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# Effect of Packaging Material on the Physicochemical and Microbiological Quality of Refrigerated Tiger Nut Milk (*Cyperus esculentus*)

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**Abstract:** The effect of packing material on the physicochemical and microbiological quality of tiger nut milk in refrigeration storage was investigated. Milk extracted from fresh and dry tiger nut was pasteurized, packaged in polyethylene terephthalate (PET) and glass bottles and stored in the refrigerator at 4.4±2°C for 3, 5, 8, 10, 12 and 15 days. pH in glass bottle was 5.59 - 3.89 and 6.91 – 4.06 for fresh and dry tiger nut milk and 5.35 – 3.98 and 7.05 – 4.07 in PET bottles. Decrease in sugar content in glass bottle (2.85 and 2.53%) for dry and fresh tiger nut milk was significantly ($P \leq 0.05$) greater than PET bottle (1.87 and 0.79%). Mould and *Salmonella* were not found in the samples. *Escherichia coli* was below detection limit in the dry tiger nut milk but was within marginally acceptable levels (1.54 - 2.62 and 2.02 - 2.71 $Log_{10}$CFU/ml respectively in glass and PET bottles) in the fresh tiger nut milk on the first 5 days. Total coliform count ($Log_{10}$CFU/ml) in the PET bottle (≤4.30) was significantly (P≤0.05) greater than glass bottle ≤3.13. Total aerobic mesophiles ($Log_{10}$CFU/ml) in PET bottles varied from 3.30 – 4.89 and 1.79 – 5.02 respectively, for the fresh and dry tiger nut milk while in glass bottles the values were 3.11 – 5.09 and 3.01 – 4.94. Tiger nut milk in glass bottles had significant (P≤0.05) reduction in pH and lower microbial count than PET bottles and microbiological quality deteriorated significantly (P≤0.05) on day 8. Glass bottle will be a better storage material for tiger nut milk with a recommended shelf-life of 3-5 days under refrigeration temperature of 4.4±2ºC.

**Keywords:** Tiger nut milk; packaging material; physicochemical and microbiological quality; refrigeration storage

## 1. Introduction

Tiger nut (*Cyperus esculentus*) belongs to the Family – *Cyperaceae*, and was introduced by the Arabs, first in the Valencia region (Ibrahim et al., 2016). It is native to most of the Western Hemisphere as well as Southern Europe, Africa, Madagascar, the Middle East and the Indian Subcontinent (Abaejoh et al., 2006). Tiger nuts can be eaten raw, roasted, dried, or baked (Belewu and Abodunrin, 2006; Oladele and Aina, 2007; Rita, 2009). In Nigeria, tiger nut milk or "*kunu aya*" is mostly consumed in the afternoon to cool the body from the hot weather, it is cheap and popular; available, affordable, drink of both the poor and the rich (Bamishaiye and Bamishaiye, 2011). Tiger nut milk is a nutritive and energetic drink both for old and young people (Abaejoh et al., 2006; David, 2010). It is a rich source of starch, fat, sugar and protein, minerals (phosphorus, potassium) and vitamins E and C (Belewu and Belewu, 2007; Oladele and Aina, 2007).

The milk is underutilized due to its short shelf life and lack of information on its nutritional potentials (Omode et al., 1995; Cortes et al., 2005). Apart from use as a beverage, tiger nut milk is thought to be beneficial to diabetic patients (Anderson et al., 2009) and those seeking to reduce cholesterol or lose weight (Oladele and Aina, 2007; Borges et al ., 2008). Tiger nut milk has a very short shelf life of often less than 24 hours depending on the condition of storage (Akoma et al., 2006). High temperature and humidity significantly reduces the shelf life of the product (Nutso, 2014), as tiger nut milk is often associated with significant microbial contamination, including bacteria and moulds (Onovo and Ogaraku, 2007; Nutso, 2014). Although, tiger nut milk has contributed to the upliftment of the living conditions of people especially women, where most women have developed the skills for commercial production of the milk (Musa and Hamza, 2013), the short shelf life of raw tiger nut milk hinders widespread consumption of the beverage due to the deteriorating effects of some microorganisms on the milk (Abaejoh et al., 2006).

Microbial quality of beverage drink, dairy products and other food is determined by significant total microbial count, coliform count and presence of pathogenic microorganisms. Microbial quality determination is completely used to reflect hygienic practice in food production. Milk analogue can serve as a good medium for the growth of many microorganisms especially bacterial pathogens; therefore, its quality control is considered essential to the health and welfare of a community. As reported by Foster (1990), the threat posed by diseases spread through contaminated food is well known and the epidemiological impact of such diseases is considerable. The presence of pathogenic microorganisms in beverages developed from under-utilized crops can emerge as a major public health concern especially for consumers.

Tiger nuts from the farm to the point of sale are likely to be exposed to all forms of microbial contaminations (Ibenyassine, et al., 2007). Some diverse microbial species associated with tiger nuts and its products include *Bacillus subtilis, Staphylococcus aureus Escherichia coli, Samonella, Shigella, Pseudomonas, Aspergillus flavus, Aspergillus niger, Fusarium solani, Saccharomyces cerevisiae, Saccharomyces fubiligera* and *Candida pseudotropicalis* (Adejuyitan, 2011; Badau et al., 2018). It has been revealed that lack of effective microbial control and treatment at any stage from planting to consumption could result to the introduction of pathogenic microorganisms to the final food product (Cheesbrough, 2006). There have been challenges on storage stability of tiger nut extract. This has been worsened by the use of recycled and unsealed polyethylene terephthalate (PET) bottles as packaging materials (Udeozor and Awonorin, 2014). The use of these PET bottles which may not be adequately sterilized renders the product susceptible to microbial contamination thereby reducing the shelf life of the product. Earlier researchers have made attempt to delay its spoilage by addition of natural tropical preservatives and some physical methods such as short temperature long time pasteurization and refrigeration (Nwobosi et al., 2013), but with little success that could allow long distance transportation of the product and enhance shelf-live. This work was thus aimed at evaluating the effect of packing material (glass and PET bottles) on the physicochemical and microbiological quality of refrigerated milk extracted from fresh and dry tiger nut.

## 2. Materials and Methods

### *2.1. Sample and Sample Preparation*

The yellow variety of fresh and dry tiger nuts used for this study was sourced from the fruit market in D/Line, Port Harcourt, Rivers State, Nigeria. The tiger nuts after sorting to remove foreign matters, sand, broken and bad nuts were washed with tap water. About 1 Kg of the dry tiger nuts was soaked with 5 litres of tap water for 48 h. Thereafter, the nuts were washed again with clean tap water.

*2.2. Extraction of Milk from the Fresh and Dry Tiger Nuts*

Tiger nut milk was extracted according to the method described by Udeozor and Awonorin, (2014), with some modifications as reported by Obinna-Echem et al., (2018). Briefly, about 1kg of the cleaned tiger nut was blended five times for 5min each into slurry with 2 L of potable water in a Kenwood blender (Model BL440, UK). The milk from the slurry was extracted using muslin cloth. The milk extract was pasteurized at 72ºC for 10mins and allowed to cool to a constant temperature of 25ºC. The pasteurized milk was packaged in 100 ml PET and glass bottles and stored at refrigerated temperature (4.4±2ºC) for 15 days.

*2.3. Sampling for Analysis*

The initial sample was aseptically withdrawn after bottling before storage from the bottles designated as initial. Then on day 3, 5, 8, 10, 12 and 15 of storage, the bottles for each day were removed from storage and samples withdrawn aseptically for analysis.

*2.4. Analysis*

**Physicochemical Properties**

pH and Titratable acidity (TTA) were determined using AOAC (2012) standard methods. Briefly, the pH of 10 mL of the milk sample was determined with a pH meter (TS 652, Germany). Thereafter, the 10 mL of the sample was titrated against 0.1 mol L-1 NaOH with phenolpthaline as indicator to determine the amount of acid in the sample as total titratable acidity (TTA). The result was expressed as % lactic acid.

Determination of sugar was carried out using Lane and Eynon method as described by James (1999). Briefly, 5 ml of the milk sample was measured into a beaker and 100ml of warm water was added. This was transferred was into a conical flask; 10ml of dilute HCL was added and boiled for 5mins. It was allowed to cool and the solution was neutralized with 10% NaOH and made up to volume in a 250 volumetric flask. The solution was used for titration against Fehling's solution. The reading was taken and the sugars calculated as follows:

$$\% \text{ Total Sugars} = \frac{2.5FD}{10TW}$$

Where:

F = 4.95 mg glucose (Glucose factor for 1 ml of Fehling's solution as the milk was extracted from tuber not an animal source)

D = 250 ml dilution

T = Titre

W= Weight of sample

**Microbiological Analysis**

One millilitre (1 ml) of the aseptically withdrawn samples was pipetted into 9 mL of sterile peptone water in a sterile 20 mL tubes, vortexed for 3-5 s and serially diluted to $10^{-8}$ in the same diluent following the procedure described by Harrigan, 1998. Aliquots (100 μL) of the dilutions were spread-plated on appropriate microbial media for each microorganism.

Total *Coliform* and *Escherichia coli, Mesophiles, and Salmonella* were respectively enumerated on Chromogenic Coliform Agar (CCA), Plate Count Agar (PCA) and Salmonella Shigella Agar (SSA) incubated at 37ºC for 24h. Mould was enumerated on Potato Dextrose Agar (PDA) incubated at 25ºC for 48 hr.

*2.5. Statistical Analysis*

Data collected were subjected to analysis of variance (ANOVA): general linear model (GLM) using IBM SPSS (Release 2015) software for windows version 23.0, (Amrmonk, Ny: IBM Corp). The Duncan's multiple range test was used to determine the significant difference between mean values. Statistical differences were established at ($P \leq 0.05$).

## 3. Results and Discussion

*3.1. Physicochemical Properties of Tiger Nut Milk Packaged in PET and Glass Bottles and Stored in the Refrigerator at 4.4±2ºC for 15 days*

**pH**

pH of tiger nut milk (extracted from fresh and dry tiger nut) packaged in PET and glass bottles and stored in the refrigerator at 4.4±2ºC for 15 days are shown in Table 1. pH of the fresh tiger nut milk in PET and glass bottles varied from 5.35 – 3.98 and 5.59 -3.89 respectively. pH of the dry tiger nut milk in PET and glass bottles varied from 7.05 – 4.07 and 6.91 – 4.06 respectively. The pH of all samples (3.99 – 3.96) did not differ significantly ($P \leq 0.05$) on day 8 except for the fresh tiger nut milk packaged in PET bottle with significantly ($P \leq 0.05$) the least pH of 3.83. There were significant ($P \leq 0.05$) variations in the initial pH of the milk. Milk from the dry tiger nuts had significantly ($P \leq 0.05$) higher initial pH than milk from the fresh tiger nuts. The specific changes in pH from the initial pH of the samples over the storage period are shown in Figure 1. The changes in pH of the samples relative to the initial pH revealed that the change in pH of the fresh tiger nut milk packaged in glass bottle from day 5 was significantly (P≤0.05) higher than PET bottles. The changes in pH of the dry tiger nut milk packaged in PET bottles were significantly (P≤0.05) higher than the glass bottles, except on days 10 and 12,

**Table 1.** pH of tiger nut milk packaged in PET and glass bottles and stored in the refrigerator at 4.4±2ºC for 15 days

| Samples | Time (Days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 3 | 5 | 8 | 10 | 12 | 15 |
| $F_{GB}$ | 5.59±0.42[c] | 4.59±0.18[c] | 4.99±0.03[c] | 3.97±0.05[a] | 3.90±0.02[c] | 4.12±0.20[a] | 3.97±0.04[b] |
| $F_{PB}$ | 5.35±0.06[d] | 5.25±0.08[b] | 5.24±0.28[b] | 3.83±0.02[b] | 3.98±0.00[b] | 4.03±0.00[a] | 3.98±0.02[b] |
| $D_{GB}$ | 6.91±0.03[b] | 6.54±0.01[a] | 6.53±0.06[a] | 3.96±0.04[a] | 4.00±0.02[b] | 4.04±0.06[a] | 4.06±0.06[a] |
| $D_{PB}$ | 7.05±0.03[a] | 6.61±0.00[a] | 6.57±0.01[a] | 3.99±0.04[a] | 4.09±0.09[a] | 4.16±0.03[a] | 4.07±0.01[a] |

Values are mean ± SD of 4 replications
Means with the same superscript in the same column do not differ significantly (P≤0.05).
$F_{GB}$ - Fresh tiger nut milk packaged in glass bottle
$F_{PB}$ - Fresh tiger nut milk packaged in PET bottle
$D_{GB}$ - Dry tiger nut milk packaged in glass bottle
$D_{PB}$ - Dry tiger nut milk packaged in PET bottle

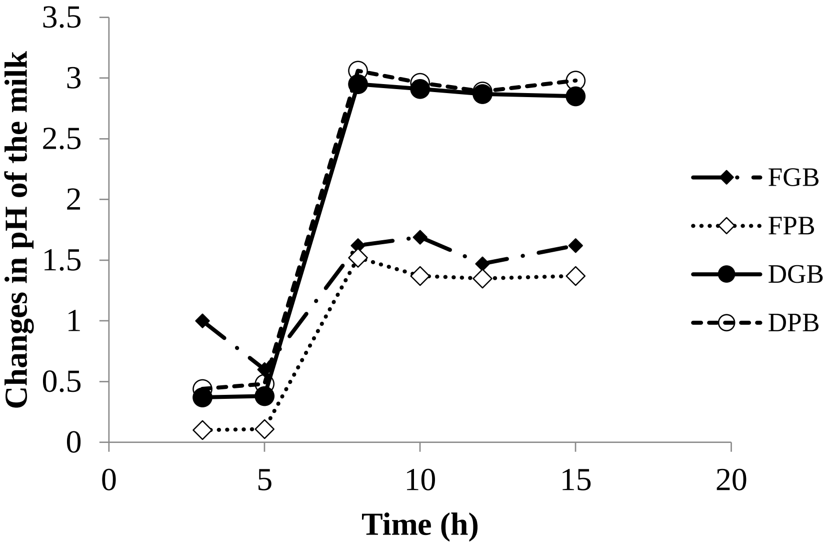


**Figure 2.** Changes from the initial pH of tiger nut milk packaged in PET and glass bottles and stored in the refrigerator at 4.4±2ºC for 15 days

$F_{GB}$ - Fresh tiger nut milk packaged in glass bottle
$F_{PB}$ - Fresh tiger nut milk packaged in PET bottle
$D_{GB}$ - Dry tiger nut milk packaged in glass bottle
$D_{PB}$ - Dry tiger nut milk packaged in PET bottle

Milk from the dry tiger nuts had significantly (P≤0.05) higher initial pH than milk from the fresh tiger nuts. This could be attributed to the pre-treatment given to the nuts. There may have been some reactions in the presence of moisture in the fresh nuts that have resulted in the significantly (P≤0.05) lower initial pH. There was significant (P≤0.05) decrease in pH with storage time. Due to this significant difference in the initial pH of the samples, the changes in pH relative to the initial pH were considered important. The changes in the pH of the dry tiger nut milk were significantly (P≤0.05) higher than changes in the fresh tiger nut milk irrespective of the storage material. The effect of the storage material may be a function of the type of tiger nut used rather than that of the packaging material and also depends on the initial pH of the milk. Due to the higher rate of decrease in the pH of the milk from the dry tiger nut, there was no significant (P≤0.05) difference in the pH of the samples at day 8 of storage. The decrease in pH with the increase in storage time agreed with results from earlier researchers (Efiuvwevwere and Akoma, 1995; Akoma et al., 2006) and indicated there were activities of microorganisms. The change in pH with storage in glass bottle for the fresh tiger nut milk was significantly (P≤0.05) higher than the change in the PET bottle throughout the storage period except on day 8. Hence for the milk from the fresh tiger nut, glass bottle can be considered a better storage material than PET bottle.

**Changes in Titratable acidity**

Titratable acidity (% Lactic acid) of the tiger nut milk packaged in PET and glass bottles and stored in the refrigerator at 4.4±2ºC is shown in Table 2.

**Table 2.** Titratble acidity (% Lactic acid) of tiger nut milk packaged in PET and glass bottles and stored in the refrigerator at 4.4±2ºC for 15 days

| Samples | Time (Days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 3 | 5 | 8 | 10 | 12 | 15 |
| $F_{GB}$ | 1.06±0.09$^{a}$ | 0.05±0.05$^{a}$ | 0.01±0.00$^{a}$ | 0.80±0.09$^{a}$ | 1.15±0.00$^{a}$ | 0.54±0.03$^{a}$ | 0.55±0.06$^{ab}$ |
| $F_{PB}$ | 0.64±0.15$^{b}$ | 0.01±0.00$^{b}$ | 0.01±0.00$^{a}$ | 0.69±0.04$^{b}$ | 1.13±0.05$^{a}$ | 0.58±0.05$^{a}$ | 0.59±0.01$^{a}$ |
| $D_{GB}$ | 0.34±0.09$^{c}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{a}$ | 0.55±0.01$^{c}$ | 1.07±0.05$^{a}$ | 0.58±0.06$^{a}$ | 0.60±0.01$^{a}$ |
| $D_{PB}$ | 0.13±0.06$^{d}$ | 0.00±0.00$^{b}$ | 0.01±0.00$^{a}$ | 0.50±0.04$^{c}$ | 1.03±0.16$^{a}$ | 0.47±0.02$^{b}$ | 0.52±0.02$^{b}$ |

Values are mean ± SD of 4 replications
Means with the same superscript in the same column do not differ significantly (P≤0.05).
$F_{GB}$ - Fresh tiger nut milk packaged in glass bottle
$F_{PB}$ - Fresh tiger nut milk packaged in PET bottle
$D_{GB}$ - Dry tiger nut milk packaged in glass bottle
$D_{PB}$ - Dry tiger nut milk packaged in PET bottle

The titratable acidity (TTA) of the samples differed significantly (P≤0.05). The dry tiger nut milk had significant (P≤0.05) increase on day 8 (≥0.13 - ≤0.55%) which peaked on day 10 (≥1.03%). The fresh tiger nut milk had significant (P≤0.05) increase on day 10. In all the samples the TTA was significantly (P≤0.05) highest on day 10 which decreased significantly (P≤0.05) thereafter. The increase in glass bottles on day 8, 10 was significantly (P≤0.05) greater than the PET bottles in the milk from fresh and dry nuts and on day 12 and 15 in the milk from dry tiger nut. The TTA result agreed with earlier investigation by Nwobosi et al., (2013) and Ukwuru et al., (2008). Similar to the pH, the change in TTA may be a function of the type of tiger nut used and the microbial activities rather than that of the packaging material. The variation in the acidity on the different storage days indicates that other short chain fatty acids may be responsible for the change in pH rather than the lactic acid. Acidity plays important role in retardation of growth of spoilage microorganisms.

**Sugar Content of tiger nut milk packaged in PET and glass bottles and stored in the refrigerator at 4.4±2ºC for 15 days**

The sugar content of the tiger nut milk packaged in PET and glass bottles and stored at 4.4±2ºC for 15 days are shown in Figure 2.

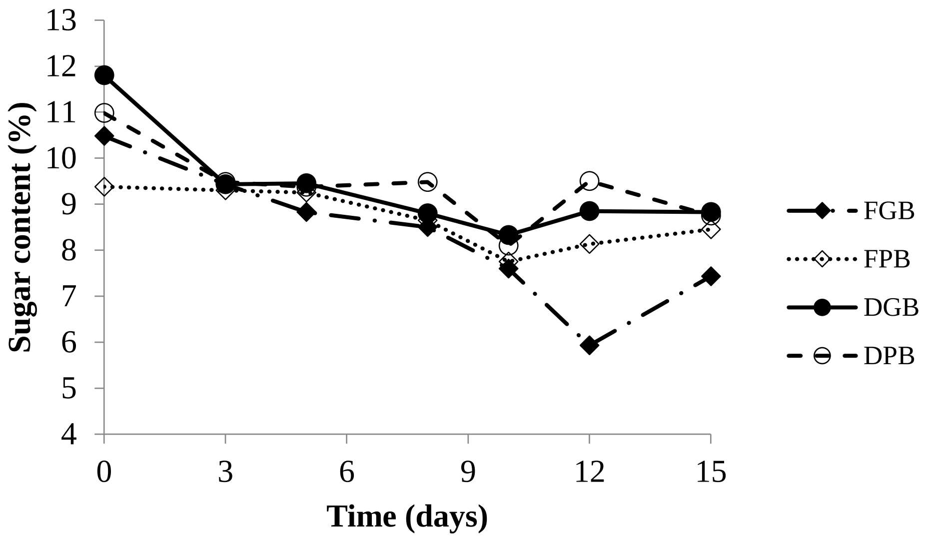


**Figure 2.** Sugar content of tiger nut milk packaged in PET and glass bottles and stored in the refrigerator at 4.4±2ºC for 15 days

$F_{GB}$ - Fresh tiger nut milk packaged in glass bottle
$F_{PB}$ - Fresh tiger nut milk packaged in PET bottle
$D_{GB}$ - Dry tiger nut milk packaged in glass bottle
$D_{PB}$ - Dry tiger nut milk packaged in PET bottle

Generally, there was a significant (P≤0.05) decrease in the sugar content of the samples with increase in storage time. The sugar content for fresh tiger nut milk decreased from 10.48 – 5.93% in glass bottle and 9.38 – 8.13% in PET bottle on day 12 with an increase on day 15. In the dry tiger nut milk, the sugar content decreased from 11.80 – 8.33% in glass bottle and 10.98 – 8.10% in PET bottle on day 10 with an increase on day 12 and decrease on day 15. The mean decrease in sugar content of the tiger nut milk in glass bottle (2.85 for dry and 2.53 for fresh tiger nut milk) was significantly (P≤0.05) greater than the PET bottle (1.87 for dry and 0.79 for fresh tiger nut milk). Hence, the milk packaged in glass bottles had significantly (P≤0.05) higher decrease in sugar content than the PET bottles. The decrease peaked on day 10. The decrease in sugar content of the milk samples with increase in storage time was in agreement with the findings of Ukwuru et al., (2008) and Musa and Hamza (2013). Sugar is a substrate for microbial fermentation, utilization resulted in a decrease with an increased in storage time. Sugar can be converted into different products depending on the organism involve and the growth conditions. The peak decrease in the sugar content on day 10 further explains the significantly (P≤0.05) lower pH on day 10. The decrease in sugar could be attributed to microbial activity in the conversion of the organic substrate to short chain fatty acids as evidenced in the TTA value and the reduced pH on day 10. The effect is significantly (P≤0.05) more with the glass bottles than the PET bottles. The observed increase though unclear may be attributed to breakdown of polysaccharide contents to simple sugars by the activity of the microorganisms or their enzymes.

### *3.2. Microbiological Quality of Tiger Nut Milk Packaged in PET and Glass Bottles and Stored in the Refrigerator at 4.4±2°C for 15 days*

**Mould, Salmonella and *Escherichia coli count***

In all the tiger nut milk samples, mould and *Salmonella* were not found. Some authors have identified mould and *Salmonella* in commercially sold tiger nut drinks and attributed it to poor hygiene practices, non-aseptic handling, processing method, utensils and water used during preparation and prolonged storage of the product at ambient temperature (Umar et al., 2014; Badua et al., 2018).

*Escherichia coli* was below detection limit in the dry tiger nut milk but was detected at a very low level in the fresh tiger nut milk for the first 5 days. For the fresh tiger nut milk, the initial, day 3 and 5 *E. coli* counts were 2.62±0.01, 1.99±0.54 and 1.54±0.76 $Log_{10}$CFU/ml respectively for the samples packaged in glass bottle and 2.71±0.01, 2.25±0.03 and 2.02±0.03 $Log_{10}$CFU/ml for the samples packaged in PET bottles. Similar result was obtained by Sabastia et al., (2012) in tiger nut beverages sold in Valentia. *E. coli* is a gram-negative, facultative anaerobic rod shape bacteria that are common part of the normal intestinal flora of humans and other warm-blooded animals. Its presence in Chromogenic Coliform Agar (CCA) was differentiated from coliform by its dark blue to violet colour due to the cleavage of 5-bromo-4-chloro-3-indoxyl-β-D-glucuronide component of the agar by the enzyme -D-glucuronidase which is characteristic of *E.coli*, while -D-galactosidase characteristic of coliform cleaves to the 6-chloro-3-indoxyl-β-D-galacto-pyranoside component to give a pink to salmon-red coloration (Lange et al., 2013). *E. coli* presence in the fresh tiger nut milk samples is an indication of the contamination of the nuts either from handling, storage, display for sale and/or the water used in preserving the freshness by the sellers. The survival of *E. coli* in the sample despite the heat treatment implied heavy microbial load of the fresh raw materials or contamination during processing. The significant decrease from the initial count on day 3 and 5 indicates the effect of the storage temperature. The values on day 3 and 5 were not significantly (P≤0.05) difference showing that the organism was still on its lag phase. *E.coli* count on the first 5 days was within the recommended acceptable microbiological level ≤ $10^2$ CFU/ml (FAO/WHO, 2002; ICMSF, 2011). Its absence on the rest of the storage days implies that there was no growth in the sample under the storage condition. The absence of Mould and *Salmonella* and the safe levels of *E.coli* on the first 5 days and its absence thereafter is a good indication of microbiological safety and imply that the samples are safe for consumption.

## Coliform Count

Total coliform count in tiger nut milk packaged in PET and glass bottles and stored in the refrigerator at 4.4±2ºC for 15 days is shown in Table 4

**Table 4.** Total coliform count ($log_{10}$cfu/ml) in tiger nut milk packaged in PET and glass bottles and stored in the refrigerator at 4.4±2ºC for 15 days

| Samples | Storage Time (Days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 3 | 5 | 8 | 10 | 12 | 15 |
| $F_{GB}$ | 2.63±0.01$^b$ | 2.25±0.03$^a$ | 3.07±0.01$^b$ | ND | ND | 3.69±0.03$^b$ | ND |
| $F_{PB}$ | 2.71±0.00$^a$ | 1.99±0.54$^a$ | 3.40±0.00$^a$ | ND | 2.91±0.86$^{ab}$ | 3.60±0.03$^b$ | ND |
| $D_{GB}$ | ND | 1.95±0.01$^a$ | 2.96±0.03$^b$ | ND | ND | 3.13±0.07$^c$ | ND |
| $D_{PB}$ | 2.32±0.00$^c$ | 2.40±0.07$^a$ | 2.84±0.08$^c$ | 3.78±0.35$^a$ | 3.70±0.10$^a$ | 4.02±0.04$^a$ | 4.30±0.00$^a$ |

Values are mean ± SD of 4 replications
Means with the same superscript in the same column do not differ significantly (P≤0.05).
$F_{GB}$ - Fresh tiger nut milk packaged in glass bottle
$F_{PB}$ - Fresh tiger nut milk packaged in PET bottle
$D_{GB}$ - Dry tiger nut milk packaged in glass bottle
$D_{PB}$ - Dry tiger nut milk packaged in PET bottle
ND = Not detected

There was a significant (P≤0.05) variation in the coliform count of the samples. Coliform count for fresh and dry tiger nut milk in glass bottle was ≤3.07 $Log_{10}$CFU/ml on day 5 and ≤3.13 $Log_{10}$CFU/ml on day 12 while in PET bottle it was ≤3.60 $Log_{10}$CFU/ml on day 12 and ≤4.30 $Log_{10}$CFU/ml on day 15 respectively, for fresh and dry tiger nut milk. Total coliform count in the PET bottle was significantly (P≤0.05) greater than the glass bottle. The level of coliform in the samples in PET bottles was 1.79 – 3.60 $Log_{10}$CFU/ml and 2.32 – 4.30 $Log_{10}$CFU/ml for the fresh and dry tiger nut milk respectively. While the values for samples in the glass bottles were 2.63 – 3.69 $Log_{10}$CFU/ml and 1.95 – 3.13 $Log_{10}$CFU/ml in the fresh and dry tiger nut milk respectively. These values were less than what was obtained in tiger nut drinks prepared and sold by vendors in Maiduguri (Badau et al., 2018) and those obtained in Valentia (Sebastia et al., 2012). The initial coliform count in the fresh tiger nut milk was significantly (P≤0.05) higher that the dry tiger nut milk. For all samples, coliform was below detection limit on day 8 and 15 except for dry tiger nut milk packaged in PET bottles. The fresh tiger nut milk in PET bottles had significantly (P≤0.05) higher coliform count on day 5 and 10, while milk from fresh tiger nut in glass bottles had no coliform count on day 8, 10 and 15. Dry tiger nut milk in PET bottle had significant (P≤0.05) increase in coliform count with increase in storage time and the values range from 2.32 – 4.30 $Log_{10}$CFU/ml while in glass bottle, there was no detectable level on day 8, 10 and 15. Though the levels of Coliform in the samples exceeded the acceptable limit (<$10^2$ CFU/ml), they were within the marginal limit ($10^2$ -$10^4$ CFU/ml) (ICMSF 2011). Coliform is gram-negative, non-spore forming food borne pathogen and are found in human or animal intestinal tracts, as well as plant and the environment (Frazier, 1995). The presence of coliform in many foods does not necessarily indicate unsatisfactory hygiene measure as they are part of the normal flora of many raw foods. Also their presence in food does not necessarily indicate faecal contamination as they can survive and grow in food processing environment where other pathogenic Enterobacteriaceae may not. Therefore, their presence at high levels provides a warning that unhygienic food handling may have occurred. Sebastia` et al., (2012) recommended the application of correct and strict HACCP system(s) during manufacturing and storage of products to check high contamination level in home-made tiger-nut beverages. The higher initial level of Coliform in the milk from fresh tiger nut is an indication of the contamination of the fresh material during handling and storage. The increase in coliform count of the dry tiger nut milk in the PET bottle suggests favourable conditions and could be discouraging in the consideration of PET bottles for packaging.

### Total aerobic mesophilic count

Presented in Table 5 is the Total aerobic mesophilic count in tiger nut milk packaged in PET and glass bottles and stored in the refrigerator at 4.4±2ºC for 15 days.

**Table 5.** Total mesophilic Count ($Log_{10}$cfu/Ml) in tiger nut milk packaged in PET and glass bottles and stored in the refrigerator at 4.4±2ºC for 15 days

| Samples | Time (Days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 3 | 5 | 8 | 10 | 12 | 15 |
| $F_{GB}$ | $3.11±0.00^b$ | $3.40±0.01^b$ | $3.85±0.00^a$ | $5.01±0.01^a$ | $5.08±0.00^a$ | $5.09±0.01^a$ | $4.51±0.05^b$ |
| $F_{PB}$ | $3.30±0.01^a$ | $3.33±0.02^b$ | $3.66±0.00^{ab}$ | $4.03±0.05^b$ | $4.80±0.02^d$ | $4.89±0.01^b$ | $4.44±0.01^b$ |
| $D_{GB}$ | $3.01±0.02^c$ | $4.68±0.01^a$ | $3.52±0.06^c$ | $4.46±0.72^{ab}$ | $4.89±0.04^c$ | $4.89±0.02^b$ | $4.94±0.02^a$ |
| $D_{PB}$ | $1.79±0.02^d$ | $3.01±0.14^c$ | $3.29±0.71^{bc}$ | $4.06±0.02^b$ | $5.00±0.00^b$ | $5.02±0.06^a$ | $5.02±0.01^a$ |

Values are mean ± SD of 4 replications
Means with the same superscript in the same column do not differ significantly (P≤0.05).
$F_{GB}$ - Fresh tiger nut milk packaged in glass bottle
$F_{PB}$ - Fresh tiger nut milk packaged in PET bottle
$D_{GB}$ - Dry tiger nut milk packaged in glass bottle
$D_{PB}$ - Dry tiger nut milk packaged in PET bottle

Total aerobic mesophiles are the total number of bacteria in a food substance that grow in the presence of oxygen (aerobic) and at moderate temperature (mesophilic). There was significant (P≤0.05) increase in with increase in storage time in all the samples. The increase followed a normal growth curve for microorganism. The increase peaked on day 10, was stationary on 12 and decreased in some samples on day 15. In the PET bottles values varied from 3.30 – 4.89 $Log_{10}$CFU/ml and 1.79 – 5.02 $Log_{10}$CFU/ml for the fresh and dry tiger nut milk respectively. In the glass bottles the values were 3.11 – 5.09 $Log_{10}$CFU/ml and 3.01 – 4.94$Log_{10}$CFU/ml for the fresh and dry tiger nut milk respectively. This values are similar to the Total Bactria Count (2.26 – 9.93 x $10^4$ CFU/ml equivalent to 4.35 -5.00 $Log_{10}$CFU/ml) *kunu aya* (Tiger nut drink) sold in Universities in Mudugiri (Badau et al., 2018). The increased in mesophilic count with increase in storage time throughout the storage period for all samples agrees with the report of Nwobosi, et al., (2013). This increase further explains the decrease in pH and increase in titratable acidy of the samples. The growth may likely be in succession of different organisms whose metabolic activities resulted in the changes in acidity. Aerobic mesophilic count was within the acceptable level of $<10^3$-$10^4$ for the first 5 days according to Codex Alimentarius (FAO/WHO, 2002) and International Commission on microbiological specification for food (ICMSF, 2011). Generally, growth in the glass bottle was significantly (P≤0.05) higher than the PET bottles. This implies that the glass bottles tend to favour more mesophilic growth than the PET bottles and a disadvantage in the use of glass bottles.

## 4. Conclusions

The result revealed that decrease in pH content of the tiger nut milk followed an increase in TTA and a decrease in sugar content. Decrease in sugar content in glass bottle for dry and fresh tiger nut milk was significantly (P≤0.05) greater than PET bottle. Mould and *Salmonella* were not found in the samples. *Escherichia coli* was below detection limit in the dry tiger nut milk but was within marginally acceptable levels in the fresh tiger nut milk on the first 5 days. Total coliform and aerobic mesophilic count in the PET bottle was significantly (P≤0.05) greater than glass bottle. Samples in PET bottle had significantly (P≤0.05) higher pH and microbial count than glass bottle and the microbiological quality of tiger nut milk deteriorated significantly (P≤0.05) on day 8. The use of glass bottle will be a better storage material for tiger nut milk with a recommended shelf-life of 3-5 days under refrigeration temperature of 4.4±2ºC.

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