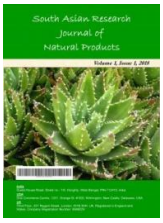

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Production of Fermented Drink from Milk Extract of Tigernut (*Cyperus esculentus*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author ALO designed the study and performed the statistical analysis, author TTM wrote the protocol and the first draft of the manuscript while author JEM managed the literature and analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

The study was aimed at producing fermented drink from milk extract tiger nut (*Cyperus esculentus*) using lactic acid bacteria isolated from locally fermented milk (nono), and to compare it with a yoghurt brand at New Karu, Nasarawa State, in terms of physicochemical and consumers' sensory properties. The extracted tiger nut milk was fermented with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in an anaerobic chamber, and the product was subjected to proximate and sensory evaluations. The result of the proximate analysis show that moisture, ash and carbohydrate were not significant ($p>0.05$), however crude fibre protein and fat were significant ($p<0.05$). The pH of all samples ranged from 4.0 – 4.2 and titrable acidity between 0.94 – 0.95. On the base of a preliminary consumers' sensory evaluation texture was not significant for all the samples, appearance, aroma, taste, consistency and overall acceptance were significant. The result of the study revealed that tiger nut milk can be a good alternative to other source of milk for the production of fermented drink.

Keywords: Fermentation; tiger nut milk; starter culture; Lactobacillus bulgaricus and Streptococuss thermophilus.

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1. INTRODUCTION

Fermented milks are widely produced in many countries. The process of fermenting milk is one of the oldest methods used to extend the shelf-life of milk, and has been practised by human beings for thousands of years [1]. Fermented milk is the collective name for products such as yoghurt, ymer, kefir, cultured buttermilk, filmjölk (Scandinavian sour milk), cultured cream and koumiss (a product based on mares' milk). The generic name of fermented milk is derived from the fact that the milk for the product is inoculated with a starter culture which converts part of the lactose to lactic acid. Dependent on the type of lactic acid bacteria used carbon dioxide, acetic acid, diacetyl, acetaldehyde and several other substances are formed in the conversion process, and these give the products their characteristic fresh taste and aroma [2].

Recently importance has been given to produce fermented milk with improved health attributes particularly the therapeutic properties of these products such as alleviation of lactose intolerance, protection against gastrointestinal infection, anti-carcinogenic effect, immune system stimulation, antihypertensive activity, lowering of serum cholesterol and anti-allergic qualities [3]. Starter culture organisms used in fermentation belong to a family of bacteria collectively known as the Lactic Acid Bacteria (LAB) which are widely distributed in nature and occur naturally as indigenous micro-flora in raw milk and fermented milks.

Tiger nut (*Cyperus esculentus*) is a cosmopolitan perennial crop located all over the world. Its common names are; tiger nut, Aya(hausa), chuffa sedge(Spanish), yellow nut sedge and earth almond. The nut was discovered to be rich in myristic acid, oleic acid and linoleic acid [4]. Tiger nut possesses a high content of soluble glucose and oleic acid, along with high energy content (starch, fats, sugars and proteins),they are rich minerals such as phosphorous and potassium and in vitamins E and C. Tiger nut are believed to help prevent heart attacks, thrombosis and cancer especially of the colon. They are thought to be beneficial to diabetics and those seeking to reduce cholesterol or lose weight. The very high fibre content combined with its delicious taste makes tiger nut ideal for healthy eating [5].

Tiger nut milk is derived from the sweetened water extract of tiger nut tubers (*C. esculentus*), with the addition of sugar to taste [6]. It can serve as a superb alternative to traditional cow milk with a natural sweet taste. The production requires a soaking process of the tiger nuts of about 8 hours, the grinding of the nuts, pressing of the mass, and mixing with sugar [7]. The objective of the research was to produce the fermented milk drink from tiger nut extract and to compare it physicochemical and sensory properties with yoghurt brand.

2. MATERIALS AND METHODS

2.1 Sample Collection

Tiger nut was purchased from Masaka market in New Karu, Nasarawa State, Nigeria. They were collected in a clean polythene bag and taken to the laboratory for processing and analysis. Also, a common yoghurt brand in New Karu, Nasarawa State was obtained and refrigerated.

2.2 Source of Inoculums

Inocula were isolated from nono (naturally fermented cow-milk) purchased from Fulani women in their settlement at New Karu, Nasarawa State. Collection of nono samples were taken to the laboratory for further analysis in a sterile container [8].

2.3 Microbiological Analysis

Seven (7) nono samples were collected in sterile bags from different locations in New Karu, Nasarawa State. Samples were transported to the laboratory in a cold box and stored in a refrigerator until they were used for the isolation of starter culture. MRS (De Mans, Rogosa and Sharpe) agar and broth medium were prepared according to manufacturer's instruction (TITAN Biotech Ltd., India) and sterilised at 121°C and 15 PSI for 15 minutes before cooling. Samples were homogenised in sterile normal saline. Serial dilution up to 10^{-6} was made using a sterile pipette by transferring 1 ml from 10 ml of the normal saline culture into 9 ml of diluent in sterile test tubes [9].

2.3.1 Enumeration of lactic acid bacteria

Enumeration of Lactic Acid Bacteria was done by plating appropriate dilutions using MRS agar. The Plates were incubated inside an anaerobic chamber at 37°C for 48 hours. Distinct colonies were sub-cultured and purified by streaking on agar plates repeatedly. The isolates were

characterised after 48 hours of incubation using: macroscopic examination for shape, size, elevation and pigmentation; microscopic examination by gram staining; growth at 15°C, 37°C and 45°C and biochemical methods such as indole, catalase, citrate and methyl red test were carried out [10]. API 50 CHL system (Biomerieux® France) was used for further identification of LAB strains. The identity of each isolate was also confirmed by comparing their characteristics with those of known taxa using Bergery's manual of bacteriology [11].

2.3.2 Identification of isolates

Identification of isolates from locally fermented milk (nono) was done using gram staining technique and biochemical test (catalase, citrate, indole and methyl red test).

Gram staining: Fixation of smear was carried out by spreading loopful of isolate on a glass slide and passing it over low flame 3 times. Smear was covered with 1% crystal violet, Lugol's iodine solution and washed with 95% ethanol and stained with 2% safranin before being observed under light microscope.

Catalase test: One drop of 3% hydrogen peroxide (H_2O_2) was added to a loopful of LAB culture.

Citrate test: The LAB culture was inoculated on slants of Simmon's citrate agar then incubated at 37°C for 24 hours.

Indole test: LAB was inoculated in 5 ml of tryptone broth and incubated at 37°C for 24 hours. Five (5) drops of 0.5% Kovac's reagent was added after incubation and mixed by gently shaking.

Methyl red test: LAB cultures were inoculated in 5 ml glucose phosphate peptone water and incubated at 37°C for 24 hours. Following incubation, drops of 0.02% methyl red solution were added.

API 50 CH kit (Biome-rieux) was used for rapid identification of LAB isolates to differentiate at the strain level. Wells in the incubation trays were filled with sterile distilled water to create a humid atmosphere, and the strips were placed on the trays accordingly. Pure culture incubated for 24 hours were harvested into ampoules containing sterile peptone water. Bacterial suspension in the ampoule (2.0 McFarland) was dispensed into the media then into the strip's microtubules using pipette avoiding bubbles formation. Wells were covered with sterile mineral oil to achieve anaerobic condition and incubated at 37°C for 48 hours. Reaction based on changes in the colour of each well was studied and interpreted as negative, positive. Identification was determined after result patterns were analysed with the numerical pro-file using api webTM (Version 5.1).

The Identified strains were later transferred into fresh medium and sub cultured every 2 weeks for proper maintenance of isolates.

2.4 Extraction of Milk from Tiger Nut

The method of Belewu & Abodunrin [12] was used. After proper picking of nuts to remove stones and infected nuts, 1 kg of the tiger nut was washed and soaked in 8 litres of distilled water for 24 hours. They were washed again with distilled water and blended several times with a blender. A muslin cloth was used to filter meaty part of the milled tiger nut to separate the milk from the insoluble chaff.

The filtered tiger nut milk was transferred into a container and pasteurised at 95°C for 15 minutes and later cooled to a temperature of 43°C.

2.5 Production of the Fermented Milk

Two starter cultures of tiger nut milk were prepared. One of the starter culture contained *Lactobacillus bulgaricus* and the other contained *Streptococcus thermophilus*. Two adiabatic jars, each containing one litre of tiger nut milk were labelled sample A and B for fermentation. At temperature 43°C, Sample A was inoculated with 4% v/v of starter culture containing *Lactobacillus bulgaricus* and sample B was inoculated with 4% v/v of starter culture containing *Streptococcus thermophilus*. The jars were covered and incubated at 42°C for 4 hours [13]. The resulting fermented tigernut-milk samples were compared with Sample C containing a common yoghurt brand in New Karu, Nasarawa State.

2.6 Proximate and Physicochemical Analysis

Moisture, ash, carbohydrate, protein, fat and crude fibre content were determined according to methods described in AOAC [14]. pH measurement was carried on all samples with a standardised pH meter and titratable acidity was

determined by titration of samples against 0.1 NaOH also according to methods described in AOAC [14].

2.7 Consumers' Sensory Evaluation

For a preliminary consumers' sensory evaluation, a 9 point hedonic Scale was used to determine the Sensory qualities (Appearance, Aroma, Texture, Taste, Consistency and General Acceptability) of the samples [15]. Ten untrained panel members were selected and they comprised of Lecturers, Laboratory technologists and Students of the Department of Microbiology who are very familiar with yoghurt. The Samples were served to all members of the panel with a glass of Water to rinse their mouth during the tasting exercise. The Scale used were 1; Dislike extremely, 2; Dislike very much, 3; Dislike moderately, 4; Dislike slightly, 5; Neither like nor dislike, 6; Like slightly, 7; Like moderately, 8; Like very much and 9; Like extremely [16].

2.8 Statistical Analysis

Data were subjected to analysis of variance(Anova) where it was appropriate and means separated by Duncan's Multiple Range test (DMRT) at 0.05 level of significance [16] using the statistical package for social sciences version 16 (SPSS).

3. RESULTS AND DISCUSSION

3.1 Lactic Acid Bacteria Isolates

One specie (1) of genus Streptococci and three (3) specie of lactobacillus were successfully isolated from samples of fermented cow milk (nono) using MRS media. The isolates were identified using conventional bio-chemical methods as presented in Table1. All the isolates were Gram positive and lack the ability to utilise citrate (negative reaction). Catalase test indicated that all isolates were non-catalase producing bacteria.

Further identification was carried out using standard API-50 CHL system represented in Table 2. The first tube lacked any active carbohydrate substrate and was used as negative control. Entire isolated microorganisms fermented glucose, fructose and galactose except LAB 4 that fermented lactose, which were indicated by the change of color from purple to pale yellow. However, there was variation in fermentation pattern of other substrates. LAB 1 was identified as *Lactobacillus acidophilus* after fermenting melibiose and raffinose LAB 2 was identified as *Lactobacillus bulgaricus* because it did not produce a darker color during Esculin hydrolysis, where as other isolates did. LAB 3 was identified as *Lactobacillus lactis*. Lab 4 was identified as *Streptococcus thermophilus* because it did not ferment lactose. Identified isolates were further maintained at 4ºC on MRS agar slants.

3.2 Proximate and Physicochemical Properties

The results of the proximate composition of tiger nut yoghurt (A) and common yoghurt brand (B) in New Karu, Nasarawa State are shown in Table 3. Sample A represents fermented tigernut-milk containing *Lactobacillus bulgaricus,* Sample B represents fermented tigernut-milk containing *Streptococcus thermophilus* as starter culture, and Sample C representing common yoghurt brand in New Karu Nasarawa State. From the proximate analysis result, no significant difference ($p>0.05$) was recorded between the moisture, ash and carbohydrate content of all samples, but there was significant difference ($p<0.05$) between the fat, protein and fibre content of sample C to that of samples A and B. The reason for the significant difference in the protein content of sample C from that of Samples A and B may be due to the high protein content of diary milk which is almost similar with the research done by Ogbonna et al. [17]. Also the reason for the significant difference in the fibre and fat content of sample A and B from that of sample C may be due to the high fibre and fat content of tiger nut milk which agrees with work done by Ogbonna et al. [17].

The values for pH and titratable acidity recorded no significant difference ($p>0.05$) for all of the samples, as the values were in line with the recommended pH of 4.0 to 4.4 and titratable acidity of 0.85 to 0.95 percent for fresh yoghurt [18].

3.3 Consumers' Sensory Evaluation

The preliminary consumers' sensory evaluation result from the 10 panelist for all samples is shown in Table 4. The result recorded no significant difference ($P>0.05$) in texture for all samples except for appearance, aroma, taste, consistency and overall acceptance. The significant difference ($P<0.05$) in appearance and overall acceptance of samples A and B could be

Table 1. Morphological and biochemical characteristics of isolated microorganism

Isolates	Characteristics on agar medium	Microscopic characteristics	Growth @ 10 °C	Growth @ 37 °C	Growth @ 45 °C	Catalase test	Citrate test	Indole test	Methyl red test
LAB 1	Small, fussy and flat	Gram positive, singly and tampering end	-	+	+	-	-	-	-
LAB 2	Circular, Irregular and off-white	Gram positive Chained rod	-	+	+	-	-	-	-
LAB 3	Small, flat, Creamy white	Gram positive Short chained rod	-	+	+	-	-	-	-
LAB 4	Small raised colonies and White	Gram positive and Cocci	-	+	+	-	-	-	-

Key: (+) Positive Reaction; (−) Negative Reaction

Table 2. Identification of isolated microorganisms using ApiWeb (v5.1) system

Isolates	Specie identified	Identification (%)
LAB 1	*Lactobacillus acidophilus*	96.9
LAB 2	*Lactobacillus bulgaricus*	86.3
LAB 3	*Lactobacillus lactis*	93.7
LAB 4	*Streptococcus thermophilus*	79.4

Key: (LAB) Lactic acid bacteria

Table 3. Proximate and physicochemical properties of fermented tigernut-milk and a common yoghurt brand in New Karu, Nasarawa State

Parameters	Sample A	Sample B	Sample C
Moisture %	55.73 ± 1.19[a]	55.89±0.19[a]	57.02±2.02[a]
Ash %	0.26 ± 0.01[a]	0.24±0.06[a]	0.23±0.01[a]
Carbohydrate %	28.89± 0.55[a]	29.32±0.38[a]	33.20±0.53[a]
Protein %	3.64± 0.05[a]	3.19±0.06[a]	5.12±0.13[b]
Fat %	8.18± 0.07[a]	8.71±0.09[a]	3.20±0.34[b]
Crude fiber %	3.65± 0.06[a]	3.43±0.05[a]	1.42±0.35[b]
pH	4.1 ± 0.57[a]	4.0±0.11[a]	4.20±0.15[a]
TA %	0.94± 0.01[a]	0.95±0.01[a]	0.94±0.02[a]

Each value is a mean ± standard deviation of triplicate determinations. Mean value in a row not sharing a common superscript letters are significantly (p<0.05) different as assessed by Duncan multiple Range Test.
Key:TA= titratable acidity
Sample A= Fermented tigernut- milk produced using Lactobacillus bulgaricus
Sample B= Fermented tigernut- milk produced using Streptococcus thermophilus
Sample C= Common yoghurt brand in New Karu, Nasarawa State

Table 4. Consumers' sensory evaluation score of fermented tigernut-milk and a common yoghurt brand in New Karu, Nasarwa State

Samples	Appearance	Aroma	Texture	Acceptability	Taste	Consistency
A	6.10±0.73[a]	6.10±0.87[a]	7.0±0.67[a]	5.70±0.48[a]	7.10±0.56[a]	5.40±0.51[a]
B	6.80±0.63[a]	5.30±0.48[a]	6.70±0.51[a]	5.20±0.42[a]	5.60±0.69[b]	5.30±0.48[a]
C	7.30±0.83[b]	8.40±0.76[b]	7.10±0.64[a]	8.10±0.65[b]	7.50±0.75[a]	7.20±0.63[b]

Each value is a mean ± standard deviation of triplicate determinations. Mean value in a row not sharing a common superscript letters are significantly (p<0.05) different as assessed by Duncan multiple Range Test.
KEY: Sample A= Fermented tigernut- milk produced using Lactobacillus bulgaricus
Sample B= Fermented tigernut- milk produced using Streptococcus thermophilus
Sample C= Common yoghurt brand in New Karu, Nasarawa State.

due to the brown colour of fermented tigernut-milk as suggested by Charles et al. [18] where as the common yoghurt brand (sample C) is white in color. Similarly, the significant difference (P<0.05) in aroma could be due to the aromatic profile of fermented tigernut-milk [13] to that of the common yoghurt brand in Keffi. For the significant difference (P<0.05) recorded for taste in sample B may be due to the low lactic acid and acetaldehyde produced by *Streptococcus thermophilus* as compared to the lactic acid and acetaldehyde produced by *Lactobacillus bulgaricus* in the study carried out by Gezginc et al. [19]. while for consistency, some of the panel members suggested that proper homogenisation of the fermented tigernut-milk may be improved upon.

4. CONCLUSION

The result obtained from the comparison of the proximate, physicochemical and preliminary consumers' sensory properties of fermented tigernut-milk (sample A and B) and a common yoghurt brand in New Karu, Nasarawa State (sample C), showed that fermented tigernut-milk if properly produced can be a good alternative to other fermented milk product.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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