

Original Research Article

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

Abstract

Yoghurt made from cow milk is popularly consumed because of its appealing taste and nutritious benefits. However, concerns about animal-based proteins call for action. This study evaluates the microbial and sensory attributes of yoghurt produced from tiger nut (*Cyperus esculentus L.*) milk and different forms of cow milk. Yoghurt was produced from tigernut milk and different forms of cow milk namely: full cream, fat-filled, and skim milk. By fermenting the milk samples at 42 °C for 8 h using a starter culture. A brand of commercial yoghurt was used as the control. The samples were evaluated for physicochemical, microbial using morphological and biochemical techniques and sensory attributes using multistage selection with 9-point hedonic scale. The pH value significantly ranged from 3.97-4.65 for tiger nut and skim milk yoghurts, respectively. While a reverse occurred with titratable acid ranging from 0.088-0.095 %. For both total plate and lactic acid bacteria count, control sample had the significantly lowest values of 1.361×10^5 and 1.063×10^5 CFU/ml, while full cream milk had the highest values of 2.123×10^5 and 1.853×10^5 CFU/ml, respectively. The isolated microorganisms were *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Overall acceptability ranged from 6.88-7.63 for skim and full-cream yoghurt, respectively. While tiger nut yoghurt scored 7.23. Therefore, tiger nut milk is a suitable plant-based alternative to cow milk for sustainable yoghurt production.

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

Comment [M1]: COMPARISON OF THE PHYSICOCHEMICAL, MICROBIAL AND SENSORY QUALITY OF YOGHURT PREPARED FROM TIGER NUT (*Cyperus esculentus L.*) AND DIFFERENT FORMS OF COW MILK

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Comment [M4]: physicochemical properties, microbial quality

Note: morphological and biochemical techniques are basically used for the identification of bacteria. Also, the identified bacteria must be provided in the abstract and the results section.

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Comment [M8]: Please provide a conclusion statement about the work base on the outcome of the study.

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Introduction

The growing global population interested in healthy food options have led to a significant push to enhance food variety and nutrition. Meeting these interests, through animal sources alone 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 [5,3].

Cow milk is known for its high protein content including a complex mix of fats, minerals and vitamins[6,7]. Full cream milk still retains its natural fat content. Skim milk is produced by completely removing milkfat from full cream milk [8]. While, fat-filled milk is formulated by blending skim milk with non-dairy fats such as vegetable oils [9]. Numerous industries presently utilize fat-filled milk in yoghurt production as a cost-effective alternative instead of full cream. 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]. Its milk content is high. However, not many studies have compared tiger nut milk, especially with fat-filled milk for yoghurt. Therefore, this research aimed to compare the microbial and sensory attributes of yoghurt produced from tiger nut and different forms of cow milk.

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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 known yoghurt to serve as the control. All were obtained from a local market in Enugu state and transported to the laboratory for analysis.

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Sample preparation

Preparation of tiger nut milk

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The method employed had slight modification [11]. Fresh tiger nuts were sorted, washed and drained. Water (2 L) was added to 500g of tiger nut, placed in a water bath at 45 °C for 12 hours, 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 (1 L) was added to form tiger nut mush. Then, strain through a cheesecloth, and the filtrate (tiger nut milk) was pasteurised for 15 min at 75 °C [2]. The milk was cooled to 45 °C for inoculation and fermentation.

Comment [M16]: The method of Oladipo et al. [11] was adopted with little modification for the preparation of tiger nut milk.

Comment [M17]: 500g of tiger nut was soaked in 2litres of water and placed in water bath at 45° C for 12 hours.

Comment [M18]: 1 liter of distilled water

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Preparation of cow milk

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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 5 min and cooled to 45 °C for inoculation, followed by fermentation. This same procedure was used for skim and fat-filled milk powders [12].

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Preparation of yoghurt with the different forms of cow milk

Comment [M24]: Did you used the same condition for the fermentation of each of the milk products.

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].

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Comment [M26]: One liter of of tiger nut, full cream, skim and fat-filled milk were immediately inoculated with 5g of a freeze-dried starter culture (yogourmet) in a separate beakers.

Physiochemical attributes of the yoghurt samples

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Determination of pH values

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

Determination of Titratable Acidity (TA)

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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].

Comment [M30]: One gram (1 g) of each yoghurt sample

Comment [M31]: indicator [14].

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

(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

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Homogenized yoghurt samples (1 ml) each were 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 colonies were counted for bacteria load. All counts were expressed as CFU/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 24 h. 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].

Comment [M34]: One milliliter (1 mL) of homogenized yoghurt sample was

Comment [M35]: and serially diluted to four-fold serial dilution.

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Comment [M37]: inoculated

Comment [M38]: What is MRS? Please write it in full first before you abbreviate it

Comment [M39]: Pure isolates

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Catalase test

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The catalase test is used to distinguish microorganisms that can produce catalase enzymes. A clear, grease-free slide was treated with 3% hydrogen peroxide (H₂O₂), 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].

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Comment [M44]: Presence of bubbles indicate positive test while absence of bubbles indicates negative test

Oxidase test

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The ability of organisms to produce cytochrome oxidase enzymes is employed in oxidase tests.

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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].

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Coagulase test

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The coagulase test is utilized for differentiating 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].

Comment [M49]: REMOVE

Comment [M50]: create a smear

Gram staining

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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].

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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 panellist 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].

Comment [M53]: A smear of the bacteria isolated was prepared and allowed to dry. The smear was covered with crystal violet FOR 30 seconds, and washed with distilled water. It was then covered with Gram's iodine for 10 seconds, after which it was washed with water. The smear was then decolorized with 95 % acetone-alcohol, and immediately washed with water. The smear was then counterstain with safranin for 30 seconds, and washed with water. The smear was then allowed to dried by air, and examined with a 100X oil immersion objective [14].

Comment [M54]: This should come before the biochemical test.

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Statistical Analysis

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

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Results and Discussion

Microbial evaluation

The physicochemical properties and microbiological loads of the yoghurt samples are presented in Table

1. Physicochemical attributes measured in this study are pH and titratable acid values, required to denote the acidity and possible shelf stability of the yoghurt.

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

SAMPLE	pH	TITRATABLE ACID (%)	TOTAL COUNT (CFU/ml)	PLATE 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 ^d

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 acidic value of 3.97, while skim milk yoghurt had the highest of 4.65.

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Also, you should separate the results of the physicochemical properties and that of the microbial quality of the samples.

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Comment [M61]: Separate the table into two:
Table 1: Physicochemical.....
Table 2: Microbial Quality.....

Titrateable acidity (TA) measures acid(s) quantity in a medium. The titrateable acid levels in the samples did not differ significantly, except for tiger 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	LAB 1	LAB 2	LAB 3
Feature			
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	<i>Lactobacillus acidophilus</i>	<i>Lactobacillus bulgaricus</i>	<i>Streptococcus thermophilus</i>

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Comment [M63]: These are not biochemical test. Here, you should separate the Table in two:
 Table 3: Morphological Characteristics of the Bacteria Isolated from the Samples.
 Table 4: Gram Reaction and Biochemical Characteristics of the
 Note: the different samples used should also be included in each table to show where the bacteria was isolated from.

After determining the LAB count in the samples, it became necessary to identify these bacteria further to indicate the probable organisms present in the samples. Microbial occurrences are indigenous to fermented foods, with Lactic Acids Bacteria being more dominant in yoghurts. *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, were identified as probable organisms after morphological and biochemical tests were conducted in all the yoghurt samples.

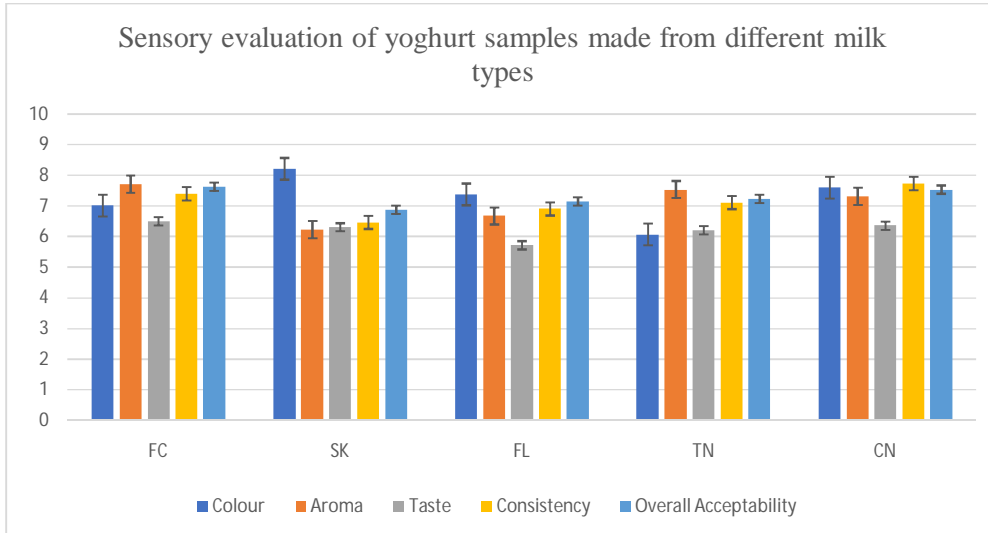
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Sensory evaluation

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The findings of sensory attributes including colour, aroma, taste, consistency and overall acceptability of the yoghurt samples are shown in Figure 1. The result exhibits that the sensory values of all measured parameters ranged from 6.07-8.22, 6.23-7.72, 5.72-6.50, 6.91-7.74 and 6.88-7.63 for colour, aroma, taste, consistency and overall acceptability, respectively. The colour values for tiger nut milk and skim milk ranged from 6.07 (lowest) to 8.22 (highest). The highest average aroma for the panelists was scored for full cream milk (7.72), followed by tiger nut milk (7.54) yoghurt. While the least score was observed for skim milk (6.23) yoghurt. For this study, it was shown that the least taste scores of 5.72 of fat-filled milk yoghurt was observed as compared to full-cream milk being the highest (6.50), followed by control (6.36), skim milk (6.31) and tiger nut milk (6.21) yoghurt. The control sample with 7.74 value showed the highest consistency. Next was the full cream sample of 7.4, then tiger nut of 7.12, while skim milk was the least (6.47). The observation indicates that the panelist preferred the full cream milk yoghurt (7.63) when compared to others, followed by the control sample (7.54), while skimmed milk (6.88) yoghurt was the least accepted. The familiarity of panelists with full cream milk used as a standard for yoghurt production may account for the observed preference. However, tiger nut milk yoghurt showed a high score of 7.23 and was the third most accepted yoghurt sample.

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Colour
Aroma
Taste
Consistency
.....



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 forms 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 previous report [21]. This similarity is desirable because it indicates that the pH level below 3.5 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 the fermentation process is expected [21]. It is recommended that TA levels fall into 0.85 - 0.95 %

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range, which agrees with the values obtained in this study (0.88 – 0.95 %). Similar results were obtained in studies by [22,11].

Quantification of LAB is critical in the yoghurt manufacturing process, necessitating the evaluation of LAB as a critical 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 [22]. 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 Lactic 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 finding is similar to that in the production of yoghurt, tiger nut milk can serve as good 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-dairy products of milk because white or creamy colour are universally seen as milk colour. Consequently, this unconsciously affects to what extent milk or its related products are accepted. Similar colour values were obtained from

tiger nut and coconut milk, ranged from 6.2-6.8 [24]. The lower tiger nut milk score from this study may be due to the brownish pigments in whole tiger nuts, which were leached during milk extraction.

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The aroma finding supports previous study for comparing tiger nut milk yoghurt 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]. While 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. Lower taste scores (4.90-5.95) were observed [11]. This result could be so because, animal protein sources have improved sensory perception of mouthfeel when consuming products like Yoghurt. Of which fat-filled and tiger nut milk yoghurt consist of plant-based fat.

Consistency displays the smoothness, thickness as well as mouthfeel of yoghurt samples. This statistic that the fat content in milk products enhances its mouthfeel and emulsifying features [11]. Therefore, skimmed milk with no fat content exhibited the least consistency by panelist scores, although it is perceived to be healthier, many consumers show preference for sensory over nutritional attributes.

Overall acceptability reveals to what extent consumers like or dislike a product on a general note putting all tested parameters into consideration [21]. Therefore, tiger nut stands a chance of being a major source for yoghurt on the shelves also taking into account its health benefits amongst other factors such as affordability and availability [2].

Conclusion

Yoghurt made from tiger nut milk and different forms of cow milk exhibited comparable microbial and sensory attributes. Yoghurt made from full-cream milk showed the highest microbial count and sensory attributes. Moreover, tiger nut milk yoghurt showed both high sensory and acceptable microbial values. Therefore, findings from this study indicate tiger nut milk as a suitable plant-based alternative to cow milk for the production of yoghurt. It is recommended that other plant based milk be looked into as alternatives to making cow milk yoghurt. This will result to sustainable varieties options.

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