small root vegetables that grow underground. They are typically small and round, with a hard, brown outer shell and a creamy white or yellow interior (Adel *et al*., 2015). Tiger nuts have a slightly sweet, nutty flavor and are used in various culinary dishes, such as desserts, drinks, and snacks. It is known in Nigeria as aya in Hausa, in Igbo as aki Hausa and in Yoruba as ofio (Akam *et al*., 2020). Three varieties are cultivated, black, yellow and brown. Among the three, only two varieties, yellow and brown are readily available in the market. The yellow variety is preferred to all other varieties because of its inherent properties like its bigger size, attractive color and fresher body. The yellow variety also yield more milk upon extraction and contains lower fats and more protein (Okafor *et* al., 2017). They live in moderate climate with a temperature between 20^0C–30^0C in all soil types except saline soil (Akam *et al*., 2020).

It has been reported that tiger nut has a lot of health benefits, it reduces the risk of colon cancer, used as a heart stimulant, has anti-inflammatory and anti-oxidant properties. They are rich in minerals, phosphorus, potassium, calcium, magnesium and iron. It is also rich in vitamin E and C and a good quantity of vitamin B1

(Chen.,2021). Tiger nut assists in stabilizing the central nervous system and helps the body to adapt to stress. It prevents constipation and diarrhoea. Vitamin C in tiger nut is a good antioxidant. Because tiger nut contains a high amount of vitamin B1, it assists in adjusting the central nervous system and encourage the body to adapt stress (Achoribo and Ong, 2017). Tiger nut was reported to be high in dietary fibre content, which is effective in the treatment and prevention of many diseases such as colon cancer, coronary health diseases, gastrointestinal disorders, obesity and diabetes (Achoribo and Ong, 2017). It is also reported to be an aphrodisiac carminative, diuretic, emmanagogue, and stimulant. It is used in the treatment of flatulence, indigestion and excessive thirst (Adejuyitan, 2011). Tiger nut has a golden brown color and a rich nutty taste, is one of the highest oleic acid contents that it has lipid and fatty acid profiles very similar to the olive oil. It has a high oleic acid and low polyunsaturated fatty acid and low acidity, which makes it excellent for the skin (Adejuyitan, 2011).

Tiger nut milk is very nutritive and serves as a good source of energy (Iboyi *et al*., 2021). It is a rich source of mineral such as iron, magnesium, and carbohydrates more than the cow's milk, it does not contain lactose, casein, sugar or proteins of the milk and therefore, an ideal drink for people who are lactose intolerance (Bamishaiye and Bamishaiye, 2011). Tiger nut contains vitamin E which delays cell aging, increases skin elasticity and helps relieve the appearance of wrinkles. Besides, it is essential for fertility in both men and women, heart stimulant, drank to heal serious stomach pain, it promotes normal menstruation (Oyetan and Oyetayo, 2021). It was also reported that tiger nut promotes bone health. Diets that are rich in calcium are associated with stronger bones. Calcium is essential for bone health and calcium helps prevent osteoporosis, a condition where bones are weak and brittle over time. In addition to promoting bone health, calcium also plays a role in blood clotting and muscle contraction. It is crucial for proper nerve function and heart rate regulation (Akam *et al*., 2020). The family Enterobacteriaceae includes gram-negative bacilli and belongs to Class Gammaproteobacteria. Members of the family Enterobacteriaceae are mostly nonpathogenic and present in the lower part of the intestine of their human and other mammalian hosts as commensals and symbionts (Victor *et al.,* 2020). They are part of the normal gut flora, providing by synthesizing vitamin K, which the hosts are unable to synthesize on their own. Nevertheless, some of them may become opportunists or pathogenic, causing diseases such as gastroenteritis, urinary tract infections, etc Oyetan and Oyetayo, 2021). Therefore, became necessary to evaluate locally produced tiger nut drinks in Abakaliki for its nutritional stability and the presence of these intestinal bacteria species.

II. Materials And Methods

Study Area

This study was carried out within Abakaliki Metropolis, in Ebonyi State Nigeria. Abakaliki is the Capital city of Ebonyi State located in the South eastern part of Nigeria. The state has a total land mass of 5,533km^3 and a population of over 1.8 million people, majority of whom are Igbo speaking people. It has a mean elevation of 400m above sea level. The indigenes of this area are predominantly farmers, with few traders and civil and public servants. They are leading producers of foods such as rice, yam, cassava, potatoes and others. Ebonyi has several mineral resources including lead, crude oil and natural gas, but few large scale commercial mining/mines. Ebonyi is called the "Salt of the Nation" for its huge salt deposit at Okposi and Uburu salt lakes. The towns and villages have their sources of water from boreholes, streams and ponds. Many fresh foods such as vegetables, tomatoes are grown around streams and ponds. English and Igbo are officially spoken, alongside other indigenous languages (Evelyn *et al.,* 2021).

Sample Collection

One hundred and sixty (160) freshly prepared and packaged tiger nut drinks were randomly purchased from five different locations Nwokpo, Mile 50, International Market, Kpiri Kpiri market and Azugwu. The samples were labelled and transported to the laboratory immediately on ice parked cooler in Abakaliki metropolis, Ebonyi state Nigeria. The samples were collected in sixteen (16) batches, with a total of ten (10) samples, with two samples from each location.

Acquisition and identification of tiger nut

Big yellow tiger nut seed (the most commonly used for preparation of tiger nut milk), was purchased from ogbe Hausa in Abakaliki , Ebonyi state Nigeria. It was identified and authenticated by a taxonomist in the Department of Applied biology, Ebonyi State University, Abakaliki.

Preparation of tiger nut

Tiger nut seeds were sorted to remove broken nuts, rotten nuts, stones, pepples and other dirty material rinsed in water to remove adhering soils. Other ingredients used in the milk preparation (coconut, date and ginger), were processed before use. The shell of the coconut was removed with knife, and the flesh of the

coconut cut into smaller pieces. The seeds of the dates were removed and discarded. The entire ingredients were thoroughly washed in warm water (Sandra *et al.,* 2018).

Tiger Nut Milk Preparation

Four thousand gram (g) of tiger nuts was soaked in 10 liters of boiled water at 60^{oc} for 6 hours. After washing, the nuts were mixed with 400g of coconut. Eight hundred gram (g) of date, Twenty gram (g) of ginger, and then the mixture was blended with 6 liters of cooled boiled water several times with engine motar. The mixture was pressed using muslin cloth to extract the milk. The extracted milk was transferred into sterile container.

Analysis of Tiger Nut Drinks for Bacteria Species

A loop full of the sample (tiger nut) was obtained using sterilized wire loop, and inoculated on XLD (Xylose lysine Deoxycholate) agar plate and MRS agar. Samples were cultivated in an incubator at the temperature of 37°C for 48 hours.

Cultural isolation and identification

The bacteria growth was characterized based on colonial morphology and biochemical characteristics. Streaking method was employed to isolate discrete colonies of bacteria from the mixed culture. Xylose Lysine Deoxycholate (XLD) and Man Rogosa and sharpes agars (MRS) were employed.

Gram staining

A loop full of normal saline was placed on a clean grease free glass slide. The wire loop was flamed, and a colony of the test organism was used to make a smear with the normal saline placed on the slide. The smear was allowed to air dry, after which it was heat fixed by passing it gently over the burnsen flame. The slide was flooded with crystal violet for 1 minute, and then rinsed with water. It was flooded with Lugol's iodine for 1 minute, and then rinsed with water. The slide was decolorized with acetone for few seconds, then rinsed with water. The slide was counter stained with neutral red for 2 minutes, and then rinsed with water. The slide blotted and allowed to air dry. After air drying, a drop of immersion oil was placed on the stained portion of the slide, and then be examined under the microscope using x100 objective lens as was previously described by Monica, (2009).

Biochemical identification

Biochemical test for identifying the enterobacteria was carried out in accordance with the standard procedure. Indole Test, Motility Test by Hanging Drop Method, Methyl Red Test (MR), and Triple Sugar Iron Test. All the biochemical tests were carried out according to standard microbiological procedures (CLSI, 2022).

III. Results And Discussion

Table 1: Bacteria specie isolated from tiger nut drinks (N = 213)

A total of 213 bacteria isolate were obtained from 152 samples out of 160 samples of Tiger Nut. The identified bacteria species include: *Lactobacilius plantarum, Escherichia fergusoni, Lactobacilius modestisalitoleran, Seratia marscenes, Salmonella spp* and *Shigella spp*

Bacteria Species	No Isolated	Percentage (%)
Lactobacilius plantarum	100	46.9
Escherichia fergusoni	63	29.6
Lactobacilius modestisalitoleran	20	9.4
Seratia marscenes	15	7.0
Salmonella spp	13	6.1
Shigella spp	2	0.9

From Table 2 we observed that all the samples from the five (5) different sampling locations; Azugwu, International market, kpirikpiri and Mile 50, were 100% contaminated, except samples collected at Nwokpo axis which were 80% contaminated. The association between bacteria contamination of tiger nut drink and location of production was statistically significant ($p < 0.001$) and for every one unit decrease in the rate of bacteria contamination of tiger nut, the chances of being due to location, decreases by 0.050 (Table **3**).

Table 2: Occurrence of Bacteria spp in Tiger Nut Drinks Sold in Abakaliki (n=160)

Sampling location	No of sample collected	Occurrence (%)	B-Coefficient	p-value
Nwokpo axis	40	32(80%)	-0.050	< 0.001
Kpirikpiri	20	20(100%)		
International market	40	40(100%)		
Mile 50	40	40(100%)		
Azugwu	20	20(100%)		

Total	**160**	**152 (95%)**	

From table 3 we observed that out of the 213 bacteria isolates obtained from various tiger nut drink sample, samples collected from international market yielded the highest bacterial isolates 54 (25.3%). This was followed by 49(23%) bacterial isolates obtained on samples from mile 50 axis. Also, 43(20.2%) bacteria isolates were obtained on samples from nwokpor axis, whereas 30(14.1%) and 37(17.4%) bacteria isolates each were obtained on samples collected from kpiri-kpiri market and Azugwu axis respectively. in the same vein, it was also observed that *Lactobacillus plantarum* 100(46.9%) was the most isolated bacteria species from all the tiger nut drink samples analysed. This was followed by *Escherichia fergusonii* 63(29.6%) isolates. *Lactobacillus modestisalitoran* 20(9.4%) isolates. Also, *Seratia marcescens* 15(7%) isolates, and *Salmonella* species 13(6.1%) isolates were obtained. We also observed that the least bacteria species isolated was *Shigella* species 2(0.9%).

Table 3: Distribution of bacteria species isolated from various tiger nut drink at different locations (n=213)

	No of Bacteria species isolated					
Sample location	*Lactobacillus plantarum*(%)	***L. modestisalitorans* (%)**	*Shigell*a spp (%)	*Salmonella* spp(%)	*E. fergusonii* (%)	*Serratia marcescens* (%)
Nwokpor (n=43)	30 (69.8%)	7(16.2%)	0(0)	0(0)	6(14%)	0(0)
Kpiri-kpiri (n=30)	12(40%)	4(13.3%)	0(0)	2(6.7)	9(30)	3(10)
Int'l market (n=54)	20(37%)	0(0)	0(0)	8(14.8)	16(29.6)	10(18.5)
Mile 50 (n=49)	23(46.9%)	5(10.2%)	2(4.1)	0(0)	19(38.8)	0(0)
Azugwu (n=37)	15(40.5%)	4(10.8%)	0(0)	3(8.1)	13(35.1)	2(5.4)
Total (213)	**100 (46.9)**	**20(9.4%)**	**2(0.9)**	**13(6.1)**	**63(29.6)**	**15(7.0)**

Keys; %= percentage

This study was carried out to evaluate locally prepared tiger nut drinks in Abakaliki for intestinal bacteria species, and nutritional stability. The statistical analysis revealed correlation between production factors and bacterial contamination of the tiger nut drinks. The result of the study revealed that both beneficial bacteria (*Lactobacillus plantarum* and *Lactobacillus modestisalitoleran*) and pathogenic bacteria (*Eschericha fergusoni*, *Salmonella* spp, *Serratia marscence* and shigella spp) were isolated from the samples from the five sampling locations. The result of bacteria isolates in the present study is similar to the result previously obtained by Obasi and Mani, (2023); Samuel *et al.,* (2020); and Adeyanju *et al.,* (2014) in that both studies isolated several bacteria species, but differs in the genera and species of bacteria isolated. The difference in the bacteria isolated in the two studies could be as a result of target interest. While the present study investigated for the presence of intestinal bacteria, the previous studies were open for all species of bacteria that could be implicated in tiger nut drinks. The presences of pathogenic bacteria have been reported on tiger nut drinks and are considered public health importance Samuel *et al.,* (2020). Also, the result of the present research is consistent with the findings of Nwadinma (2023), who isolated so many *Lactobacillus* species implicated with Tiger nut. In the present study, bacteria species was isolated (100% prevalence) on all the tiger nut samples analysed, except samples collected within Nwokpo axis which yielded 80% occurrence **(tab. 3).** This result is in agreement with the findings of Nwadinma (2023). This high prevalence of bacteria species on the tiger nut drinks could as a result of raw materials used in the preparation such as the source of water, processing methods and storage condition (Nwadinma, 2023).

In the present study, *Lactobacillus plantarum* 100(46.9%) was the most isolated bacteria species on the tiger nut drinks **(tab. 4).** This finding agrees with the result previously obtained and documented by Nwadinma, (2023), *Lactobacillus* bacteria, such as *Lactobacillus plantarum.* , and *Lactobacillus modestisalitoleran* have been reported in literature to have so many health benefits particularly for the gastrointestinal tract and immune system. They can help with reducing gastrointestinal infections, decreasing the risk of inflammatory bowel disease and stimulating the immune system (Igwebuike *et al.,* 2022); Nwadinma, (2022); and Maduka *et al.,* (2017). Additionally, they enhance food shelf life and improve the nutritional quality and flavor of food. They are also known for their anti-inflammatory, anti-carcinogenic and immunomodulatory properties (Adeojo, 2018). Of worthy of note is that *Lactobacillus plantarum* was the only bacteria species isolated from tiger nut washed with salt. This depicts *Lactobacillus plantarum* as a salt tolerant bacterium. This finding is consistent with the results previously obtained and documented in literature by several authors. Gambo and Da'u (2014); and Yao *et al.,* (2020) found that *Lactobacillus plantarum* could thrive in a salty environments, and is hence salt tolerance.

Also, *Escherichia fergusoni* 63(29.6%) was the second to the most isolated bacteria species in the present study. *Escherichia fergusonii* has not been isolated on tiger nut drinks, although *Escherichia coli* has been a reoccurring specie of the *Escherichia* in tiger nut drinks. Samuel *et al.,* (2020) and Obasi and Mani, (2023) found *Escherichia coli* as one the most implicated bacteria species on tiger nut drinks. The present of specie of the *Escherichia* in tiger nut drinks could be as a result of fecal contamination of the water used in preparation, or contamination of the tiger nut itself. Also, *Salmonella* spp, 13(6.1%) was isolated in the present study. The presence of *Salmonella* species in tiger nut has been reported in literature. Ire *et al.,* (2020); Obasi and Mani (2023); and Opeyemi and Obumneme (2020) found *Salmonella* species as one of the bacteria species implicated in tiger nut drinks. The present of *Salmonella* species in tiger nut drink could be as a result of fecal contamination of the, improper handling and lack of personal hygienic practices among personnel involved in the tiger nut preparation.

The local brands of bottled tiger nut drink evaluated in this study revealed that tiger nut drink has potential health benefits due to the presence of beneficial bacteria such as *Lactobacillus plantarum* and *Lactobacillus modestisalitoleran* which are probiotics that have the ability to tolerate stress factors like low high salt concentrations. Tiger nut drink is also susceptible to contamination with harmful pathogens such as *Eschericha fergusoni*, *Salmonella* spp*, Serratia marscence* and *Shigella* spp. The presence and type of bacteria can vary based on the factors like production methods, hygienic practices, and storage conditions. The quality of water used in preparing the drink can significantly impact the microbial load. Extended storage at room temperature promotes bacteria growth and increase the risk of spoilage. Improving hygienic practices and developing safer processing methods are crucial for ensuring the safety and promoting the consumption of this traditional Nigeria beverage.

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