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Production of Yoghurt from Milk Extract of Tigernut (*Cyperus esculentus*) Using Lactic Acid Bacteria Isolated from Locally Fermented Milk (Nono)

M. D. Makut[1], A. L. Olokun[1*] and R. M. Olokun[1]

[1]Department of Microbiology, Nasarawa State University Keffi, Nasarawa State, Nigeria.

Authors' contributions

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

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ABSTRACT

Aims: The aim of the study was to produce yoghurt from milk extract of tigernut (*Cyperus esculentus*) using lactic acid bacteria isolated from locally fermented milk (nono) with specific objectives of comparing the proximate composition of tigernut- yoghurt with a popular yoghurt brand in Keffi, Nasarawa State and determining the acceptance level of both products based on sensory evaluation.
Study Design: A comparative study of tiger nut yoghurt and a popular yoghurt brand in Keffi, Nasarawa State, Nigeria.
Place and Duration of Study: Department of Microbiology, Nasarawa State University Keffi, Nasarawa State, Nigeria, between December 2016 and October 2017.
Methodology: Belewu and Abodunrin's method was used for the extraction of milk from tigernut obtained in the market. The presumptive isolates from the locally fermented milk (nono) were confirmed using the API rapid identification test kit and sub-cultured to obtain pure isolates of lactic acid bacteria. The extracted tigernut milk was fermented with *Lactobacillus bulgaricus* and *Streptococuss thermophilus* in an anaerobic chamber and the resulting yoghurt was subjected to quantitative proximate analysis and sensory evaluation.

**Corresponding author: Email: lanzyko07@yahoo.com;*

Results: The result obtained from the proximate analysis showed no significant difference (p=.05) between the ash, carbohydrate and moisture content of all yoghurt samples but there were significant differences (p=.05) between the protein, crude fibre and fat content of both Samples. The pH of all samples was between 4.0-4.5 and titra-table acidity between 0.91-0.95. Also, the sensory evaluation result showed no significant difference (P=.05) in texture and taste except for appearance, aroma, consistency and overall acceptance

Conclusion: This study revealed that tiger nut milk can be a good substitute to cow milk for the production of yoghurt.

Keywords: Starter culture; fermentation; tiger nut milk; Lactobacillus bulgaricus and Streptococuss thermophilus.

1. INTRODUCTION

Yoghurt is a fermented product produced by bacterial fermentation of milk from animal and plant sources. Fermentation of the milk lactose leads to the production of lactic acid, which acts on milk protein (casein) to give yoghurt its texture and its characteristic [1]. In order to prevent ill effect resulting from consuming products containing lactose, individuals that are moderately lactose-intolerant prefer yoghurt to milk because bacteria in yoghurt can convert lactose present in milk into lactic acid [2].

Yoghurt is known to have medical uses, particularly for a variety of gastrointestinal conditions and in preventing antibiotic-associated diarrhoea. One study suggests that human consumption of yoghurt containing *Lactobacillus acidophilus* helps prevent *vulvovaginal candidiasis* [3].

Tiger nut (*Cyperus esculentus*) is a cosmopolitan perennial crop found all over the world. Its common names include; tiger nut, Aya (hausa), chuffa sedge (Spanish), yellow nutsedge and earth almond. The nut was found to be rich in myristic acid, oleic acid and linoleic acid [4]. Tiger nut is valued for the high starch dietary fibre, carbohydrate (mono, di and polysaccharides), mineral and oil content. According to [5], tiger nut has long been recognized for its health benefits as they are high in fibre, protein and natural sugars. They have 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 [6].

Tiger nut milk (also called Horchata in Spain) is a non-alcoholic beverage of milky appearance derived from the sweetened water extract of tiger nut tubers (*C. esculentus*), with the addition of sugar to taste [7]. It can serve as a superb substitute of traditional cow milk with a natural sweet taste The “horchata” 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 (between 100 and 120 g/L) [8].

2. MATERIALS AND METHODS

2.1 Sample and Sample Collection

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

2.2 Source of Inoculums

Inocula were isolated from nono (naturally fermented cow-milk). Cow milk samples were purchased from Fulani women in their settlement at Keffi, Nasarawa State. Collection of samples were done in a sterile bottle and taken to the laboratory for further analysis [9].

2.3 Microbiological Analysis

A total of five (5) nono samples were collected in sterile bags from different locations in Keffi, 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 in powdered form were prepared according to manufacturer’s instruction (TITAN Biotech Ltd., India) and sterilized at 121°C and 15 psi for 15 minutes

before cooling. Samples were homogenized 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 [10].

2.3.1 Enumeration of lactic acid bacteria

Enumeration of Lactic Acid Bacteria was performed by plating out appropriate dilutions using MRS agar. Plates were incubated in an inverted manner inside an anaerobic chamber at 37°C for 48 hours. Morphologically distinct colonies were sub-cultured and purified by streaking on agar plates repeatedly. Isolates were characterized after 48hours of incubation using: macroscopic examination for shape, elevation, size and pigmentation; microscopic examination by gram staining; growth at 15°C, 37°C and 45°C and biochemical methods such as catalase, citrate, methyl red and indole test were carried out [11]. Further identification of LAB strains by sugar fermentation was carried out using API 50 CHL system (Biomerieux® France). The identity of each isolate was also confirmed by comparing their characteristics with those of known taxa using Bergery's manual of bacteriology [12].

2.3.2 Identification of isolates

Conventional identification of isolates from locally fermented milk (nono) was done using gram staining technique and biochemical test.

For gram staining, smear fixation 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.

Biochemical test carried out were indole, citrate, catalase and methyl red test.

For indole test, the 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.

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

For catalase test, a drop of 3% hydrogen peroxide (H_2O_2) was added to a loopful of LAB culture.

For 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.

The LAB isolates were identified using rapid identification method that involved the use of API 50 CH kit (Biome-rieux) was used to differentiate LAB isolates at strains level. Wells in the incubation trays were filled with sterile distilled water to create a humid atmosphere, 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 profile using apiwebTM (Version 5.1).

Identified strains were then transferred onto fresh medium and subcultured every 2 weeks for proper storage.

2.4 Extraction of Milk from Tiger Nut

The method of Belewu and Abodunrin [13] was used. Briefly, the nuts were properly picked to remove the stone, infected nut and other debris. After which, 1 kg of the tiger nut was washed and soaked in 8 litres of distilled water for 24 hours. The total content was washed again with distilled water and blended several times with a blender. The milled tiger-nut meaty part was filtered with a muslin cloth 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 Tiger nut Yoghurt

Starter culture of tiger nut milk was prepared, containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. One litre of tiger nut milk was poured into adiabatic jars for fermentation. At temperature 43°C, tiger nut milk was inoculated with 4% v/v of starter culture containing *Lactobacillus bulgaricus* and

Streptococcus thermophilus. The jar was covered and incubated at 42°C for 4 hours [14], to coagulate into yoghurt. The resulting tigernut-yoghurt was labelled Sample A and was compared with a Sample B containing a popular yoghurt brand in Keffi, Nasarawa State.

2.6 Physicochemical Analysis

Measurement of pH was carried on all samples with a standardized pH meter and titratable acidity was determined by titration of samples against 0.1 NaOH according to methods described in AOAC [15].

2.7 Proximate Analysis

Moisture, fat, ash, protein, carbohydrate and crude fibre content were also determined according to methods described in AOAC [15].

2.8 Sensory Evaluation

A 9 point hedonic Scale was used in determining the Sensory qualities (Appearance, Aroma, Texture, Taste, Consistency and General Acceptability) of the samples [16]. Ten untrained panel members comprising of Lecturers, Laboratory technologists and Students of the Department of Microbiology who are very familiar with yoghurt. The Samples were served to the panel members with a glass of Water to rinse their mouth in the course of 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 [17].

2.9 Statistical Analysis

Data were subjected to analysis of variance where it was appropriate and means separated by Duncan's Multiple Range test (DMRT) at 0.05 level of significance [18].

3. RESULTS AND DISCUSSION

3.1 Lactic Acid Bacteria Isolates

Three (3) species of genus Lactobacillus and one specie (1) of genus Streptococci 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 utilize 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 colour from purple to pale yellow. However, there was variation in fermentation pattern of other substrates. LAB 1 was identified as *Lactobacillus bulgaricus* because it did not produce a darker colour during Esculin hydrolysis, whereas other isolates did. LAB 2 was identified as *Lactobacillus lactis*. LAB 3 was identified as *Lactobacillus acidophilus*after fermenting melibiose and raffinose. Lab 4 was identified as *Streptococcus thermophilus* because it did not ferment lactose. Identified isolates were maintained at 4°C on MRS agar slants.

Images of the produced tigernut-yoghurt are presented in Fig. 1 and Fig. 2.

Fig. 1. Front view of Tigernut-Yoghurt (end product)

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	Methyl red test	Citrate test	Catalase test	Indole test
LAB 1	Circular, Irregular and off-white	Gram positive Chained rod	-	+	+	-	-	-	-
LAB 2	Small, flat, Creamy white	Gram positive Short chained rod	-	+	+	-	-	-	-
LAB 3	Small, flat and fussy	Gram positive, singly and tampering end	-	+	+	-	-	-	-
LAB 4	Small raised colonies and White	Gram positive and Cocci	-	+	+	-	-	-	-

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

Fig. 2. Top view of tigernut-yoghurt (end product)

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

Isolates	Specie identified	Identification (%)
LAB 1	*Lactobacillus bulgaricus*	97.9
LAB 2	*Lactobacillus lactis*	84.3
LAB 3	*Lactobacillus acidophilus*	92.7
LAB 4	*Streptococcus thermophilus*	78.9

KEY: (LAB) Lactic acid bacteria

3.2 Proximate and Physicochemical Properties

The results of the proximate composition of tiger nut yoghurt (A) and popular yoghurt brand (B) in Keffi, Nasarawa State are shown in Table 3. Sample A represents tigernut- yoghurt containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as starter culture and Sample B representing popular yoghurt brand in Keffi. From the result, there is no significant difference (p=.05) between the ash, carbohydrate and moisture content of samples A & B, but there was significant difference (p=.05) between the protein, fat and fibre content of sample B to that of sample A. The reason for the significant difference in the protein content of sample B from that of Sample A may be attributed to the high protein content of diary milk. Also the reason for the significant difference in the fibre and fat content of sample A from that of sample B may be attributed to the high fibre and fat content of tiger nut milk.

The values for pH and titratable acidity showed no significant difference (p=.05) for all samples, as the values were so close, which is 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.].

Table 3. Proximate and physicochemical properties of tigernut-yoghurt and popular yoghurt brand in Keffi, Nasarawa State

Parameters	Sample A	Sample B
Moisture %	54.56 ± 1.92^{a}	55.49 ± 2.00^{a}
Ash %	0.29 ± 0.01^{a}	0.21 ± 0.01^{a}
Protein %	3.45 ± 0.06^{a}	4.98 ± 0.15^{b}
Fat %	7.63 ± 0.07^{b}	4.84 ± 0.43^{a}
Carbohydrate %	30.03 ± 1.07^{a}	32.75 ± 0.41^{a}
Crude fiber %	3.24 ± 0.12^{b}	1.90 ± 0.45^{a}
pH	4.10 ± 0.10^{a}	4.20 ± 0.15^{a}
TA %	0.91 ± 0.02^{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=.05) different as assessed by Duncan multiple Range Test.
Key: TA=titratable acidity
Sample A=Tigernut-Yoghurt
Sample B=Popular yoghurt brand in Keffi, Nasarawa State

Table 4. Sensory evaluation score of tigernut-yoghurt and popular yoghurt brand in Keffi samples appearance aroma texture acceptability taste consistency

Samples	Appearance	Aroma	Texture	Acceptability	Taste	Consistency
A	6.10±0.88[a]	6.0±0.82[a]	7.30±0.67[a]	5.80±0.63[a]	7.20±0.63[a]	5.50± 0.53[a]
B	8.10±0.73[b]	7.30±0.67[b]	7.30±0.66[a]	8.30±0.65[b]	7.40±0.70[a]	7.30±0.67[b]

Each value is a mean ± standard deviation of triplicate determinations. Mean value in a row not sharing a common superscript letter are significantly (p=.05) different as assessed by Duncan multiple Range Test.
KEY: Sample A=Tigernut-Yoghurt
Sample B=Popular yoghurt brand in Keffi, Nasarawa State.

3.3 Sensory Evaluation

Sensory evaluation result from the 10 panellist for all samples is shown in Table 4. The result showed no significant difference (P=.05) in texture and taste for all samples except for appearance, aroma, consistency and overall acceptance. The significant difference (P=.05) in appearance and overall acceptance could be attributed to brown coloured tigernut-yoghurt whereas the popular yoghurt brand is white. Similarly, the significant difference (P=.05) in aroma could be attributed to aromatic profile of tigernut-yoghurt and the popular yoghurt brand in Keffi. The fact that tigernut-milk and dairy milk is plant and animal sourced, respectively could also contribute to the significant difference in aroma of the two yoghurt types. For consistency, some of the panel members suggested that effective homogenization of the tiger nut yoghurt be improved upon especially after fermentation.

4. CONCLUSION

From the comparison of the proximate, physicochemical and sensory properties of tigernut-yoghurt (sample A) and popular yoghurt brand in keffi (sample B),tigernut-yoghurt if properly produced can be a good substitute to diary yoghurt. However, to realise the full potential of tiger nut yoghurt, there is a need for further study on the shelf life.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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