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Full Length Research Paper

Physicochemical and microbial evaluation of tiger-nut milk sold in selected eateries in Awka, Anambra State

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This study was carried out to evaluate the physicochemical and microbial load of tiger-nut milk sold in selected eateries in Awka, Anambra State. A total of seven samples were collected from major eateries in Awka Anambra State. The self-processed sample served as control. Standard methods were used to analyze the tiger-nut milk for physicochemical and microbial loads. The result of the physicochemical analysis showed that the pH of the tiger-nut milk samples was within the range of 4.62 - 6.62. Total titratable acidity ranged from 0.66 - 0.79%, 11.93 - 18.87% for total solid, and 2.02 - 9.79 for viscosity. The total viable count of the tiger-nut milk samples ranged from 6.73 x10^5 CFU/ml - 8.20 x10^5 CFU/ml. Coliform counts were present in three samples (TSF, TSS, and TSM) which ranged from 1.00 x10^5 CFU/ml - 1.10 x10^5 CFU/ml and total fungal count ranged from 7.43 x10^5 CFU/ml to 8.67 x10^5 CFU/ml. The high microbial load of the samples is as a result of the fact that tiger-nut milk does not undergo any form of heat treatment during its production; it is also an indication that they were contaminated and this can pose a health hazard to the consumers. Hence, effort should be geared towards improving the sanitation and hygienic practices during production to minimize contamination and spoilage.

Keywords: Total titratable acidity, microbial load, fungal count, spoilage

INTRODUCTION

Milk is a nutrient-rich, white liquid food produced by the mammary glands of mammals like cow, sheep, and goat (Hajirostamloo, 2009). It is the primary source of nutrition for infant mammals. It is slightly acidic having a pH of around 6.5 - 6.7 (Helmenstine, 2012). Milk is highly valued because it is a source of many nutrients essential for the proper development and maintenance of the body; it is a complex product containing more nutrients than any other single food (Turler-Inderbitzin, 2012). Its exceptional nutrient profile includes water, protein, fat, carbohydrates, cholesterol, minerals, vitamins and energy (Turler-Inderbitzin, 2012). Hence, it is regarded as a complete diet. Milk has been recognized as an important food for infants and growing children (Obizoba and Anyika, 2006). In developing countries, the cost of dairy milk and their products such as cheese, yoghurt, ice-cream are expensive. This dramatic increase in the composition of milk and milk products are stimulated in part the processing of milk and milk from different seeds and nuts (Belewu and Belewu, 2007). Today, as dairy milk sales have declined due to economy global melt down, non-dairy alternative plant-based beverage have seen a strong increase. The meaning of milk gives more consideration to its constituent substances than its source of origin owning to the discovery of soy and other milk of plant origin such as almond, coconut, rice and tiger-nut. This plant milk has been shown to be very nutritious, beneficial to health and cheap (Sacks *et al*., 2006). Plant milk have been proposed as substitutes for animal milk in diets to combat diseases as well as

malnutrition in poor regions of the world where animal meats are scarce and not affordable (Santo *et al*., 2010). One of such plant milk in Africa and other developing economy is the tiger-nut milk.

Tiger-nut milk is a non-diary milks obtained from the *Cyperus esculentus* plant which is of the family *Cyperaceae* (Adejuyitan, 2011). The tiger-nut is called 'Ajemumi/Ofio' in Yoruba tribe while Igbo tribe called it 'Aki hausa'. It resembles dairy milk in colour and texture. This plant is cultivated for its small tuberous rhizome which is eaten raw or roasted, pressed for its juice to become beverage or milk, extracted of non-drying oil or used as hog feed (Belewu and Belewu, 2007). Tiger-nut milk is widely consumed in the northern parts of Nigeria for its nutritional and medicinal properties especially during dry season (Adejuyitan, 2011). It is a very refreshing and high in dietary fiber which could be effective in the cure and prevention of several diseases (Adejuyitan, 2011). Coconut, pineapple and date fruits may be added to flavour the tiger-nut milk (Kayode *et al*., 2017). This drink has a very short shelf-life at the prevailing weather conditions of tropical Africa and Nigeria and therefore should be consumed within 2-4 hours after production (Akoma *et al*., 2006). However, shelf life of the drink can be extended by pasteurization and addition of ginger, garlic and citric acid (Nwobosi *et al*., 2013). Its production in Nigeria has been hampered due to the presence of microorganisms that deteriorate the milk and cause spoilage as a result of unhygienic preparation, use of contaminated raw materials and crude utensils (Osuntogun and Abiola, 2004). It is therefore necessary to evaluate the quality characteristics of this drink sold at different locations to address the safety of its consumers in Awka, Anambra State.

MATERIALS AND METHODS

Collection of raw materials

Cyperus esculentus (Tiger-nut), *Cocos nucifera* (Coconut), *Phoenix dactylifera L* (Date) and spice such as *Zingerber officinale Roscoe* (Ginger) were purchased from Eke-Awka market in Awka South Local Government area of Anambra State, Nigeria. The materials were kept in clean containers before they were used.

Production of tiger-nut milk

Tiger-nut milk was produced following the modified method of (Belewu and Abodunrin, 2006). The tiger-nut was sorted to remove the unwholesome nut and washed thoroughly in water so as to remove any adhering soil and soaked in water in a clean bowl overnight to soften them. The dried date was cracked to remove the seed and was also soaked in 1.2 litres of water overnight. 2000 g of the tiger-nut was milled with the addition of 1000 g of date, 300 g ginger and 2 whole coconuts with 1.8 litres of water to almost smooth slurry. This was later sieved using a muslin cloth with gentle pressure applied to the content as 7 litres of water was added gradually so as to facilitate maximum liquid extraction. The resulting milk was bottled and refrigerated (Figure 3). The image of the tiger-nut seed and date seed are shown in (Figure 1 and Figure 2).

Figure 1. Tiger-nut seed

Figure 2. Date seed

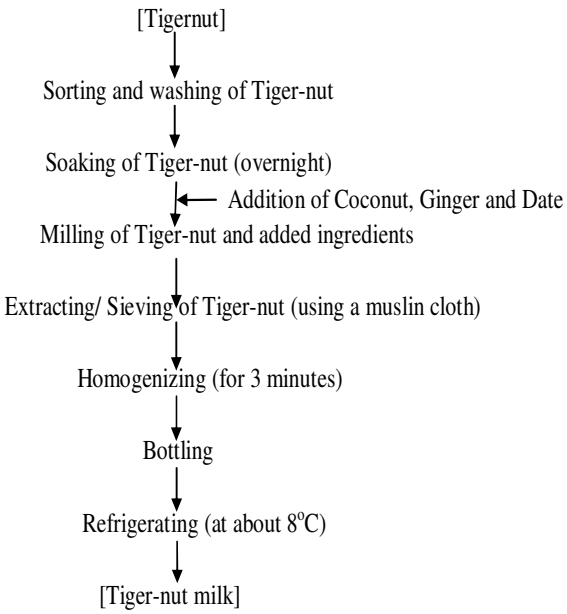

Figure 3. Flow chart for the production of tiger-nut milk. Source: Belewu and Abodunrin, (2006).

Collection of tiger nut milk samples

A total of seven freshly prepared tiger-nut milk samples which were packaged in plastic bottles were collected from seven major eateries in Awka, Anambra state (Kasorina, Five star, Stanel, Cosmilla, Mummy's pot, Divine favour and Ofiaku restaurant).

Method of analysis

Physicochemical analysis

The pH of the samples was determined using a Metler Toledo pH meter. Total titratable acidity (TTA) and total solids (TS) were determined according to the method described by AOAC (2010). Viscosity of the tiger-nut milk samples was determined using an Ostwald Viscometer, a pycnometer and a stop watch.

Microbial analysis

The bacteria load of the tiger-nut milk samples were determined following the method described by Akintunde and Souley, (2009). Serial dilutions of the various tiger-nut milk samples were made up to 10^{-7} using peptone water. 0.1 ml of each dilution was evenly spread on nutrient agar and incubated at 28°C for 24 h. Plates were screened for discrete colonies after incubation period and the actual numbers of bacteria (total bacteria count) were estimated as colony forming unit per ml (CFU/ml) of the sample. Total coliform count (TCC) and total fungal count (TFC) were performed in similar manner using Mac Conkey Agar and Sabouraud Dextrose Agar (SDA) mediums respectively. In each case plating was done in triplicates and counts taken from plates. The colonies were examined closely and distinct colonies were taken and sub cultured in fresh sterile medium and incubated at 37°C for 24 h to obtain pure cultures.

Statistical analysis

The data obtained were analyzed according to a completely randomized design with three replicates. Data were subjected to one way analysis of variance and the difference between means were evaluated by Duncan's multiple range tests using SPSS statistical program version 23.0. Significant difference was expressed at $p<0.05$.

RESULTS AND DISCUSSION

Physiochemical properties of tiger-nut milk samples

The mean values of the physiochemical properties of the tiger-nut milk samples are presented in (Table 1). The samples varied significantly ($p<0.05$) from each other with sample TSM having the highest TTA value of 0.79 and TSS having the lowest value of 0.66. The TTA measures the fermentation stability of food substrates. Thus, TSS with the lowest TTA would be more stable to fermentation while TSM would ferment more readily. The total solid content of the samples ranged from 11.93% - 18.87%. The control sample (TSN) had a total solid content of 15.16%. The highest pH value of 6.62 was found in the tiger-nut milk sample TSK while sample TSM had the lowest value of pH 4.62. The control sample had a pH value of 5.52 which was significantly difference ($p<0.05$) from other samples. The pH values showed that the samples were neither acidic nor alkaline and this values are within the range for standard water which is safe for human consumption. The variations in the pH of tiger-nut milk samples may be attributed to incidence of microbiological activities and chemical reactions during fermentation processes. The pH values showed that the samples were neither acidic nor alkaline so it is safe to drink. This acidity can be attributed to the production of lactic acid by some species of lactic acid bacteria such as *Lactobacillus leichmanni* and *Lactobacillus fermentum* (Akoma *et al*., 2006). The viscosity of the tiger-nut milk samples ranged from 2.02 in sample TSS to 9.79 in sample TSD. The control sample (TSN) had a viscosity value of 2.99 and there was significant difference ($p<0.05$) between the control sample and other samples. Viscosity is an important factor in determining the consistency of a product. The quantity of water used during production of tiger-nut milk and quantity of date added may attribute to variations in viscosity (Kayode *et al*., 2017).

Microbial load of tiger-nut milk samples

The microbial load of tiger-nut milk samples sold in selected eateries in Awka, Anambra State, is presented in (Table 2). The value of bacteria growth ranged from 6.73×10^5 CFU/ml in the control sample TSN to 8.20×10^5 CFU/ml in sample TSO. Total coliform count ranged from 1.00×10^5 CFU/ml in sample TSM to 1.10×10^5 CFU/ml in sample TSF and total fungal count ranged from 7.43×10^5 CFU/ml to 8.67×10^5 CFU/ml. The study revealed that most of the samples collected contained high bacteria count higher than those reported by Aboh and Oladosu, (2014) which ranged from 2.00×10^5 CFU/ml to 5.10×10^5 CFU/ml. The high total viable count observed in the tiger-nut milk samples could be attributed to the fact that the production process of tiger-nut milk does not involve any heat treatment or pasteurization that could reduce or kill microorganisms present in the beverage. Also, this could be as a result of contaminations from milling method, water used in the processing as well as personal hygiene of the handlers which agrees with the report by Musa and Hamza, (2013). It could also be due to how well the sorting and washing of the added ingredients (ginger, date and coconut) and the tiger-nut itself were carried out using potable water. The coliforms present in the drink are considered as an indication of bacteria pollution of human and animal origin which may have been introduced during processing (Badua *et al*., 2018). There was no coliform detected in the control sample (TSN) and samples TSD, TSC, TSO and TSK.

Table 1. Physicochemical composition of tiger-nut milk samples.

SAMPLE	TTA	TS	pH	VISCOSITY
TSD	$0.76^{ab}\pm 0.03$	$18.87^{a}\pm 0.42$	$6.08^{d}\pm 0.04$	$9.79^{a}\pm 0.01$
TSF	$0.78^{a}\pm 0.02$	$16.86^{b}\pm 0.34$	$5.71^{e}\pm 0.03$	$2.94^{d}\pm 0.02$
TSM	$0.79^{a}\pm 0.05$	$12.12^{e}\pm 0.21$	$4.62^{g}\pm 0.03$	$2.52^{f}\pm 0.02$
TSN	$0.67^{c}\pm 0.01$	$15.16^{c}\pm 0.41$	$5.52^{f}\pm 0.14$	$2.99^{c}\pm 0.00$
TSC	$0.77^{ab}\pm 0.04$	$11.93^{e}\pm 0.02$	$5.98^{d}\pm 0.02$	$2.63^{e}\pm 0.03$
TSS	$0.66^{c}\pm 0.02$	$13.61^{d}\pm 0.59$	$6.21^{c}\pm 0.01$	$2.02^{g}\pm 0.02$
TSO	$0.72^{b}\pm 0.02$	$14.20^{d}\pm 0.41$	$6.43^{b}\pm 0.06$	$2.51^{f}\pm 0.01$
TSK	$0.77^{ab}\pm 0.01$	$12.28^{e}\pm 0.31$	$6.62^{a}\pm 0.02$	$3.23^{b}\pm 0.03$

Values represent the mean of triplicate determinations ± SD. Samples in the same column bearing different superscript differ significantly ($p<0.05$).
Keywords:
TSD =Tiger-nut milk obtained from Divine Favour Restaurant.
TSF = Tiger-nut milk obtained from Five Star Restaurant.
TSM = Tiger-nut milk obtained from Mummy's Pot Restaurant.
TSN = Self Processed Tiger-nut milk sample (control).
TSC = Tiger-nut milk obtained from Cosmilla.
TSS = Tiger-nut milk obtained from Stanel.
TSO = Tiger-nut milk obtained from Ofiaku Restaurant.
TSK = Tiger-nut milk obtained from Kasorina Restaurant and Bar.
TTA=Total Titratable Acidity.
TS=Total Solid.

Table 2. Total microbial load of tiger-nut milk samples (CFU/ml $x10^5$).

SAMPLE	TVC	TCC	TFC
TSD	$7.10^{c}\pm 0.26$	NG	$7.43^{e}\pm 0.15$
TSF	$7.63^{b}\pm 0.15$	$1.10^{a}\pm 0.10$	$8.27^{c}\pm 0.15$
TSM	$6.97^{cd}\pm 0.21$	$1.00^{b}\pm 0.00$	$8.67^{a}\pm 0.15$
TSN	$6.73^{d}\pm 0.06$	NG	$7.43^{e}\pm 0.15$
TSC	$7.47^{b}\pm 0.15$	NG	$8.40^{bc}\pm 0.20$
TSS	$6.80^{cd}\pm 0.10$	$1.07^{ab}\pm 0.06$	$7.77^{d}\pm 0.15$
TSO	$8.20^{a}\pm 0.10$	NG	$7.53^{de}\pm 0.06$
TSK	$6.97^{cd}\pm 0.21$	NG	$8.53^{ab}\pm 0.06$

Values represent the mean of triplicate determinations ± SD. Samples in the same column bearing different superscript differ significantly ($p<0.05$).

Keywords:
TSD =Tiger-nut milk obtained from Divine Favour Restaurant.
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TSM = Tiger-nut milk obtained from Mummy's Pot Restaurant.
TSN = Self Processed Tiger-nut milk sample (control).
TSC = Tiger-nut milk obtained from Cosmilla.
TSS = Tiger-nut milk obtained from Stanel.
TSO = Tiger-nut milk obtained from Ofiaku Restaurant.
TSK = Tiger-nut milk obtained from Kasorina Restaurant and Bar.
TVC=Total Viable Count.
TCC=Total Coliform Count.
TFC=Total Fungal Count.
NG=No Growth.

The absence of coliform is a good indication of the good manufacturing practices employed by the manufacturers. The increase in yeast and mould count loads of the tiger-nut milk may be due to contamination through water, atmosphere, packaging materials, utensils and processing environment. Other sources of contamination could be attributed to handling and storage practices. A study conducted by Chukwu *et al*. (2013) indicated that fungus (*Aspergillus niger, Aspergillus flavus* and *Aspergillus terreus*) were present in both fresh and dried tiger-nuts and they can possibly endure processing treatment. The fungal count of tiger-nut milk samples has been associated to the spoilage of the storage microflora which is manifested primarily by the development of off-flavours. Therefore, tiger-nut milk obtained from Mummy's Pot (TSM) is more likely to have a rancid taste

when compared to others.

Authors' declaration

We declared that this study is an original research by our research team and we agree to publish it in the journal.

Conclusion

This study has shown the physicochemical and microbial evaluation of different tiger-nut milk samples sold in selected eateries in Awka, Anambra State. The pH of the tiger-nut milk samples were within the range of 4.62 - 6.62. All samples contained high bacteria and high fungal count with coliforms present in three samples (TSF, TSM and TSS). The control sample (TSN) had lesser microbial load and there was no coliform count in the sample, indicating that it was more hygienically prepared compared to the commercial samples. However, there is need to employ standard hygienic measures during production for the commercial samples since the production process does not involve any thermal treatment.

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