Original Research Article

COMPARISON OF YOGHURT ATTRIBUTES PREPARED FROM TIGER NUT (*Cyperus esculentus L.*) AND DIFFERENT FORMS OF COW MILK

ABSTRACT

Yoghurt made from cow milk is popular due to its delicioustaste and nutritional benefits. However, concerns about animal-based proteins have led to the evaluation of alternative options. This study examines thephysiochemical, microbial and sensory characteristics of yoghurt made from tiger nut (*Cyperus esculentus L.)* milkcompared to various forms of cow milk. Yoghurt was produced from tigernut milk, full cream, fat-filled, and skim milk by fermenting the milk samples at 42 °C for 8 hours,using a starter culture. A commercial brand was used as a control. The samples were analyzed for physicochemicalproperties, microbial content and sensory attributes using a 9-point hedonic scale. The pH values ranged from 3.97 to 4.65 for tiger nut and skim milk yoghurts,respectively, while titratable acid ranged from 0.088 to 0.095 %. The control sample had the lowesttotal plate and lactic acid bacteria count values of 1.361x10^5 and 1.063 x10^5 CFU/ml, while full cream milk had the highest values of 2.123x10^5 and 1.853x10^5 CFU/ml, respectively. The identified microorganisms were *Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Streptococcus thermophilus.*Overall acceptabilityscores ranged from 6.88to7.63 for skim and full-cream yoghurt, withtiger nut yoghurt scoring 7.23. Therefore, tiger nut milk shows promise as a plant-basedalternative for sustainableyoghurt production.

Keywords: Yoghurt, plant-based, milk, probiotics, microbial, sensoryevaluation.

INTRODUCTION

The growing global population interested in healthy food options hasled to a significant push to enhance food variety and nutrition. Meeting theseinterests through animal sourcesalone is far-fetched, raising the need for sustainable plant-based alternatives. Also, plant-based diets have fewer calories required to address the challenges associated with high blood pressure, obesity, lactose intolerance and ethics of vegetarian diets [1,2].

Yoghurt is a fermented milk beverage listed as one of the healthiest food products, resulting mainly from its nutritional composition and probiotic quality[3, 4].Probiotics are living microorganisms that, when consumed, have positive effects on the host, such as improved digestibility, enhancement of microflora, and immune stability[5].

Cow milk is known for its high protein content, including a complex mix of fats, minerals and vitamins[6,7]. Fullcream milk still retains its natural fat content.Skim milk is produced by completely removing milkfat from fullcream milk [8]. Fat-filled milk is formulated by blending skim milk with non-dairy fats such as vegetable oils [9]. Numerous industries presently utilizefat-filled milk in yoghurt production as a cost-effective alternative insteadof fullcream.However, the affordability of plant-based milk tends to be more promising [10].

Tiger nut (*Cyperus esculentus* L.) an underutilized nut, is chosen for its high levels of essential nutrients, health benefits, affordability and availability[2,11,4]. Itsmilk content is high. However, few studieshavecompared tiger nut milk, especially with fat-filled milk for yoghurt. Therefore, this research aimed to compare the physiochemical, microbial and sensory attributes of yoghurt produced from tiger nuts and different forms of cow milk.

MATERIALS AND METHODS

The samples used for this research were Dano milk powder (full cream, skim and fat-filled), fresh tiger nut tubers, freeze-dried starter culture (yogourmet) and a commercially knownbrand of yoghurt to serve as the control. All were obtained from a local market in Enugu state and transported to the laboratory for analysis.

Sample preparation

Preparation of tiger nut milk

The method employed had slight modifications [11]. Freshtiger nuts were sorted, washed and drained.Water (2 L) was added to 500gof tiger nutandplaced in a water bath at 45 °C for 12 h, to enhance softening, milling and milk extraction. The hydrated nuts were drained and milled using a blender at 1800 rpm for 5 min.After that, distilled water (1L)was added to form tiger nut mush. Then, strainthrough a cheesecloth, and the filtrate (tiger nut milk) was pasteurized for15min at 75 °C [2]. The milk was cooledto 45 °C for inoculation and fermentation.

Preparation of cow milk

Full cream powdered cow milk (600 g) was dissolved in 1.5 L of distilled water and stirred properly. It was pasteurized at 85 °C for 5min and cooled to 45 °C for inoculation,followed by fermentation.This same procedure was used for skim and fat-filled milk powders [12].

Preparation of yoghurt the yoghurt

Each sample (1 L) of tiger nut, full cream, skim and fat-filled milk was immediately inoculated with 5g of a freeze-dried starter culture(yogourmet), in separate beakers.After inoculation, samples were tightly covered and placed in an incubator at 42 °C for 8 h to ferment. The fermented samples (Yoghurt) were refrigerated at 4 °C for further analysis [13].

Physiochemical attributes of the yoghurt samples

Determination of pH values

The pH values of each sample were measuredusing a pH meter. The pH meter was standardized by testing the buffer solutions of known pH.This aimed to test the acidity level of the yoghurt samples [14].

Determination of Titratable Acidity (TA)

Each yoghurt sample (1g) was mixed with 10 ml of hot distilled water (90 °C) and titrated to a faint colour with 0.1N NaOH comprising 0.5% phenolphthalein as an indicator. The percentage of lactic acid produced by fermentation in the sample was determined as follows [14].

$$\text{Titre value} \times 0.09 \times 100\ \%$$

(Where the Titre value is the Volume of yoghurt sample solution used; 0.09 is a conversion factor).

Microbial and biochemical determination

Determination of microbial count

Homogenized yoghurt samples (1 ml) eachwere aseptically transferred into a corresponding sterile test tube containing 9 ml of distilled water up to a four-fold serial dilution. Using the pour plate method, dilutions 2 and 4 were cultured on MRS and Nutrient Agar,the plates were incubated at 37°C for 24h and the colonieswere counted forbacteria load. All counts were expressed asCFU/ml. Pure cultures were obtained by sub-culturing on sterile fresh MRS and nutrient agar plates for Lactic acid bacteria count and Total coliform count, respectively. The plates were incubated at 37°C for 24h. The obtained pure culture was stored on agar slants and refrigerated at 4°C. Isolates were identified using morphological, biochemical and gram-staining tests [15,12].

Catalase test

The catalase test is used to distinguish microorganisms that can produce catalase enzymes. A clear, grease-free slide was treated with 3% hydrogen peroxide (H_2O_2), and a small amount of each bacterial isolate was placed on the glass slide using a sterile inoculating loop, allowing for the isolate's bubbles to develop.Bubbles show catalase positive, while the absence means catalase negative [16].

Oxidase test

The ability of organisms to produce cytochrome oxidase enzymes is employed in oxidase tests. The oxidase reagent was freshly prepared into a 1% solution, and filter paper strips were immersed. The culture was scratched with the inoculating wire loop. Positive reactions are indicated by a vivid, deep-purple hue that appears within 5–10 seconds, while adverse reactions are indicated by a lack of colour [17].

Coagulase test

The coagulase test differentiates pathogenic from non-pathogenic test organisms through their ability to coagulate blood plasma. Two distinct grease-free slides each received a few drops of saline, and a loop of the bacterial isolates was emulsified on slides to create two suspensions. A sterile Pasteur pipette was used to collect a drop of human plasma, which was then gently mixed on the slides. The glass was checked for clumping after 5–10 minutes. The presence of clumping indicated coagulase-positive, while the absence showed coagulase-negative[14].

Gram staining

This test was employed to test the organisms microscopically and differentiate Gram-negative from Gram-positive using coloured stains. Smear was created, placed on a spotless glass slide, and stained for 30 seconds with crystal violet. The smear was then cleaned with distilled water. Gram's iodine was applied for 10 seconds, after which the smear was washed with tap water, decoloured with 95% acetone alcohol, and dyed with safranin for 30 seconds. The smear was then rinsed with tap water, dried by air, and examined with a 100X oil immersion objective [14].

Determination of Sensory attributes of the yoghurt samples

The yoghurt samples were coded and evaluated for sensory attributes by 100 individuals. Multistage selection was applied, such that various age groups, genders, occupations, and social strata were randomly chosen among students and staff of the university. A glass of water was given to each panelist so they could rinse their mouths after tasting each sample. They were also given questionnaires to score the yoghurt samples for appearance, colour, aroma, taste, consistency and overall acceptability, using a 9-point hedonic scale ranging from 0 (extremely dislike) to 9 (extremely like) [18].

Statistical Analysis

All analyses were performed in triplicate and presented as mean ± standard deviation. Statistical Analysis of Variance (ANOVA) using SPSS version 28 (SPSS, Inc., USA) was applied for means variation,while theDuncan Multiple Range Test (DMRT) at an acceptable level of $p \leq 0.05$ was utilized for the separation of means [19].

RESULTS AND DISCUSSION

Microbialattributes

The physiochemical properties and microbiological loads of the yoghurt samples are presented in Table 1. Physicochemical attributesmeasured in this study are pH and titratable acid values, which arerequired to denote the acidity and possibleshelfstability of the yoghurt.

Table 1: Physiochemical and microbial count of yoghurt samples made from tiger nut milk and different forms of cow milk.

SAMPLE	pH	TITRATABLE ACID (%)	TOTAL PLATE COUNT (CFU/ml)	LAB COUNT (CFU/ml)
FC	4.31±0.076^{b}	0.090±0.002^{a}	2.123x10^{5}±0.035^{d}	1.853 x10^{5}±0.025^{d}
SK	4.65±0.035^{d}	0.088±0.001^{a}	1.960 x10^{5}±0.010^{c}	1.570 x10^{5}±0.026^{b}
FL	4.36±0.030^{b}	0.092±0.003^{a}	1.747 x10^{5}±0.015^{b}	1.092 x10^{5}±0.028^{a}
TN	3.97±0.017^{a}	0.095±0.010^{b}	1.750 x10^{5}±0.030^{b}	1.630 x10^{5}±0.026^{c}
CN	4.53±0.035^{c}	0.091±0.002^{a}	1.361 x10^{5}±0.029^{a}	1.063 x10^{5}±0.047^{a}

Means in the same column with same superscript letters are not significantly different at $p \leq 0.05$. FC- Full cream milk yoghurt; Sk – Skim milk yoghurt; FL – Fat-filled milk yoghurt; TN – Tiger nut milk Yoghurt; CN – Commercial Yoghurt (Control), LAB – Lactic Acid Bacteria.

The microbial attributes evaluated are Total Plate Count (TPC) and Lactic Acid Bacterial (LAB) count of the yoghurt samples.This indicates the general active culture and expected probiotic loads in each sample.pH indicates the extent of acidity or alkalinity of a medium.The pH values differed significantly ($P \leq 0.05$). Tiger nut milk yoghurt exhibited the significantly lowest acidicvalue of 3.97,while skim milk yoghurt had the highestat 4.65.

Titratable acidity (TA)measures acid(s) quantity in amedium. The titratable acid levels in the samples did not differ significantly, except fortiger nut milk yoghurt, which had the highest significant value of 0.95 %.

Microbiological loads, as shown in Table 1, reveal a total microbial count ranging from 1.36×10^5 to 2.13×10^5 CFU/ml, while lactic acid bacteria (LAB) ranged from 1.063×10^5 to 1.85×10^5 CFU/ml.

Table 2: Morphological and biochemical features of yoghurt samples made from tiger nut milk and different forms of milk.

Biochemical Feature	**LAB 1**	**LAB 2**	**LAB 3**
Shape	Coci	Rod	Rod
Cell Setting	Single/ short	Circular	Paired/ long
Texture	Dry	Moist	Wet
Colour	Creamy	Off-white	Whitish
Elevation	Flat	Irregular	Raised
Appearance	Opaque	Opaque	Shinny
Catalase	-	-	-

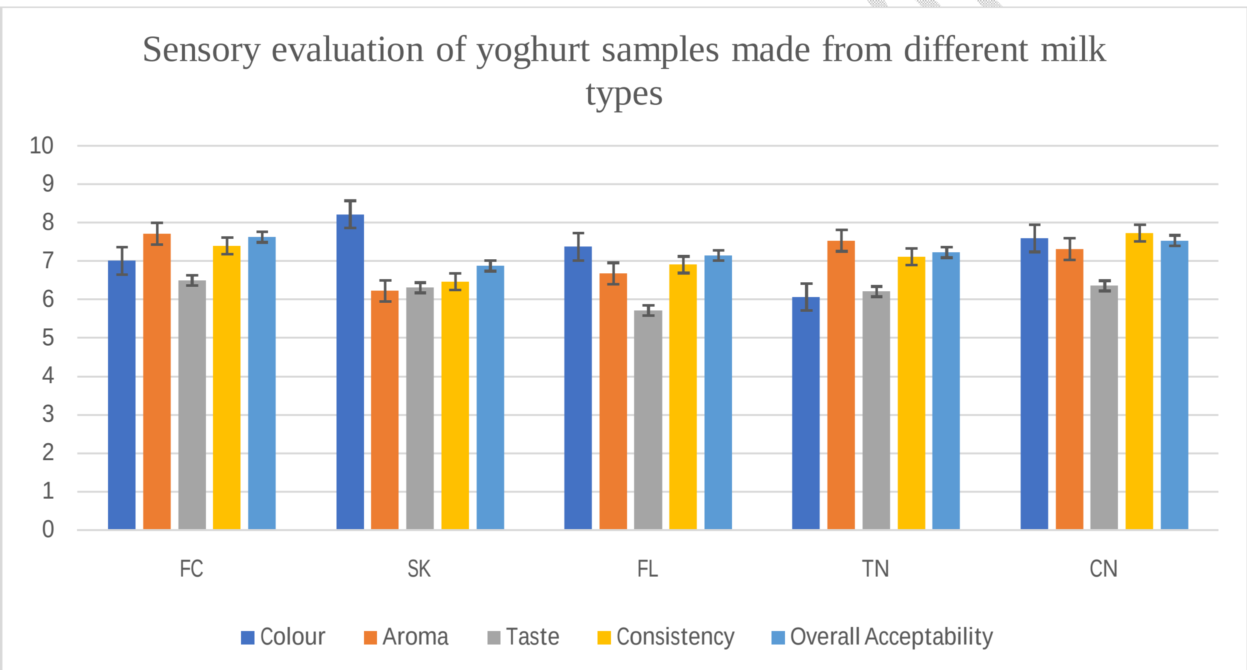
Oxidase	-	-	-
Coagulase	+	+	+
Gram staining	+	+	+
Probable Organism	*Lactobacillus acidophilus*	*Lactobacillus bulgaricus*	*Streptococcus thermophilus*

After determining the LAB count in the samples, it became necessary to identify these bacteria further to indicate the probable organisms in the samples.Microbialoccurrences are Indigenous to fermented foods, with LaticAcids Bacteria being more dominant in yoghurts.*Lactobacillusacidophilus*, *Lactobacillus bulgaricus* and *Streptococcusthermophilus*, were identified as theprobable organisms after morphological and biochemical tests were conductedin all the yoghurt samples.

Sensory evaluation

The sensory attributes of colour, aroma, taste, consistency and overall acceptability of yoghurt samples made from tiger nut milk and different forms of cow milkare presented in Figure 1. The sensory values for all parameters ranged from 6.07 to 8.22 for colour, 6.23 to 7.72 for aroma, 5.72 to 6.50 for taste, 6.91 to 7.74 for consistency and 6.88 to 7.63 foroverall acceptability.

Tiger nut milk and skim milk had the highest and lowest colour values of 6.07 and 8.22. The highest aroma score was for full cream milk yoghurt (7.72), followed by tiger nut milk yoghurt(7.54), with skim milk yoghurt scoring the lowest (6.23).Taste scores ranged from 5.72 for fat-filled milk yoghurt to 6.50 for full-cream milk yoghurt, with the control (6.36), skim milkyoghurt (6.31) and tiger nut milkyoghurt (6.21) falling in between. The control sample hadthe highest consistency score (7.74), followed by full cream milk (7.4) and tiger nut (7.12) yoghurts, while skim milk had the lowest (6.47). Panelists preferred full cream milk yoghurt (7.63) over others, followed by the control sample (7.54), with skim milk yoghurt being the least accepted(6.88). The familiarity of panelists with full cream milk yoghurtmay explain the preference. Tiger nut milk yoghurt received a high score of 7.23 and was the third most accepted yoghurt sample.



FC- Full cream milk yoghurt; Sk – Skim milk yoghurt; FL – Fat-Filled milk yoghurt; TN – Tiger nut Yoghurt;

CN – Commercial Yoghurt (Control)

Figure 1: Sensory evaluation of yoghurt samples made from tiger nut milk and differentforms of cow milk.

Discussion

The yoghurt samples examined in this study are within the recommended pH range of 4-4.50. However, it is essential to note that the pH of the skim milk sample differs slightly from this range, measuring at 4.65. The pH values of this study were found to be comparable to aprevious report[21]. This similarity is desirable because it indicates that a pH level below 4.5 may be sufficient to effectively prevent the proliferation of undesirable microorganisms, thereby extending its shelf life [11].

A progressive rise in titratable acid levels and a corresponding decrease in pH values during fermentation is expected [21]. It is recommended that TA levels fall into 0.85 - 0.95 % range, which agrees with the values obtained in this study (0.88 – 0.95 %). Similar results were obtained in studies by [3,11].

Quantification of LAB is critical in the yoghurt manufacturing process, necessitating the evaluation of LAB as a crucial factor in determining the overall microbial composition of yoghurt products. The full cream milk yoghurt had the highest significant count in both cases, while the control sample showed the lowest. The control sample may contain chemical preservatives to regulate the microbial load and extend the shelf life of the yoghurt [21]. The observed microbial counts in this study indicate a significant level of microbial load in the fermented yoghurts, which is thought to be a positive characteristic of the presence of active probiotic organisms [2]. The values found in this study are slightly lower than a previous study on yoghurt derived from public school sources[3]. This could have resulted from the operating procedures since the yoghurts in this study were produced in a laboratory adhering to strict hygiene control measures.

Microbial occurrences are Indigenous to fermented foods, with Latic Acids Bacteria being more dominant in yoghurts. This is because as yoghurt fermentation progresses pH decreases and inhibits the growth of many microorganisms, while LAB dominates [1]. This indicates thattiger nut milk can serve as agood substitute for cow milk if probiotic composition is the main interest [23].

The sensory values show to what extent the yoghurt samples are accepted by consumers and the possible sensory features that call for improvement [1].

Colour is a required attribute for measuring dairy and non-diary products of milk because white or creamy colour are universally seen as milk colour. Consequently, this unconsciously affects the extent to whichmilk or related products are accepted. Similarcolour values were obtained from tiger nut and coconut milk, ranging from 6.2-6.8 [23]. The lower value of tiger nut milk yoghurt fromthis study may be due to the brownish pigments in whole tiger nuts, which were leached during milk extraction.

The aromavalues supportthe previous studies comparing tiger nut milk yogurt with fresh cow milk and other milk forms. The observation could be attributed to the diverse array of volatile aromatic chemicals and acetaldehyde produced by microbes present in tiger nut yoghurt during the breakdown of carbohydrates, enhancing its aroma [21]. The absence of fat in the skim milk could have been a limiting factor to its aroma values.

The taste of a product determines to what extent a product appeals to the sweetness or sourness of the yoghurt[11]. Lower taste scores (4.90-5.95) were observedin this study. This observation could be becauseanimal protein sources have improved sensory perception of mouthfeel when consuming products like yoghurt. Of which fat-filled and tiger nut milk yoghurt consists of plant-based fat.

Consistency displays the smoothness, thickness, and mouthfeel of yoghurt samples. The fat content in milk products enhances its mouthfeel and emulsifying features [11]. Consequently, skim milk with no fat content exhibited the least consistency. Although skim milk is perceived to be healthier, many consumers prefer sensory over nutritional attributes.

Overall acceptability reveals to what extent consumers like or dislike a product on a general note,considering all tested parameters [21]. Therefore, tiger nut stands a chance of being a suitableoption for making yoghurt,considering its acceptability, health benefits, affordability and availability [2].

CONCLUSION

Yoghurt made from tiger nut milk and various forms of cow milk displayed similarphysicochemical, microbial and sensory characteristics. The yoghurt made from full-cream milk had the highest microbial count and sensory attributes. Additionally, tiger nut milk yoghurt exhibited favourablesensoryqualities and acceptable microbial values. These findings suggest that tiger nut milk is a viable plant-based substitute for cow milk in yoghurtproduction. It is advisable to explore other plant-based milk alternativesfor making yoghurtinstead of cow milk. This will lead to a wider range of sustainable options.

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