Assessment of Microbiological and Nutritional Quality of Some Local Brands of Tigernut-Milk Sold in Benin City, Nigeria

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ABSTRACT

The quality of tigernut milk consumed by the general public could impact human health. Thus, this study is aimed at evaluating the level of nutrients and microbial contamination of some local brands of tigernut milk available in Benin city. A total of fifteen (15) samples of tigernut milk were randomly sampled. They were subjected to microbiological and proximate analysis; minerals and vitamins contents were also determined using Standard methods. Total heterotrophic bacterial count and total fungal count of the samples was within the range $1.35\pm0.026 \times 10^9$ - $6.50\pm0.040 \times 10^9$ and $1.85\pm0.066 \times 10^7$ - $7.95\pm0.076 \times 10^7$ CFU/ml, respectively. There was no significant difference ($p>0.05$) in the crude protein (0.71 ± 0.011 - 0.75 ± 0.048 %) and ash content (0.30 ± 0.03 - 0.44 ± 0.07 %) among the samples. The highest quantities of Ca, K, Mg, Na, Mn, P, Fe and Cu encountered in the tigernut milk was 6.79 ± 0.51, 259.27 ± 1.78, 8.32 ± 0.52, 3.10 ± 0.22, 0.49 ± 0.15, 47.10 ± 0.31, 8.52 ± 0.54 and 0.03 ± 0.01 mg/100 g, respectively. Gross energy, vitamin A and C content of the tigernut milk was within the range 73.87 - 114.58 Kcal, 20.47 ± 0.44 - 28.20 ± 0.28 µg 100^{-g} and 5.37 ± 0.42 - 7.76 ± 0.39 mg 100^{-g}, respectively. In summary, the samples of tigernut milk contain reasonable quantities of nutrients which could be beneficial to human health, but the products were contaminated by microorganisms. Therefore, pasteurization of tigernut milk, good manufacturing practices (GMP) and good hygienic practices (GHP) are recommended to ensure that the products are safe for human consumption.

Keywords: *Non-dairy milk, Cyperus esculentus, Artisanal drinks, Vitamins, Minerals*

1. INTRODUCTION

Tigernut milk is a good substitute to cow milk. Other plant-based milk which is an alternative to cow milk include soymilk, oat milk, almond milk, quinoa milk, hazelnut milk, cashew milk, rice milk, sesame milk, Brazil nut milk and almond milk-like beverage (Sethi *et al*., 2016; Silva *et al*., 2020). In developing countries, dairy milk and dairy products such as yoghurt, cheese and ice-cream are expensive. Since non-dairy milks are cheaper than cow milk, the demand for plant-based alternative milk is increasing over the years (Maduka, 2017a; Ayuba *et al*., 2020; Victor-Aduloju *et al.,* 2020; Zandona *et al*., 2020). Different types of non-dairy milk are appreciated by vegetarians because the product lack cholesterol and lactose (Ismail, 2015). Notwithstanding the rising acceptability of plant-based milk by consumers, Haas *et al*. (2019) reported that cow milk still has a better product image.

Tigernut milk also known as tigernut beverage is an aqueous extract obtained from fresh tigernut tubers (*Cyperus esculentus*) (Maduka *et al*., 2017; Maduka and Ire, 2018). In appearance, tigernut milk is a light brownish liquid. It has a low viscosity and sweet-acidic taste (Kizzie-Hayford *et al*., 2015). Addition of date palm (*Phoenix dactylifera* L.), coconut (*Cocos nucifera*), spices such as cloves (*Syzygium aromaticum*), ginger (*Zingiber officinale* Roscoe) etc during preparation of tigenut milk is a personal choice which influences the flavour of the product (Eke-Ejiofor and Beleya, 2018; Samuel *et al*., 2020; Victor-Aduloju *et al*., 2020; Victor *et al.,* 2021). Tigernut milk is regarded as imitation milk or beverage from a plant source (Maduka, 2017b). The product is popular, affordable and nutritious (Sabah *et al*., 2019; Akakpo *et al.,* 2019a; Samuel *et al*., 2020). Tigernut milk has a short shelf life (less than 24 hours) which could be extended depending on the storage condition (Obinna-Echem *et al*., 2019a). According to Nwaiwu *et al.* (2020), tigernut milk is among the unregulated artisanal drinks popularly consumed in Nigeria. It is listed among the beverages which constitute a hazard associated with foodborne diseases (Oduori *et al.,* 2022).

During the process of extracting tigernut milk from tigernut tubers, the nutritional content of the product could be affected (Kizzie-Hayford *et al*., 2015; Ogunka-Nnoka *et al*., 2020). Yellow/brown variety of tigernut tubers contain moisture (3.50/3.78 %), crude fibre (6.26/5.62 %), ash (3.97/4.25 %), carbohydrates (46.99/41.22 %), lipid (32.13/35.43 %) and crude protein (7.15/9.70 %), respectively (Oladele and Aina, 2007). Research findings by Oluwakemi *et al*. (2021) revealed that tigernut tubers is rich in sodium (956.38 mg/kg), potassium (4478.76 mg/kg) and iron (45.617 mg/kg). The vitamin B_1, C, and D content is 2.38 mg/100g, 5.76 mg/100 g and 22.79 µg/100 g, respectively. Opeyemi and Obuneme (2020) reported that moisture, crude protein, ash, crude fiber, crude fat and carbohydrate content of homemade tigernut milk is within the range 79.50-81.30, 6.84 - 7.10, 1.87 - 2.01, 0.70 - 1.24, 4.66 - 5.62 and 2.0 - 4.50 %, respectively.

Health benefits associated with consumption of tigernut tubers which include prevention of diabetes, colon cancer, obesity, and coronary heart diseases could also be derived by drinking tigernut milk (Adejuyitan, 2011; Bashir *et al*., 2014). Tigernut milk is recommended for diabetic patients and people whose body system cannot tolerate gluten or allergic to cow milk (Maduka and Ire, 2017; Opeyemi and Obuneme, 2020). No allergy has been reported as a result of consuming tigernut milk (Suleiman *et al*., 2018; Rebezov *et al*., 2021). Since tigernut milk contain digestive enzymes (amylase, lipase, and catalase), it is suitable for those experiencing digestion disorders, flatulence and diarrhoea. The consumption of tigernut milk help in preventing arteriosclerosis as a result of high amount of oleic acid and arginine. Other health benefits associated with consumption of tigernut milk include reduction in low density lipoprotein (LDL) cholesterol and increase high density lipoprotein (HDL) cholesterol (Ogodo *et al*., 2018; Victor-Aduloju *et al.,* 2020; Iboyi *et al*., 2021).

Despite the numerous health and nutritional benefits associated with tigernut milk (Oke *et al*., 2019; Rebezov *et al.*, 2021), high level microbial contamination of the product especially homemade tigernut milk usually produced under unhygienic conditions could be a threat to public health (Maduka and Ire, 2019; Pandukur *et al*., 2019; Ire *et al*., 2020a). Polyethylene terephthalate (PET) bottles used in packaging alcoholic and non-alcoholic drinks are reused several times by local producers of tigernut milk (Obinna-Echem *et al*., 2019a). In Nigeria, small scale industries licensed to produce tigernut milk are few. On the contrary, there are many industries in developed countries involved in large scale production of tigernut milk (Pondei and Ariyo, 2021).

Okwelle (2020) reported that tigernut drink sold in a university campus were contaminated with *Staphylococcus* sp., coliforms, mould and yeast. Musa and Hamza (2013), Ike *et al*. (2017), Ire *et al.* (2020a), Opeyemei and Obuneme (2020) reported the presence of bacterial genera which include *Staphylococcus* sp., *Proteus* spp., *Escherichia* sp., *Salmonella* sp., *Pseudomonas* sp., *Klebsiella* sp., *Bacillus* sp., *Streptococcus* sp., *Micrococcus* sp., *Enterobacter* sp. and *Corynebacterium* sp. in homemade commercially available tigernut milk while fungal genera encountered in the product include *Rhizopus* sp., *Penicillium* sp., *Saccharomyces* sp., *Aspergillus* sp., *Fusarium* sp. Majority of these microorganisms are associated with tigernut tubers and surfaces of equipment used in processing the tubers into tigernut milk (Gambo and Da´u, 2014; Pondei and Ariyo, 2021).

Many research works on microbiological and nutritional quality of locally produced and bottled tigernut milk sold to the public without a product label have been reported. However, there is dearth of information on locally produced tigernut milk sold in plastic bottles carrying labels which the general public regard as a quality product. Therefore, this study is aimed at evaluating the microbiological and nutritional quality of some local brands of tigernut milk sold in Benin City.

2. MATERIALS AND METHODS

A total of fifteen (15) samples of bottled tigernut milk which comprise of three (3) samples of each local brand of the product were randomly purchased from vendors in Benin City. Shown in Figure 1 is the map of Benin City, Edo State. The brand name of the tigernut milk was abbreviated: FNF, SJG, REM, VLM and GTD. Visual inspection and expiry date of the tigernut milk was checked before sampling. All the samples were put inside icepack and quickly transported to the Microbiology Laboratory, Wellspring University, Benin City, within four (4) hours for analyses.

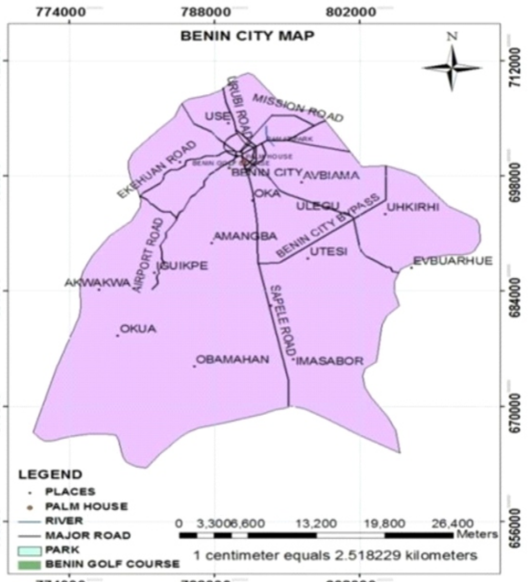

Figure 1: Map of Benin City

Source: Edo State Ministry of Lands and Surveys, Benin City

Source: Edo State Ministry of Lands and Surveys, Benin City

2.1 Serial dilution

Nine millilitre (9 ml) of sterile distilled water was dispensed into ten (10) sterile test tubes. Exactly 1 ml of each sample of tigernut-milk was transferred into the first test tube using a sterile pipette which represent dilution 10^{-1}. Stepwise transfer into other test tubes were made using a sterile pipette for each transfer until dilution 10^{-7} was reached.

2.2 Microbiological analysis

2.2.1 Total heterotrophic bacterial count

Exactly 0.1 ml dilution 10^{-7} of each sample of tigernut-milk was inoculated into sterile Petri dishes followed by molten sterilized Nutrient Agar (NA) using pour plate method. The inoculated plates were gently rocked anticlockwise and allowed to solidify. The NA inoculated plates were incubated at 37 °C for 24 h. After incubation, total number of colonies on the plates were counted manually and recorded. The bacterial population of each sample was expressed as colony forming unit per millilitre (CFU/ml) using the formula below:

$$\text{CFU/ml} = \text{no. of colonies} \ \frac{1}{\textit{dilution factor}} \times \frac{1}{\textit{volume plated}}$$

2.2.2 Total fungal count

Exactly 0.1 ml dilution 10^{-5} of each sample of tigernut milk was inoculated into sterile Petri dishes followed by pouring of molten sterilized Potato Dextrose Agar (PDA). The inoculated plates were gently rocked anticlockwise and allowed to solidify. The plates were incubated at room temperature (25±2 °C) for 5 days. The fungal population of each sample was expressed as colony forming unit per millilitre (CFU/ml) using the formula below:

$$\text{CFU/ml} = \text{no. of colonies} \ \frac{1}{\textit{dilution factor}} \times \frac{1}{\textit{volume plated}}$$

2.2.3 Isolation of pure isolates

Representative bacterial and fungal colonies on the culture plates were subcultured using streak method into freshly prepared NA and PDA plates, respectively. The NA and PDA plates were incubated at 37 °C for 24 h for bacterial growth and 25±2 °C (room temperature) for 5 days for fungal growth, respectively. The pure isolates obtained were transferred into agar slants and stored in a refrigerator (4±2 °C).

2.2.4 Identification of bacterial isolates

The bacterial isolates were identified based on their cultural and morphological characteristics. Gram staining of the bacterial isolates were carried out in order to group the organisms into two large groups – Gram positive and Gram negative bacteria. While viewing the stained bacterial cells under the microscope, the cell morphology and arrangement were noted. Biochemical tests which include oxidase test, urease test, indole production test, citrate utilization test, catalase test, and sugar fermentation test were carried out on the bacterial isolates (Shoaib *et al.*, 2020).

2.2.5 Identification of fungal isolates

Based on morphological appearance and microscopic examination, the fungal isolates were identified. A wet mount of the fungal isolates was prepared. A small portion of the fungal isolate was dropped on a clean glass slide containing few drops of normal saline. After gentle mixing, a cover slip was placed on the content of the glass slide and viewed under the microscope using x10 and x40 magnifications (Isu and Onyeagba, 2002).

2.3 Proximate analysis

The proximate composition of the tigernut-milk drink which involves moisture, crude protein, ash, and crude fibre content were determined using AOAC (2007) methods. Carbohydrate content of the samples were determined by difference method.

2.4 Gross energy

The energy content of the tigernut milk samples were calculated using the Atwer factors of 4, 9,

4 for protein, fat and carbohydrate, respectively.
Calorific value = (% carbohydrate x 4) + (% crude fat x 9) + (% crude protein x 4)

2.5 Mineral assay
The concentrations of zinc, calcium, magnesium an iron in branded tigernut milk samples were determined using Atomic Absorption Spectrophotometer (Solar 969 Unicam series) after acid digestion. Spectrophotometric method was used in analyzing for Ca, K, Mg, Na, Mn, P, Fe and Cu content of branded tigernut milk as described by AOAC (2007).

2.6 Determination of vitamins
Vitamin A and C content of branded tigernut-milk were determined using the procedure described by Rutkowski and Grzegorczyk (2006).

2.7 Statistical analysis
Data generated were subjected to statistical analysis which include One-Way Analysis of Variance (ANOVA) at 95 % confidence level. Separation of means in situations where significant differences existed was made possible using the Duncan New Multiple Range Test. IBM Statistical Package for Social Sciences (SPSS) software version 22 was employed in the statistical analysis.

1. RESULTS
Presented in Table 1 is the total heterotrophic bacterial count (THBC) of five (5) local brands of tigernut milk. The brand of tigernut milk that recorded the highest and least THBC was REM (6.50 x 10^9 ±0.040 CFU/ml) and SJG (1.35 x 10^9 ±0.026 CFU/ml), respectively. The total fungal count (TFC) of five (5) local brands of tigernut milk is presented in Table 2. The total fungal count (TFC) of FNF and VLM brands of tigernut milk which was 7.95 x 10^7 ± 0.076 and 1.85 x 10^7 ± 0.066 CFU/ml accounted for the highest and least values, respectively. Depicted in Figure 2 is the percentage occurrence of bacterial species encountered in the tigernut milk. *Pseudomonas aeruginosa* (30 %) and *Bacillus subtilis* (30 %) accounted for the bacterial species which had the highest percentage occurrence. On the contrary, bacterial species which had the least percentage occurrence are *Micrococcus* sp. (20 %) and *Lactobacillus acidophilus* (20 %). The percentage occurrence of fungal isolates encountered in the tigernut milk is depicted in Figure 3. *Mucor* sp. (22 %) and *Sacharromyces cerevisiae* (7 %) accounted for the highest and least percentage occurrence, respectively.

Table 1. Total heterotrophic bacterial count of local brands of tigernut milk.

Sample	CFU/ml
FNF	4.60±0.025 x 10^9
SJG	1.35±0.026 x 10^9
REM	6.50±0.040 x 10^9
VLM	1.55±0.066 x 10^9
GTD	3.40±0.070 x 10^9

Table 2. Total fungal count of local brands of tigernut milk.

Sample	CFU/ml
FNF	7.95±0.076 x 10^7
SJG	2.15±0.085 x 10^7
REM	2.25±0.080 x 10^7
VLM	1.85±0.066 x 10^7
GTD	2.50±0.072 x 10^7

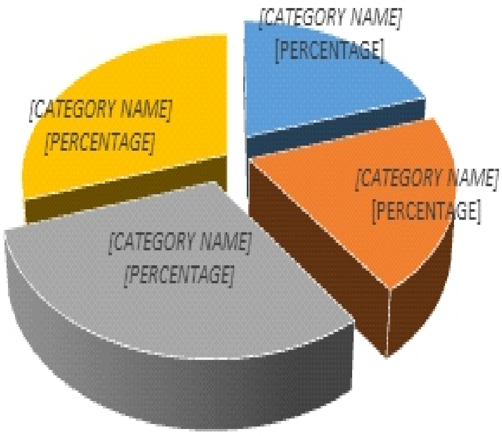

Figure 2. Percentage occurrence of bacterial isolates from local brands of tigernut milk.

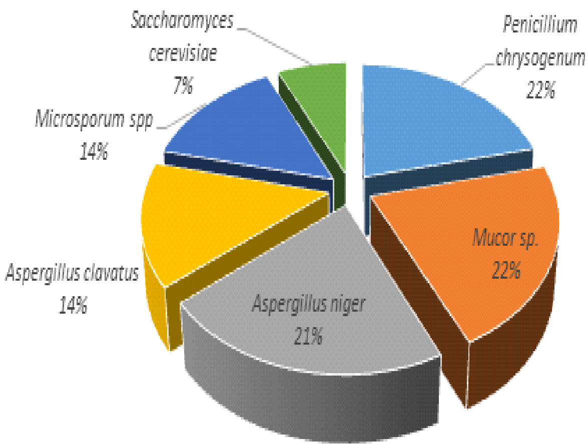

Figure 3. Percentage occurrence of fungal isolates from local brands of tigernut milk

Presented in Table 3 is the proximate composition of five (5) local brands of tigernut milk. The moisture, crude protein, crude fat, crude fibre, ash and carbohydrate content of the tigernut milk was within the range 76.45±2.24 - 82.65±1.20, 0.71±0.011 - 0.75±0.048, 2.32±0.27 - 4.46±0.09, 0.18±0.04 - 1.63±0.17, 0.30±0.03 - 0.44±0.07 and 12.01±0.30 - 17.87±0.84 %, respectively.

Presented in Figure 4 is the gross energy content of five (5) local brands of tigernut milk. The result obtained showed that 114.58 and 73.87 Kcal recorded for REM and GFD brand of tigernut milk accounted for the highest and least value, respectively.

Presented in Table 4 is the concentration of minerals in five (5) local brands of tigernut milk. The minerals include calcium (1.06±0.20 - 6.79±0.51 mg/100 g), potassium (164.84±1.06 - 259.27±1.78 mg/100 g), magnesium (4.25±0.37 - 8.32±0.52 mg/100 g), sodium (1.64±0.25 - 3.10±0.22 mg/100 g), manganese (0.40±0.12 - 0.49±0.15 mg/100 g), phosphorus (43.86±0.37 - 47.10±0.31 mg/100 g), iron (6.88±0.26 - 8.52±0.54 mg/100 g) and copper (0.02±0.00 - 0.03±0.01 mg/100 g).

Vitamin A and C content of five (5) local brands of tigernut milk is depicted in Table 5. The result showed that vitamin A and C content of the tigernut milk samples was within the range 20.47±0.44 - 28.20±0.28 µg 100^{-g} and 5.37±0.42 - 7.76±0.39 mg 100^{-g}, respectively.

Table3. Proximate composition of local brands of tigernut milk.

Brand	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Ash (%)	Carbohydrate (%)
REM	76.45 ± 2.24^{a}	0.74 ± 0.019^{a}	4.46 ± 0.09^{c}	0.18 ± 0.04^{a}	0.30 ± 0.03^{a}	17.87 ± 0.84^{c}
VLM	78.07 ± 0.44^{ab}	0.73 ± 0.010^{a}	4.28 ± 0.41^{c}	1.22 ± 0.10^{c}	0.30 ± 0.03^{a}	15.40 ± 0.57^{b}
FNF	80.23 ± 1.60^{bc}	0.75 ± 0.048^{a}	3.27 ± 0.24^{b}	0.78 ± 0.11^{b}	0.39 ± 0.09^{a}	14.58 ± 0.38^{b}
GFD	82.65 ± 1.20^{c}	0.72 ± 0.017^{a}	2.55 ± 0.46^{ab}	1.63 ± 0.04^{d}	0.44 ± 0.07^{a}	12.01 ± 0.30^{a}
SJG	81.73 ± 1.20^{c}	0.71 ± 0.011^{a}	2.32 ± 0.27^{a}	1.63 ± 0.17^{d}	0.44 ± 0.06^{a}	13.17 ± 0.28^{a}

Values show means of triplicate analysis ±SD. Values with different superscript down the column are significantly different (P = 0.05).

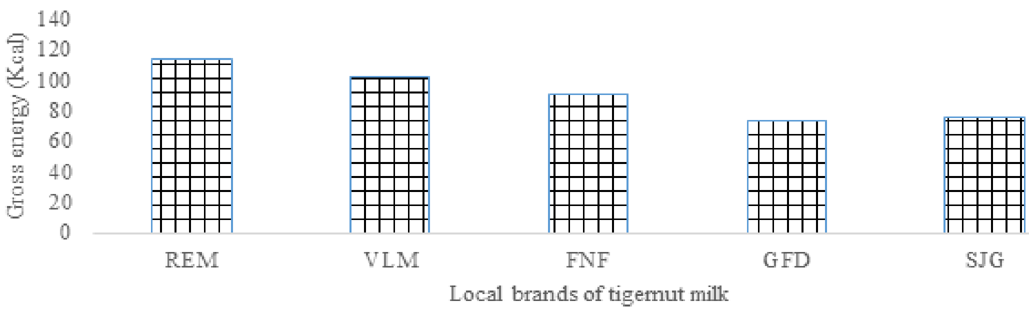

Figure 4. Gross energy content of local brands of tigernut milk.

Table4. Minerals content of local brands of tigernut milk.

Brand	Ca (mg/100 g)	K (mg/100 g)	Mg (mg/100 g)	Na (mg/100 g)	Mn (mg/100 g)	P (mg/100 g)	Fe (mg/100 g)	Cu (mg/100 g)
REM	1.06 ± 0.20^{a}	209.42 ± 1.11^{c}	5.22 ± 0.70^{ab}	3.10 ± 0.22^{b}	0.40 ± 0.12^{a}	44.32 ± 0.87^{a}	8.12 ± 0.25^{bc}	0.03 ± 0.01^{a}
VLM	3.20 ± 0.28^{b}	188.22 ± 1.30^{b}	7.43 ± 0.61^{cd}	2.02 ± 0.22^{a}	0.41 ± 0.12^{a}	46.06 ± 0.90^{bc}	8.52 ± 0.54^{c}	0.02 ± 0.01^{a}
FNF	3.72 ± 0.29^{b}	164.84 ± 1.06^{a}	4.25 ± 0.37^{a}	1.97 ± 0.12^{a}	0.49 ± 0.15^{a}	43.86 ± 0.37^{a}	7.26 ± 0.45^{ab}	0.03 ± 0.01^{a}
GFD	6.79 ± 0.51^{d}	259.27 ± 1.78^{d}	8.32 ± 0.52^{d}	1.95 ± 0.15^{a}	0.46 ± 0.13^{a}	47.10 ± 0.31^{c}	6.88 ± 0.26^{a}	0.03 ± 0.01^{a}
SJG	4.97 ± 0.28^{c}	189.72 ± 1.06^{b}	6.23 ± 0.40^{bc}	1.64 ± 0.25^{a}	0.43 ± 0.06^{a}	45.33 ± 0.40^{ab}	7.42 ± 0.26^{ab}	0.02 ± 0.00^{a}

Values show means of triplicate analysis ±SD. Values with different superscript down the column are significantly different (P = 0.05).

Table 5. Vitamin A and C content of local brands of tigernut milk.

Vitamin	REM	VLM	FNF	GFD	SJG
Vitamin A (μg 100^{-g})	28.20 ± 0.28^{e}	27.10 ± 0.29^{d}	21.90 ± 0.46^{b}	23.74 ± 0.29^{c}	20.47 ± 0.44^{a}
Vitamin C (mg 100^{-g})	7.76 ± 0.39^{c}	7.35 ± 0.29^{bc}	5.44 ± 0.39^{a}	5.37 ± 0.42^{a}	6.83 ± 0.34^{b}

Values show means of triplicate analysis ±SD. Values with different superscript along the row are significantly different (P = 0.05).

4. DISCUSSION

The total heterotrophic bacterial count (THBC) of tigernut milk samples was within the range $1.35\pm0.026 \times 10^{9}$ - $6.50\pm0.040 \times 10^{9}$ CFU/ml. In a related study, Sebastiá *et al.* (2012) reported that viable microorganisms in commercial tigernut beverages could not be detected. The researchers attributed the result to pasteurization, sterilization or ultrahigh temperature sterilization of the product. Bashir *et al.* (2014), Okwele (2020), Opeyemi and Obuneme (2020), and Badua *et al*. (2018) reported that bacterial population within the range 2.2×10^{4} - 1.4×10^{6}, 1.11- 2.40×10^{5}, 1.2 - 12.0×10^{4} and 9.92×10^{4} - 3.13×10^{4} CFU/ml were present in samples of tigernut milk sold within the campuses of selected tertiary institutions in Nigeria, respectively. According to Ntukidem *et al*. (2020), the THBC of tigernut milk sold in Uyo metropolis is within the range 1.1×10^{3} - 4.3×10^{5} CFU/ml. Microbiological Guidelines for Ready-to-Eat Food (2014) stipulate that aerobic colony count (ACC) of non-fermented dairy products below 10^{5} CFU/ml is satisfactory for human consumption; 10^{5} - $\leq 10^{7}$ CFU/ml is the borderline; $\geq 10^{7}$ CFU/g is unacceptable for human consumption. Based on the results obtained from this study, the samples of tigernut milk, though a non-dairy product is not microbiologically safe for human consumption.

Findings from this study showed that total fungal count (TFC) of the tigernut milk was within the range $1.85\pm0.066 \times 10^{7}$ - $7.95\pm0.076 \times 10^{7}$ CFU/ml. High fungal population in the tigernut milk could be attributed to unsanitary environment where processing of tigernut tubers into tigernut milk was carried out. Samuel *et al*. (2020) reported that TFC of tigernut milk prepared in the laboratory is 9.2×10^{5} cfu/g. In a related study, Okwele (2020) reported that total mould count (TMC) and total yeast count (TYC) of tigernut milk sold to students in a tertiary institution is within the range 1.56 - 2.23×10^{5} and 1.43 - 2.60×10^{5} CFU/ml, respectively.

The bacteria species identified in the tigernut milk were *Bacillus subtilis*, *Pseudomonas aeruginosa, Lactobacillus acidophilus* and *Micrococcus* sp. The percentage occurrence of *B. subtilis* and *P. aeruginosa* in the tigernut milk samples was 30 % each. Ire *et al*. (2020a) reported that *Bacillus* and *Pseudomonas* species recorded the highest frequency of occurrence compared with other bacterial isolates encountered in tigernut milk obtained from different locations. The report is in agreement with the result obtained in this study. In a related study, Okwelle (2020) isolated *Bacillus subtilis* and *Pseudomonas* sp., among other bacterial species from tigernut drink sold in a university campus. The presence of *Lactobacillus* sp. and *Micrococcus* sp. in samples of tigernut drink sold in Uyo metropolis was reported by Ntukidem *et al*. (2020).

Bacillus species are usually found in the soil, air, dust and water contaminated by soil. It could also be found on vegetables (Sebastià *et al*., 2012; Sa'id *et al*., 2017). *Bacillus* species are spore formers. This property of the organism could have contributed to its persistence in the tigernut milk. It is one of the bacteria that recorded highest frequency of occurrence (30 %). According to Ire *et al*. (2020a), consumption of tigernut beverage contaminated with *Bacillus* sp. could result in bacillus foodborne intoxication. Some *Bacillus* species are associated with bacteremia/septicemia and endocarditis.

Pseudomonas aeruginosa is ubiquitous. This could explain why *P. aeruginosa* was among the bacterial isolates that recorded the highest frequency of occurrence (30 %) in the tigernut milk samples. *Pseudomonas aeruginosa* is an opportunistic pathogen and mostly saprophytic. It is commonly isolated from moist environment and other areas such as water and soil. The organism is responsible for respiratory system infections, urinary tract infections and gastrointestinal infections. According to Ntukidem *et al*. (2020), contamination of beverages and food by *Pseudomonas* sp. could lead to spoilage.

According to Aneja *et al*. (2014), *Lactobacillus* species is among the microorganisms implicated in the spoilage of acidic products. Victor-Aduloju *et al.* (2020) reported that pH of tigernut milk is within the range 4.62 - 6.62. According to Samuel *et al*. (2020), acidity level of tigernut milk could be attributed to fermentation of the product by lactic acid bacteria (*Lactobacillus leichmanni* and *L. fermentum*). In a related study, Oyekan and Oyetayo (2021) reported the presence of *L. plantarum*, *L. composti* and *L. vini* in tigernut milk with added preservatives. The dominance of fungal species (6) compared to bacterial species (4) reported in this study could be attributed to low pH created by *Lactobacillus* sp. present in tigernut milk. The percentage occurrence of *Lactobacillus* sp. in the tigernut milk samples was 20 %.

Micrococcus species are part of the normal flora in human skin. The skin could be one of the sources of contamination of tigernut milk occasioned by poor handling during processing. Poor hygiene practices of handlers of the product could increase the risk of some species of *Micrococcus* to cause upper respiratory tract infection. Although *Micrococcus* species found in soil, water and meat products is somewhat harmless, the enterotoxins released by the bacterium could cause food poisoning (Ayoade *et al*., 2013; Akakpo *et al.,* 2019b). Therefore, the presence of *Micrococcus* sp. in the tigernut milk could pose a threat to public health. The percentage occurrence of *Micrococcus* sp. in the samples was 20 %. In a related study, Ogodo *et al.* (2018) reported that percentage occurrence of *Micrococcus* sp. in tigernut milk samples is 10 %. The percentage occurrence of fungal species in the tigernut milk samples include *Saccharomyces cerevisiae* (7 %), *Microsporum* sp. (14 %), *Aspergillus clavatus* (14 %), *A. niger* (21 %) and *Mucor* sp. (22 %). The presence of *S. cerevisiae, A. niger, and Penicillium* sp in tigernut beverage reported by Ntukidem *et al.* (2020) is substantially in agreement with the result obtained in this study. One possible source of *A. clavatus* and *A. niger* in the tigernut milk is the tigernut tubers used as a raw material (Sa´id *et al*., 2017). Aflatoxins and ochratoxins produced by *A. niger* is carcinogenic. Both toxins could cause kidney and liver disorders. The presence of *Aspergillus* sp. in the local brands of tigernut milk is a threat to public health. In a related study, Okwelle (2020) reported the presence of *Aspergillus flavus*, *A. niger, Fusarium* spp., *Rhizopus* spp., *Candida* spp. and *Saccharomyces* spp. in tigernut milk. According to Ntukidem *et al*. (2020), the use of *Saccharomyces cerevisiae* extensively in food processing has a long history. Although the organism is harmless, it also plays a role in food spoilage as a result of its osmophilic nature and fermentative ability. The ability of *S.*

cerevisiae to grow at low temperature, tolerate acid and alcohol enables the fungi to cause spoilage in food and beverages. Therefore, the presence of *S. cerevisiae* in the local brands of tigernut milk could impact on its shelf life. Recycled polyethylene terephthalate (PET) bottles often used by local producers to package tigernut milk and the environment where processing of the product took place are possible sources of *Mucor* sp. that contaminated the product (Ike *et al*., 2017). This could have contributed to highest percentage occurrence of *Mucor* species in the tigernut milk. In a related study, Samuel *et al*. (2020) reported that yeast (9.2 x 10^5 cfu/ml) had the highest frequency of occurrence in tigernut milk.

The moisture content of the tigernut milk was within the range 80.23±1.60 - 82.65±1.20 %. There was significant difference ($p<0.05$) in moisture content among the samples with the exception of sample GFD and SJG. According to Ntukidem *et al*. (2019), the stability and overall quality of food is influenced by its moisture content. Large volume of water used to extract juice from crushed tigernut tubers is the major reason tigernut milk is associated with high moisture content. Consequently, rapid microbial growth in tigernut milk during storage is likely to occur and the product will have a short shelf life. In a related study, Opeyemi and Obuneme (2020) reported that moisture content of tigernut milk sampled from different locations in a University campus is within the range 79.50-83.70 %.

The crude protein content of the tigernut milk was within the range 0.71±0.011 - 0.75±0.048 %. From the result obtained, there was no significant difference ($p>0.05$) in the protein content of the tigernut milk samples. According to Yu *et al.* (2022), tigernut tubers contain small amount of protein. Lower amount of protein in the local brands of tigernut milk compared with the values reported by some researchers should be a concern to consumers. This result could be attributed to losses that occurred during extraction of milk from tigernut tubers (Obinna-Echem *et al*., 2019b). Ogo *et al*. (2018) reported that protein content of tigernut milk prepared using fresh and dry tigernut tubers is 7.13 and 4.14 %, respectively. According to Roselló-Soto *et al.* (2019), the protein content of tigernut beverage prepared separately using tigernut tubers obtained from Nigeria and Ghana is within the range 2.34 - 2.51 and 0.47 - 0.54 g/100 g, respectively. The differences in protein content of tigernut milk could be attributed to the variety of tigernut tubers and processing methods used in preparing the product.

Crude fat content within the range 2.32±0.27 - 4.46±0.09 % was encountered in the tigernut milk samples. The values were significantly different ($p<0.05$) among the tigernut milk samples with the exception of sample REM and VLM. In a related study, Opeyemi and Obuneme (2020) reported that crude fat content of tigernut milk samples is within the range 4.66 - 6.00 %. The geographical location where the tigernut tubers were cultivated; variety of tigernut tubers used in preparing local brands of tigernut milk could be responsible for variations in crude fat content of the product.

The crude fibre content of the tigernut milk was within the range 0.18±0.04 - 1.63±0.17 %. There was significant difference ($p<0.05$) in crude fibre content among the samples with the exception of GFD and SJG local brand of tigernut milk. In a related study, Opeyemi and Obuneme (2020) reported that crude fibre content of tigernut milk consumed by University students is within the range 0.70 - 1.24 %. It is important to note that digestion is aided by high intake of crude fibre (Oke *et al.,* 2019).

Ash content of any food sample is an indication of the level of minerals present in the food (Ogo *et al*., 2018). The ash content of the tigernut milk samples was within the range 0.30±0.03 - 0.44±0.07 %. There was no significant difference ($p>0.05$) in ash content of all the samples of tigernut milk. In a related study, Obinna-Echem *et al*. (2019b) reported that ash content of pasteurized and unpasteurized milk from fresh and dry tigernut tubers is within the range 0.01-0.04 %.

The carbohydrate content of local brands of tigernut milk was within the range 12.01±0.30 - 17.87±0.84 %. It was revealed that carbohydrate content of VLM and FNF brands of tigernut milk; GFD and SJG brands of tigernut milk are not significantly different ($p>0.05$). However, the values were significantly different ($p<0.05$) from carbohydrate content of REM brand of tigernut milk. The range of carbohydrate content of local brands of tigernut milk (12.01±0.30 - 17.87±0.84 %) was higher than 2.0 - 4.5 and 2.74 - 7.16 % reported by Opeyemi and Obuneme (2020) and Obinna-Echem *et al*. (2019b), respectively.

Our results indicate that gross energy content of the tigernut milk was within the range 76.4 - 114.58 kcal. Low energy value of tigernut milk reported in this study compared with the report of Obinna-Echem *et al*. (2019b) could be as a result of processing methods and the varieties of tigernut tubers used as a raw material. Obinna-Echem *et al*. (2019b) reported that gross energy content of pasteurized and unpasteurized tigernut milk prepared using fresh tigernut tubers is 373.22 and 488.68 KJ/g, respectively. As for the pasteurized and unpasteurized tigernut milk prepared using dry tigernut tubers, the values obtained were 379.53 and 425.92 KJ/g, respectively.

It is worthy to note that local brands of tigernut milk was rich in potassium (164.84±1.06 - 259.27±1.78 mg/100 g). The product also contains reasonable amount of phosphorus (43.86±0.37 - 47.10±0.31 mg/100 g). This result is in agreement with the report of Ariyo *et al*. (2021) which stated that potassium and phosphorus content of tigernut milk is 211.03±0.78 and 46.54±0.07 mg 100 g^{-1}, respectively. There was significant difference ($p<0.05$) in the values reported for each of the minerals in the tigernut milk samples. In a related study, Obinna-Echem *et al*. (2019b) reported that potassium content in tigernut milk is within the range 1048.34±13.34 - 1181.67±213.34 mg/100 g. Potassium is essential for normal functioning of skeletal muscle fibers, amino acid and protein synthesis, and enzyme catalyzed reactions. Potassium help in preventing heart attack and hemorrhages (Rebezov *et al.,* 2021). Phosphorus play a role in bone and teeth formation (Akram *et al.,* 2020; Opeyemi and Obuneme, 2020). Daily requirement of macro-nutrients which include potassium, phosphorus, magnesium, sodium and calcium on average is above 100 mg/day for adults (Akram *et al*., 2020).

The magnesium content of the tigernut milk was within the range 4.25±0.37 - 8.32±0.52 mg/100 g. There was significant difference ($p<0.05$) in the magnesium content of the tigernut milk samples. In a related study, Opeyemi and Obuneme (2020) reported that magnesium content of tigernut milk samples is within the range 1.14 -1.32 mg/L. The recommended dietary allowance (RDA) for magnesium in healthy adult females and males is within the range 310 - 320 and 400 - 420 mg/day, respectively (Ire *et al*., 2020b). It is worthy to note that magnesium content of local brands of tigernut milk analyzed in this study was considerably lower than the RDA. According to Akram *et al*. (2020), the availability of magnesium in the body ensures that growth of bones is maintained. The mineral is involved in regulating cardiac cycle and also ensures that muscles and nerves are functioning normally.

Our results indicate that amount of sodium in tigernut milk was within the range 1.64±0.25 -

3.10±0.22 mg/100 g. There was no significant difference ($p>0.05$) in the values reported for sodium content of the tigernut milk samples with the exception of REM brand of tigernut milk. The quantity of sodium in tigernut milk patronized by some students in a tertiary institution is within the range 0.23 - 0.64 mg/L (Opeyemi and Obuneme, 2020). In a related study, Ariyo *et al*. (2021) reported that sodium content of tigernut milk prepared using fresh tigernut tubers is 4.15±0.00 mg 100 g^{-1}. Sodium plays a role in the control of blood (Akram *et al*., 2020). According to Opeyemi and Obuneme (2020), low amount of sodium in tigernut milk makes the product suitable for patients suffering from diabetes and hypertension).

Obinna-Echem *et al*. (2019b) reported that calcium content of pasteurized and unpasteurized tigernut milk prepared using fresh and dry tigernut tubers is within the range 1692.94±552-1921.99±154.33 mg/100g. In a related study, Ariyo *et al*. (2021) reported that calcium content of tigernut milk prepared using fresh tigernut tubers is 1.07±0.04 mg 100 g^{-1}. The result is in agreement with the findings from this study which reported that calcium content of tigernut milk was within the range 1.06±0.20 - 6.79±0.51 mg/100 g. There was significant difference ($p<0.05$) in calcium content of the tigernut milk samples with the exception of VLM and FNF brands of tigernut milk. According to Ire *et al.* (2020b) and Akram *et al*. (2020), the recommended daily allowance (RDA) of calcium is one gram per day. It should be a thing of concern that calcium content of local brands of tigenut milk evaluated in this study is considerably lower than the RDA. Calcium is vital for adequate growth and development of bones in humans. It plays an important in the clotting of blood, functioning of the nerve, and contraction of the muscles.

Iron content of the tigernut milk samples was within the range 6.88±0.26 - 8.52±0.54 mg/100 g. There was significant difference ($p<0.05$) in the values reported for iron content of the tigernut milk samples. The result is in agreement with the report of Ariyo *et al*. (2021) which stated that iron content of tigernut milk is 9.26±0.07 mg 100 g^{-1}. The recommended dietary allowance (RDA) of iron for every individual is 10 - 18 mg/day (Ire *et al*., 2020b). In a related study, Opeyemi and Obuneme (2020) reported that iron content of tigernut milk is within the range 0.61 - 0.86 mg/L. Diet rich in iron is recommended for pregnant women in the interest of the developing fetus. High demand of iron is required during the last trimester of pregnancy. Iron plays a vital role to ensure that oxygen is transported to red blood cells and enzymes such as flavoprotein (Akram *et al*., 2020).

The amount of copper recorded for each of the tigernut milk samples was within the range 0.02±0.00 - 0.03±0.01mg/100 g. In a related study, Ariyo *et al*. (2021) reported that copper content of tigernut milk prepared using fresh tigernut tubers is 0.03±0.01 mg 100 g^{-1}. On the contrary, Opeyemi and Obuneme (2020) did not report any value in the samples of tigernut milk which they analyzed. There was no significant difference ($p>0.05$) in the copper content of the tigernut milk samples. Copper is essential in the production of red blood cells, formation of bones and hematopoiesis (Akram *et al*., 2020).

The manganese content of the tigernut milk samples was within the range 0.40±0.12 - 0.49±0.15 mg/100 g. There was no significant difference ($p>0.05$) in the values reported for manganese content of the tigernut milk samples. In a related study, Ariyo *et al*. (2021) reported that manganese content of tigernut milk prepared using fresh tigernut tubers is 0.43±0.04 mg 100 g^{-1}. According to Akram *et al*. (2020), the human body contain 15 mg of manganese and requires 4 - 10 mg daily. Manganese is involved in blood clotting. Alongside vitamin K, the mineral plays a role in hemostasis (Akram *et al*., 2020).

Our results indicate that vitamin A and C content of tigernut milk was within the range 20.47±0.44 - 28.20±0.28 μg 100^{-g} and 5.37±0.42 - 7.76±0.39 mg 100^{-g}, respectively. There was significant difference ($p<0.05$) in the values reported for each of the vitamins in the tigernut milk samples. In a related study, Opeyemi and Obuneme (2020) reported that vitamin A and C content of tigernut milk is within the range 1.23 - 1.78 IU/L and 1.23 - 5.21 mg/L, respectively. According to Akram *et al*. (2020), RDA of vitamin A and C (ascorbic acid) is 8000 - 1000 μg/day and 60 μg/day, respectively. Vitamin A help to improve vision in a dim light. It gives protection to the eyes when there is infection. Vitamin A is required for the growth of bones. The functionality of immune system is enhanced by vitamin A and C. Wound healing is a well-known function of vitamin C.

5. CONCLUSION

The local brands of bottled tigernut milk evaluated in this study were contaminated with high population of bacteria and fungi. Nutritional analysis revealed that samples of the tigernut milk had low protein and ash content. With regards to minerals, vitamin A and C content of the tigernut milk samples, this study showed they were in reasonable quantities.

Limitations of the Study

(i) The varieties of tigernut tubers used in preparing the tigernut milk could not be obtained from the producers to further investigate its influence on proximate composition, minerals and vitamins content of the products.

(ii) The processing conditions and methods adopted by the producers of local brands of tigernut milk which could also influence the level of microbial contamination and nutritional composition of the products were not properly ascertained and evaluated due to restrictions.

Recommendations

(i) Pasteurization of tigernut milk at 72 °C for 10 minutes, strict implementation of good manufacturing practices (GMP) during production of tigernut milk and good hygienic practices (GHP) among the workers are measures recommended to reduce the microbial load of the product.

(ii) The use of newly manufactured polyethylene terephthalate (PET) bottles which have been sterilized to package tigernut milk is highly recommended.

(iii) The results of microbial analysis and nutritional composition of batches of tigernut milk produced should be within standard specifications before the products could be released into the markets.

(ii) The National Agency for Food and Drug Administration and Control (NAFDAC) should increase her level of monitoring of local brands of tigernut milk in the market to ensure the products are safe for human consumption.

Disclaimer

All the products used for this research are commonly and predominantly used in the area and country where the research was carried out. To the best of our knowledge, there is absolutely no conflict of interest that exist between the researchers and the producers of tigernut milk used in this study. The authors have no intention whatsoever to use any of the products for litigation except for the purpose of knowledge advancement. We categorically state that this research was not funded by producers of the tigernut milk. Instead, the authors personally funded the research.

Competing interests

The authors have declared that no conflict of interests exist.

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