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ASSESSMENT OF THE NUTRITIONAL AND MICROBIOLOGICAL QUALITY OF VENDED TIGER NUT (*Cyperus esculentus*) DRINK IN CHOBA, RIVERS STATE, NIGERIA

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ABSTRACT

The study was aimed at determining the nutritional and microbiological quality of tiger nut milk (Kunun-aya) sold by three vendors for a three-week period in Choba, Rivers State, Nigeria. Standard methods were employed in determining the proximate, mineral composition, total heterotrophic bacterial counts and presence of *Staphylococcus* and *Escherichia* species. Moisture content was consistently highest, ranging from 78.04±2.24 to 87.34±1.24, followed by carbohydrate (7.41±1.88 to 15.14±2.33) and the least was ash (0.11±0.03 to 0.26±0.14). The most abundant of the five minerals was potassium (K) with values ranging from 837.98±15.26 to 931.43±33.30, followed by sodium (Na) with values ranging from 587.39±15.58 to 663.78±34.65 and the least was iron (Fe) with values ranging from 8.50±3.57 to 13.44±3.47. Microbial counts results reveal the following for total heterotrophic bacteria (1.4 to 9.5×10^7 cfu/ml), *Staphylococcus* (1.6×10^3 to 5.5×10^4 cfu/ml) and *Escherichia* spp. (0 to 5×10^3 cfu/ml). The results obtained in this study has revealed the presence of valuable nutrient needed for healthy living as well as high microbial loads and presence of etiologic agents of human infections in vended tiger-nut milk sold in Choba. This underscores the need for the milk to be aseptically prepared to forestall the transmission of pathogenic bacteria to consumers.

Keywords: *Cyperus esculentus*, plant milk, nutrition, proximate composition, potassium

INTRODUCTION

Tiger nut (*Cyperus esculentus*) is a creeping perennial tuberous rhizome crop belonging to the sedge family, Cyperaceae which is often eaten raw as an unprocessed snack due to its rich flavour and texture or in its processed (roasted, dried, baked or made into a refreshing drink) forms (Musa and Hamza, 2013; Adenowo and Kazeem, 2020; Bazine and Arslanoğlu, 2020). Tiger nut is known by several names in Nigeria based on the various ethnic groups such as 'aya' (Hausa), 'aki-Hausa' (Igbo) and 'ofio' (Yoruba). (Bamishaiye *et al*., 2010; Adejuyitan, 2011; Ekeanyanwu and Ononogbu, 2010). It is an underutilized tuber comprising three cultivars; yellow, brown and black cultivars that are rich in many essential nutrients including proteins, carbohydrates, vitamins, minerals (Mohdaly, 2019), phytochemicals, oil and fibre, (Ihenetu *et al*., 2021). Sabah *et al*. (2019) reported the following proximate composition for carbohydrate (45.73%), protein (5.08%), lipid (30.01%), ash (2.23%) and crude fibre (14.80%) while Ismaila *et al*. (2020) reported a protein content of 7.90 and 10.25% in yellow and black cultivars, respectively. A 77.49 to 80.01% and 31.32 to 43.03 mg/100g of essential fatty acids and essential amino acids have also been reported in tigernut (Ijarotimi *et al*., 2018). The tuber is rich in disaccharide; D-saccharose, which yielded D-glucose, Dgalactose, D-xylose and D-arabinose upon hydrolysis (Marchyshyn *et al*., 2021). It is also rich in phosphorus and calcium (Roselló-Soto *et al*., 2019). Ismaila *et al*. (2020) reported calcium, sodium, phosphorus, potassium, iron, zinc and copper in both yellow and black cultivars while Ekeanyanwu and Onogbugbu. (2010) reported that all cultivars are rich in essential minerals such as calcium, magnesium, sodium, phosphorous, and potassium. Tigernut cultivars possessed different physicochemical properties (Ayo *et al*., 2016; Nina *et al*., 2019; Ayaşan *et al*., 2020) and functional properties (Nina *et al*., 2019; Ismaila *et al*., 2020). They are also rich in many endogenous hydrolytic enzymes including proteolytic and lipolytic enzymes (Owuama and Owuama, 2020).

Tiger nut milk (*Kunun-aya*), an aqueous extract of the tuber is a traditional non-alcoholic beverage blended with coconut (*Cocos nucifera*), date (*Phoenix dactylifera* L.), and spices [such as cloves (*Syzygium aromaticum*) and ginger (*Zingiber officinale* Roscoe)], and commonly consumed in Nigeria most especially in the Northern part of Nigeria and other West-African countries (Ibrahim *et al*., 2016; Kayode *et al*., 2017; Adesakin and Obiekezie, 2020). In Nigeria, the yellow and brown variants are readily available in the markets. The yellow variety is preferred to all other varieties because of its inherent properties like its bigger size and attractive colour. The Yellow variety also yields more milk upon extraction, contains lower fat and more protein and possesses less anti-nutritional factors especially polyphenols (Okafor and Nwachukwu, 2003). It is a good substitute for vegetarians and persons with lactose intolerance (Amponsah *et al*., 2017).

Microbial contamination of tiger nut which occurs during harvesting, storage, processing, packaging or retailing can have detrimental effects on the quality and safety of the end products (Maduka and Ire, 2019). According to Samuel *et al*. (2020) commercial samples of locally processed tigernut milk are unfit for human consumption due to poor personal hygiene that leads to microbial contamination; corroborating the assertion by Ayandele. (2015), that local drinks may serve as vehicles for food-borne diseases or pathogens. A number of authors have reported the presence of *Escherichia coli, Staphylococcus aureus, Salmonella* sp., *Klebsiella* sp., *Proteus* sp., *Pseudomonas* sp., *Bacillus* sp., *Micrococcus* sp., *Streptococcus* sp., *Enterobacter* sp., *Corynebacterium* sp., *Shigella, Acinetobacter* sp., *Enterobacter* sp., *Neisseria* sp., *Vibrio* sp., *Aeromonas* sp., *Saccharomyces cerevisiae, Rhizopus oryzae, Candida albicans, Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp. in locally prepared commercial tigernut milk (Ibrahim *et al*., 2016; Badau *et al*., 2018; Ire *et al*., 2020; Adesakin and Obiekezie, 2020).

Tiger nut milk is among the unregulated artisanal drinks popularly consumed and sold on Nigerian streets usually in pre-used polyethylene terephthalate (PET) bottles of soft drinks and water containers (Ntukidum *et al*., 2020; Nwaiwu

et al., 2020). There is no processing step to destroy any microorganism from the tubers and during the processing of the milk drink, which could result in short shelf life and public health concerns. Therefore, the study was aimed at assessing the nutritional and microbiological safety of tiger nut drink sold by three vendors in Choba, Port Harcourt, Nigeria.

MATERIALS AND METHODS

Samples collection

Forty samples of ready-to-drink tiger nut drinks were purchased over a three-week period in September, 2023 from three designated vendors [1 (15 samples), 2 (15 samples) and 3 (10 samples)] in Choba, Rivers State and taken to the Department of Microbiology Laboratory, University of Port Harcourt, Port Harcourt for analysis.

Proximate analysis of tiger nuts milk

Standard methods described by AOAC. (2010) were used to determine the moisture, crude protein, total ash, crude fibre, and carbohydrate contents. Carbohydrate content of the samples was determined by difference method.

Determination of minerals content of the samples

The concentrations of calcium, magnesium, potassium, sodium and iron were determined using the spectrophotometric method of the Association of Official Analytical Chemists (AOAC, 2010).

Enumeration for total heterotrophic bacteria, *Escherichia coli* and *Staphylococcus* spp. count

Ten milliliters (10 ml) of each sample were aseptically transferred into 90 ml of sterile peptone water, followed by ten-fold serial dilution. Aliquot (0.1ml) of appropriate dilutions were spread plated on nutrient, eosin methylene blue and mannitol salt agar in duplicates. The plates were incubated at ambient temperature (29±2ºC) for 18 to 24h. Discrete colonies on the nutrient agar were counted while colonies on the selective media were counted, purified on freshly prepared medium and stored in slants for confirmation. They were confirmed on the bases of their physiological (Gram's staining) and biochemical characteristics (motility, methyl-red/Voges Proskauer, indole, citrate, oxidase, catalase and coagulase).

Statistical analysis

The data obtained were subjected to Statistical Package for the Social Sciences (SPSS) ver. 25.0 (IBM, New York, USA) program. The data is given as mean ±SEM.

RESULTS AND DISCUSSION

Proximate Composition

The results of the average percentage proximate composition for composite sample from the three Vendors over a 3-week period is presented in Table 1. Moisture content was consistently highest, ranging from 78.04±2.24 to 87.34±1.24, followed by carbohydrate, 7.41±1.88 to 15.14±2.33 and the least was ash, ranging from 0.11±0.03 to 0.26±0.14. The main cause of tiger nut milk's high moisture content is the considerable quantity of water used to extract juice from crushed tiger nut tubers. The values obtained in this study are generally comparable to ranges previously reported by several authors for moisture (62.8±.0.00 to 86.51±2.10), protein (0.71±0.011 to 3.34±0.42) fibre (0.18±0.04 to 5.20±0.00), ash (0.03±0.03) carbohydrate (7.20±2.05 to 17.87±0.84) and lipid (1.95±0.00 to 21.50±0.00) in tiger nut milk (Kunun Aya) sold in Benin City, Edo State, Awka in Anambra State, Kaduna Metropolis in Kaduna and Port Harcourt Metropolis in Rivers State, Nigeria (Maduka *et al*., 2022; Ejimofor *et al*., 2023; Musa and Hamza, 2023; Obadesagbo *et al*., 2023). There was however, marked difference in the values of 8.50 to 21.50% lipid reported by Musa and Hamza (2023).

Table 1: Percentage proximate composition of tiger nut drinks from three vendors

Vendors	Moisture	Lipid	Protein	Ash	Carbohydrate	Fibre
1	85.45±1.64	2.48±0.85	1.21±0.24	0.26±0.14	7.41±1.88	3.19±1.55
2	87.34±1.24	1.66±0.63	1.49±0.29	0.11±0.03	7.61±0.65	1.79±0.42
3	78.04±2.24	2.78±0.75	1.62±0.29	0.26±0.12	15.14±2.33	2.16±0.72

Data are expressed as mean ±SE values, representing means of three determinations from each vendor.

The percentage occurrence of the proximate parameters of the tiger nut milk (Carbohydrate>fibre>lipid>protein>ash) was almost the same with the proximate composition of tiger nut tubers (Carbohydrate>lipid>fibre>protein>ash) but for the alternation of fibre and lipid as seen in reports by Madaki *et al*. (2018), Suleiman *et al*. (2018) and Sabah *et al*. (2019). The low protein in tiger nut milk may not be unconnected with the fact that a large portion of the proteins in cereals and other crops are often found in the testa and germ, which are typically sieved removed during processing (Khanum *et al*., 2001). The tiger nut milk samples in this study have a high percentage of carbohydrate and fiber, indicating a high energy content, making them a more energetic beverage (Musa and Hamza, 2013). It has been posited that continued intake of tiger nut milk with high crude fibre content aids digestion (Oke *et al.,* 2019). In this study the proportional relationship between ash content and available mineral was not established as the ash content was the least for samples from all three vendors (Musa and Hamza, 2013; Ogo *et al*., 2018).

Mineral Composition

Minerals play important roles in human metabolism; hence, they are required in reasonable quantity in daily diet. (Ejimofor *et al*., 2023). The results of the average mineral composition (ppm) for composite sample from the three Vendors over a 3-week period is presented in Table 2. The most abundant of the five minerals was potassium (K) with values ranging from 837.98±15.26 to 931.43±33.30, followed by sodium (Na) with values ranging from 587.39±15.58 to 663.78±34.65 and the least was iron (Fe) with values ranging from 8.50±3.57 to 13.44±3.47. In the body, potassium conducts electricity along with sodium chloride, calcium, and magnesium that were readily available in tiger nut milk. Potassium is essential for cardiac function and modulates smooth and skeletal muscle concentration (Ejimofor *et al*., 2023). There is need to fortify tiger nut milk with iron which is necessary for growth and development because of its central roles in supporting enzyme systems, aiding in immunity, and transporting and storing oxygen (Ejimofor *et al*., 2023).

Table 2: Mineral composition (ppm) of tiger nut drinks

Vendors	Mg	Na	K	Fe	Ca
1	448.05±92.98	600.10±26.40	847.62±16.04	8.50±3.57	28.60±10.52
2	371.19±12.54	663.78±34.65	931.43±33.30	13.44±3.47	13.45±4.74
3	407.63±25.03	587.39±15.58	837.98±15.26	9.63±1.83	46.81±21.39

Data are expressed as mean ±SE values, representing means of three determinations from each vendor.

There is paucity of information on the determination of mineral content of tiger nut milk, unlike the tuber. The high potassium and low iron content (mg/100g) of the drink is consistent with the values report for tuber in comparison with the determined minerals ranging from 259.27±1.78 to 1225.50±8.72 when compared to magnesium (8.32±0.52 to 122.00±0.93), sodium (3.10±0.22 to 218.04±3.14) iron (2.82.00±0.00 to 8.52±0.54) and calcium (0.65±0.23 to 100.0±2.65) (Ekeanyanwu and Ononogbu, 2010; Madaki *et al*., 2018; Suleiman *et al*., 2018; Maduka *et al*., 2022; Omeje *et al*., 2022). This was also the trend in the only available report for tiger nut milk/drink reported by Kanu and Obi (2021) who report the following values (mg) for potassium (349.90), magnesium (169.92), calcium (40.05), sodium (26.26) and iron (4.22) in sample purchased at an Ekwulobia market in Aguata Local Government Area, Anambra State.

Potassium content was also the highest when Madaki *et al.* (2018) compare the contents of potassium (1225.50±8.72/38600.46±32.62), sodium (218.04±3.14/139.53±2.71), iron 6.15±0.70/10.90±0.09, copper (0.40±0.23/0.45±0.12), zinc (4.10±0.20/7.85±0.34) and calcium (0.65±0.23/0.00±0.00 mg/100g), respectively in raw and processed tiger nut obtained from Bosso market Minna, Niger State.

According to Akram *et al*. (2020), sodium which was next in quantity to potassium plays a role in the control of blood but only low amount in tiger nut milk according to Opeyemi and Obuneme. (2020) makes the product suitable for patients suffering from diabetes and hypertension).

Magnesium which was third highest among the minerals determined functions as a cofactor for enzymes, aiding in the synthesis of RNA and DNA, maintenance of bone growth and also controls cholesterol levels produced by the human body (Akram *et al*. 2020; Ejimofor *et al*., 2023). The magnesium recorded in this study is above the 310 - 320 and 400 - 420 mg/day recommended dietary allowance (RDA) for healthy adult females and males, respectively (Ire *et al*., 2020b). Calcium was also available in tiger nut milk. It is vital for adequate growth and development of bones as well as clotting of blood, functioning of the nerve and muscle contraction in humans. The standard error values of samples from the same vendor in the present study is indicative of lack of standard in the preparation of the tiger nut milk.

Microbial counts

Like other raw materials, contamination which can occur during harvesting, storage, processing, packaging or retailing of tiger nut can have detrimental effects on the quality and safety of the end products (Maduka and Ire, 2019). Storage of tiger nut milk presents a number of difficulties, most likely due to fermentation and microbiological activity (Akakpo *et al*., 2019). The results of the total heterotrophic bacteria (THB), *Escherichia coli* and *Staphylococcus aureus* counts are presented in Table 3. The THB count from all three vendors ranged from 1.4 to 9.5×10^7 cfu/ml (7.15 to 7.98 $\log_{10}$cfu/ml). The values in this study are comparable with the 1.3 to 2.2×10^7 cfu/ml reported by Ogodo *et al*. (2018) in tiger nut milk (Kunun-Aya) sold in Wukari, Taraba State, Nigeria. The values were, however, higher than the range of 1.04×10^2 to 1.59×10^6 cfu/ml reported by several authors (Ikpoh *et al*., 2013; Musa and Hamza, 2013; Adesakin and Obiekezie, 2020; Ire *et al*., 2020; Ntukidem *et al*., 2020; Okwelle, 2020; Victor-Aduloju *et al*., 2020; Olofu *et al*., 2021; Ejimofor *et al*., 2023) but lower than the 1.35×10^9 to 2.8×10^{10} cfu/ml (Eke-Ejiofor and Awaji, 2018; Maduka et al., 2022).

Table 3: Total heterotrophic bacteria, *Staphylococcus* and *Escherichia* counts

Vendors	THB/Bacteria	Range of bacterial count (Cfu/ml)		
		Week 1	Week 2	Week 3
Vendor 1	THBC	ND	$1.4 - 9.5\times10^7$	$1.7 - 4.5\times10^7$
	Escherichia	$0 - 8\times10^2$	$0 - 1.4\times10^3$	$7\times10^2 - 5\times10^3$
	Staphylococcus	$1.4 - 3.9\times10^4$	$2.1 - 8.0\times10^3$	$5.6 - 7.5\times10^3$
Vendor 2	THBC	ND	$1.6 - 5.5\times10^7$	$2.6 - 5.6\times10^7$
	Escherichia	$0 - 1.5\times10^3$	$0 - 2.0\times10^3$	$1.1 - 1.2\times10^3$
	Staphylococcus	$1.6 - 5.5\times10^4$	$3.3 - 9.5\times10^3$	$7.2 - 8.4\times10^3$
Vendor 3	THBC	ND	ND	$1.8 - 6.4\times10^7$
	Escherichia	$0 - 1.6\times10^3$	ND	$0 - 1.4\times10^3$
	Staphylococcus	$2.6 - 5.4\times10^4$	ND	$1.6 - 9.3\times10^3$

ND= Not determined; THBC= Total heterotrophic bacteria count

The *Staphylococcus* counts ranged from 1.6×10^3 to 5.5×10^4 cfu/ml (3.20 to 4.74 $\log_{10}$cfu.ml). The recorded values in this study are within the range of 1.2 to 7.0×10^4 and 4.0 to 8.0×10^4 cfu/ml reported by Adesakin and Obiekezie. (2020) and Ogodo *et al*. (2018), respectively in tiger nut samples sold at Keffi, Nasarawa State and Wukari, Taraba State. The values were, however, lower than the range of 1.44 to 2.61×10^5 cfu/ml reported by Okwelle. (2020) in tiger nut sold in Ignatius Ajuru University of Education, Port Harcourt, Nigeria.

The least counts obtained in this study were those of *Escherichia coli*; with counts ranging from 0 to 5×10^3 cfu/ml (0 to 3.70 $\log_{10}$cfu/ml). This finding is comparable with the 0 to 3.3×10^3 and 1.0 to 5.0×10^3 cfu/ml of total coliform reported by Ntukidem *et al*. (2020) and Ikpoh *et al*. (2013), respectively in tiger nut milk Sold in Uyo Metropolis of Akwa Ibom State and Calabar metropolis, Cross River State, Nigeria, respectively but lower than the range of 0.8 to 6.6×10^4 and 1.54 to 3.10×10^5 cfu/ml reported by Adesakin and Obiekezie. (2020) and Okwelle. (2020), respectively. The *Escherichia* counts in this study were also lower than the total

fecal count of 1.0 to 11.0×10^4 and 1.15 to 5.13×10^4 cfu/ml reported by Adesakin and Obiekezie. (2020) and Eke-Ejiofor and Awaji. (2018), respectively.

The high total heterotrophic bacteria count and presence of *Staphylococcus aureus* and *Escherichia coli* in the examined samples were indicative of high level of bacterial contamination and could be a matter of serious public health concern and making the products unfit for human consumption. According to Okereke. (2015), the complete absence of harmful bacteria such as *Escherichia coli* and *Staphylococcus aureus* is necessary to meet the required official level for microbiological contamination of beverages or sorrel drinks. *Staphylococcus aureus* has been implicated in food borne intoxication and septic arthritis in humans (Taiwo *et al*., 2017) while a strain of *E. coli* [enterohemorrhagic *E. coli* (EHEC)] causes haemorrhagic diarrhea which can result to serious health conditions as kidney failure and even death (Ogodo *et al*., 2018). These organisms as well as other pathogenic bacterial species and strains have been reported previously by several authors in tiger nut milk and in high number (Ikpoh *et al*., 2013; Ogodo *et al*., 2018; Adesakin and Obiekezie, 2020; Okwelle, 2020). The commercial tiger-nut milk samples may have been contaminated with these organisms through the use of unsafe water, equipment used in processing and storage, handlers, or the environment due to inadequate sanitation and hygiene practices.

CONCLUSION

The result obtained in this study has revealed the presence of valuable nutrient needed for healthy living as well as high microbial loads and presence of etiologic agents of human infections in vended tiger-nut beverage sold Choba. Awareness of the importance of observing good personal hygiene and implementation of good manufacturing practices during preparation and storage will improve the safety of traditionally processed tiger nut (Kunun-aya) drink.

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